BMC Urology

RESEARCH

Open Access

Comparative study of trace metal concentration in the diagnosis of category III prostatitis

Zhidi Lin[3,7†], Muyan Li[6†], Mingjin Zhang[5†], Yimin Gong[4], Xiang Gan[1,2], Weiyuan Liang[1,2], Yanjun Tan[1,4], Chong Zhang[1,2], Qian Gao[1,2] and Xiaoli Yang[1,2*]

Abstract

Background Chronic prostatitis (CP) is one of the general diseases in urological practice, with category III prostatitis being particularly prevalent. The trace metal abnormalities might be a primary cause of prostatitis, however, their specific roles in category III prostatitis remain largely unexplored.

Method In total, 42 expressed prostatic secretion (EPS) samples from IIIa prostatitis patients, 42 from IIIb prostatitis patients, and 45 from controls were collected, along with 42 serum samples from IIIa prostatitis patients, 45 from IIIb prostatitis patients, and 50 from controls for analysis in this study. To investigate the diagnostic potential of trace metals in category III prostatitis, we analyzed the concentration of zinc (Zn), copper (Cu), calcium (Ca) and magnesium (Mg) in EPS and serum of patients with category III prostatitis and healthy controls using a flame atomic absorption spectrometer (FAAS).

Results The Results showed that the concentrations of Zn, Ca and Mg in both serum and EPS samples of all subjects with category III prostatitis were significantly different compared to controls (***all P < 0.05***), while Cu levels were significantly altered in all EPS samples (***P < 0.000***). In the category of IIIa prostatitis group, the levels of Zn, Ca, Mg in EPS, as well as Ca in serum were significantly reduced (***all P < 0.000***), whereas the serum Zn level was markedly elevated (***P < 0.000***). In the category IIIb prostatitis group, the EPS levels of Zn, Ca, Mg were decreased significantly (***all P < 0.05***), and the levels of serum Ca, Mg were markedly decreased (***all P < 0.000***), however, the EPS Cu level increased significantly (***P < 0.05***). Moreover, receiver operating characteristic (ROC) analysis showed that the levels of Mg and Zn/Mg in EPS had better diagnostic value for category IIIa prostatitis (***Area Under the ROC Curve*(*AUC*) = *0.796*, *0.791*, *respectively*, *all*** $P < 0.0001$); while Cu and Cu/Ca levels exhibited better diagnostic value for category IIIb prostatitis (***AUC = 0.880***, ***0.901***, ***respectively***, ***all P < 0.0001***).

†Zhidi Lin, Muyan Li and Mingjin Zhang contributed equally to this work and should be considered as co-first authors.

*Correspondence:
Xiaoli Yang
cncsyxl@glmc.edu.cn

Full list of author information is available at the end of the article

Conclusion Summarily, there are significant abnormalities in the concentrations of Cu, Mg, Ca, and Zn in EPS and serum samples of patients with category III prostatitis. The levels of Mg, Cu, Zn/Mg, Cu/Ca in EPS may serve as potential diagnostic markers for category III prostatitis.

Keywords Category III prostatitis, FAAS, Trace metal, Diagnostic potential

Introduction

Chronic prostatitis (CP) is a common disease in adult male patients [1]. The U.S. National Institutes of Health (NIH) classified prostatitis into four categories in 1995, with category III, known as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), being the most prevalent. It accounts for 90-95% of all prostatitis diagnoses, while only about 8% of patients are affected by other categories [2, 3]. The estimated prevalence of CP/CPPS in the male population is 15% [4–6]. According to the latest version of the NIH consensus classification, the diagnosis of CP/CPPS is based on whether presence of white blood cells (WBCs) in expressed prostatic secretion (EPS), the first voided urine (VB3) after prostate massage, or in semen analysis [7]. Within this classification, CP/CPPS is further divided into two forms: IIIa, which refers to inflammatory CP/CPPS with WBCs present in semen, EPS, or VB3, and IIIb, which refers to non-inflammatory CP/CPPS with no WBCs detected in these fluids [6].

CP/CPPS is characterized by discomfort in the pelvic, genital, and suprapubic areas and is often associated with urinary symptoms and sexual dysfunction [6, 8]. Studies suggest that CP/CPPS may be caused by various factors, including oxidative stress (OS), abnormal immune response, intra-prostatic urinary reflux, pathogen infection, neurological and endocrine system dysfunction, and Th17 cell-driven autoimmunity [7–10]. However, due to the lack of definitive causative factors, the etiology and pathogenesis of chronic prostatitis remain unclear, making diagnosis challenging for urologists, and treatment outcomes often unsatisfactory.

Metals play a vital role in various aspects of human health, as multiple metal ions, such as copper (Cu), zinc (Zn), calcium (Ca), and iron (Fe), are present in the human body. These ions are crucial components of several metalloproteins and are essential for maintaining overall health by participating in various metabolic and biochemical processes [11, 12]. Imbalanced trace metals can lead to diseases [13]. Research indicates that balanced levels of trace minerals, including Fe, Zn, selenium (Se) and Cu, are essential to prevent the progression of chronic inflammation like periodontitis [14]. Moreover, research suggests that lower blood manganese levels are associated with a higher risk of glaucoma [15]. Additionally, an imbalance of trace metals can contribute to cancer development through complex mechanisms involving DNA repair, oxidative damage, disturbance of signal transduction pathways, and maintenance of redox homeostasis, potentially leading to metal-induced carcinogenicity [16].

In recent years, studies have increasingly focused on the specific role of metal ions in various diseases, including prostatitis. It is well known that the adult male prostate accumulates significant amounts of trace metals such as zinc (Zn), calcium (Ca), and selenium (Se). Research has reported that both Zn and cadmium (Cd) are associated with the development of prostate diseases [17, 18], and trace metal abnormalities might be a primary cause of prostatitis [19]. In a healthy prostate, Zn accumulation is higher than in any other glandular organ [20], its levels are reported to decrease significantly [21]. Based on this knowledge, we aimed to investigate the concentrations of Cu, Zn, Ca, and Mg, in both EPS and serum of patients with CP/CPPS, as well as in healthy controls. Our goal was to determine whether changes in trace metal concentrations in CP/CPPS are significant compared to healthy controls and to explore potential new strategies for the diagnosis and treatment of category III prostatitis.

Materials and methods

Patients

The patients included in this study were individuals with category III prostatitis who underwent physical examinations in the Guilin area of Guangxi, China. The sample collection was approved by the Guilin Medical University Ethics and Anthropology Committee (Number: GLMC202001011). The study was conducted in accordance with the Committee's guidelines, with informed consent obtained from all participating patients.

Clinical trial number: not applicable.

All patients were diagnosed with the type III prostatitis (CP/CPPS) according to the NIH criteria. Eligibility requirements included: (1) negative results of bacterial culture of post-massage EPS; (2) no history of treatment with antibiotics and α-receptor blocker; (3) no history of urinary tract infections, benign prostatic hyperplasia, and other pelvic organ diseases.

Exclusion criteria

(1) urinary tract infection; (2) history of urinary cancer, surgery, radiotherapy, systemic chemotherapy; (3) unilateral testicular pain, active urethral stricture or bladder stone with pelvic symptoms, or any other urinary disease associated with lower urinary tract symptoms, any

neurological disease or disorder affecting the bladder; (4) did not sign informed consent.

Based on the NIH prostatitis classification standard, the participants were classified into 3 groups: category IIIa prostatitis group; category IIIb prostatitis group and control group. We obtained the serum and EPS from the same subjects at first. Thus 42 cases of IIIa prostatitis, 45 cases of IIIb prostatitis, and 50 cases of control patients were included in the experiment, and their serum and EPS were collected respectively. However, due to the limited sample volume of EPS, 5 cases of control, 3 cases of IIIb prostatitis EPS samples were consumed for pre-experiments. Therefore, 129 EPS samples (42 cases of IIIa prostatitis, 42 cases of IIIb prostatitis, and 45 cases of control) and 137 serum samples (42 cases of IIIa prostatitis, 45 cases of IIIb prostatitis, and 50 cases of control) were collected and analyzed in this study. The disease characteristics of the patients were shown in Table 1.

Blood sample collection

Blood sample collection was performed in the morning before eating. Serum was collected by allowing the blood to coagulate for 30 min, centrifuged at 1200×g for 10 min at 4 °C. And then, serum samples were stored at -80 °C until assays. All blood samples were collected prior to the administration of any diagnosis.

Table 1 Baseline of clinical and demographic characteristics of all subjects for flame absorption spectroscopy (FAAS) analysis

	Healthy control (*n* = 45)	Category IIIa prostatitis (*n* = 42)	Category IIIb prostatitis (*n* = 42)
Age[a]	36.85(6.56)	41.71(8.02)	38.83(7.29)
Height (cm)	168.92(3.82)	169.11(3.53)	166.49(4.60)
Weight (kg)	67.57(4.76)	68.34(4.23)	67.06(4.82)
NIH-CPSI[b]	-	19(4.25)	20(3.00)
WBC in EPS	-	+ (*n* = 11)	-
	-	+ + (*n* = 8)	-
	-	+ + + (*n* = 12)	-
	-	+ + + + (*n* = 11)	-
SPL in EPS	+ (*n* = 3)	+ (*n* = 3)	+ (*n* = 4)
	+ + (*n* = 2)	+ + (*n* = 9)	+ + (*n* = 6)
	+ + + (*n* = 13)	+ + + (*n* = 20)	+ + + (*n* = 22)
	+ + + + (*n* = 22)	+ + + + (*n* = 9)	+ + + + (*n* = 8)
	+ + + + + (*n* = 5)	+ + + + + (*n* = 1)	+ + + + + (*n* = 2)

WBC, white blood cell; EPS, expressed prostatic secretion; SPL, small particle of lecithin, lecithin corpuscles. + of WBC in EPS: +,10–20 leukocytes/High Power field (HP); + +, 21–30 leukocytes/HP; + + +, 31–40 leukocytes/HP; + + + +, 41–50 leukocytes/HP. + of SPL in EPS: +, none or scattered; + +, 1/4 of the visual field; + + +, 1/2 of the visual field; + + + +, 3/4 of the visual field, + + + + +, full of vision

a, mean (standard deviation)

b, medians (interquartile range)

EPS sample collection

EPS fluids were collected in 1.5 mL tubes directly from the genitals after the patients' prostates were massaged by urologist. Part of the EPS was taken for routine microscopic examination and bacterial culture and the rest of EPS was centrifugated in low speed to remove cell pellets/sediments. The supernatant aliquots were placed in a clean 1.5 mL centrifuge tube and stored at -80 °C before analysis.

Trace metal concentration detection

Cu, Zn, Ca and Mg concentration in both serum and EPS samples were determined using BH-5100 5-channel flame atomic absorption spectrometer (Bohui, Beijing, China) equipped with hollow cathode lamps (213.9 nm, 324.3 nm, 422.7 nm, 285.2 nm, respectively). The levels of Cu, Zn, Ca and Mg were calculated using standard curve method (all $r > 0.995$), which performed using several certified reference materials from General Administration of Quality, Inspection and Quarantine of the People's Republic of China (GBW(E) 080915–080917). The unit of Cu, Zn was μmol/L, while the unit of Ca and Mg was mmol/L. All serum and EPS samples were processed using special detection reagent kit for human elements by atomic absorption spectrometer (Bohui, Beijing, China): a total of 40 μL serum or EPS sample was obtained by adding 1.2 mL of Bohui multi-elemental detection reagents and shaken for inspection (the dilution was 31). Linearity of four elements were generated, the dilutions of samples were 20%, 40%, 60%, 80% and 100% respectively (all $r > 0.99$). QC measurements to the baseline stability trace elements did not exceed 0.005 Abs. Recovery rates were all >96%, and the sample carrying rate of four elements were low (all values<5%). Sensitivity was characterized by the levels of Cu, Zn, Ca and Mg which were less than 0.035, 0.015, 0.080, 0.040 mg/L/1%, respectively. The upper limit of precision was set at 0.1% (Abs>0.1). Materials used in the experiment including reagents, quality product and element standard liquid were all manufactured by Beijing Bohui Innovation Technology Co., Ltd. And the method could refer to our pervious article [22].

Statistical analysis

The SPSS software 19.0 and Medcalc software were used to perform statistical analysis. Mann-Whitney U test and Kruskal-Wallis test used to compare the concentration of Cu, Zn, Ca, Mg and the element ratio among patients with category IIIa and category IIIb prostatitis and the healthy control group. Pearson correlation was used to analyze the correlation between the levels of Cu, Zn, Ca, Mg and the element ratio in all participants' serum and EPS, the test level was $\alpha = 0.05$. And the P value < 0.05 was considered statistically significant. The receiver operating

characteristic (ROC) curve is used to determine the diagnostic value and the optimal cut-off value for each trace metal are defined as the point on the ROC curve with the largest Youden index (sensitivity + specificity-1).

Results

Parameters of descriptive statistics for the concentration of metals in serum and EPS

In this study, we analyzed the concentration of Cu, Zn, Ca and Mg in serum and EPS of CP/CPPS patients and control by flame atomic absorption spectrophotometry, respectively. Table 2 showed specific information of them. Firstly, compared to the control group, the contents of Zn, Ca and Mg in both serum and EPS samples of all subjects with CP/CPPS had significant changes, while the level of Cu changed significantly only in all EPS samples. And in the single category IIIa prostatitis group, the level of Ca and Mg decreased significantly in EPS compared with healthy controls (all $P<0.01$), same as serum Ca ($P<0.01$); serum Zn level increased ($P<0.01$), on the contrary, EPS Zn level decreased obviously ($P<0.01$); and the level Cu in EPS and serum had no palpable changes ($P>0.05$). Then in the independent category IIIb prostatitis group, just like the category IIIa prostatitis group, EPS Zn, Ca and Mg decreased markedly ($P<0.05$), also the levels of serum Ca and Mg were lessened visibly (all $P<0.01$). Differently, the EPS Cu level has an overt promotion ($P<0.01$) while Zn level of serum has no difference in category IIIb prostatitis.

Independent correlation of four metals in patients with CP/CPPS and healthy controls

As we can see from the Fig. 1, independent correlation of metals in three groups was analyzed. First of all, in the EPS samples, Zn level had a positive correlation with the levels of Ca and Mg in all three groups (all r values > 0.5, and all $P<0.01$); the level of Ca was positive correlated with Mg level obviously as well, especially in category IIIa prostatitis group, whose r value = 0.901 ($P<0.01$). In addition, EPS Cu level also correlated with EPS Ca level positively (r value = 0.35, $P=0.034$) in healthy controls while in category IIIa prostatitis group, EPS Cu

Table 2 Parameters of descriptive statistics for the content of metals in EPS and serum

			Cu (μmol/l)	Zn (μmol/l)	Ca (mmol/l)	Mg (mmol/l)
EPS	Healthy control ($n=45$)	mean	3.21	907.47	8.77	6.36
		median	2.44	928.24	8.45	6.75
		IQR	3.18	73.12	4.02	1.13
		Chi-Square[a]	12.28	13.41	14.73	19.8
		p[a]	***0.000***	***0.000***	***0.000***	***0.000***
	IIIa Group ($n=42$)	mean	3.51	794.97	6.15	4.75
		median	3.42	887.95	6.27	5.29
		IQR	3.66	162.79	4.18	2.74
		Z[b]	-0.541	-4.239	-4.154	-4.744
		p[b]	0.589	***0.000***	***0.000***	***0.000***
	IIIb Group ($n=42$)	mean	8.57	838.44	7.22	5.39
		median	9.27	910.71	6.46	5.89
		IQR	4.51	141.75	5.87	2.96
		Z[b]	-5.629	-2.056	-2.446	-2.905
		p[b`]	***0.000***	***0.040***	***0.014***	***0.004***
Serum	Healthy control ($n=45$)	mean	16.340	15.92	2.34	0.89
		median	15.730	15.29	2.35	0.88
		IQR	6.100	4.8	0.15	0.068
		Chi-Square[a]	0.094	4.772	21.758	10.706
		p[a]	0.759	***0.029***	***0.000***	***0.001***
	IIIa Group ($n=42$)	mean	17.310	19.41	2.28	0.87
		median	17.080	18.84	2.27	0.86
		IQR	3.720	5.57	0.13	0.1
		Z[b]	-1.690	-3.944	-3.673	-1.506
		p[b]	0.091	***0.000***	***0.000***	0.132
	IIIb Group ($n=45$)	mean	15.450	15.2	2.22	0.83
		median	15.130	15.88	2.24	0.83
		IQR	4.390	4.15	0.125	0.095
		Z[b]	-1.093	-0.108	-4.283	-4.023
		p[b]	0.273	0.914	***0.000***	***0.000***

a, Kruskal-Wallis test; b, Mann-Whitney U test, compared to control group; IQR: Interquartile range (IQR) calculated as the upper quartile (XU) – lower quartile (XL)

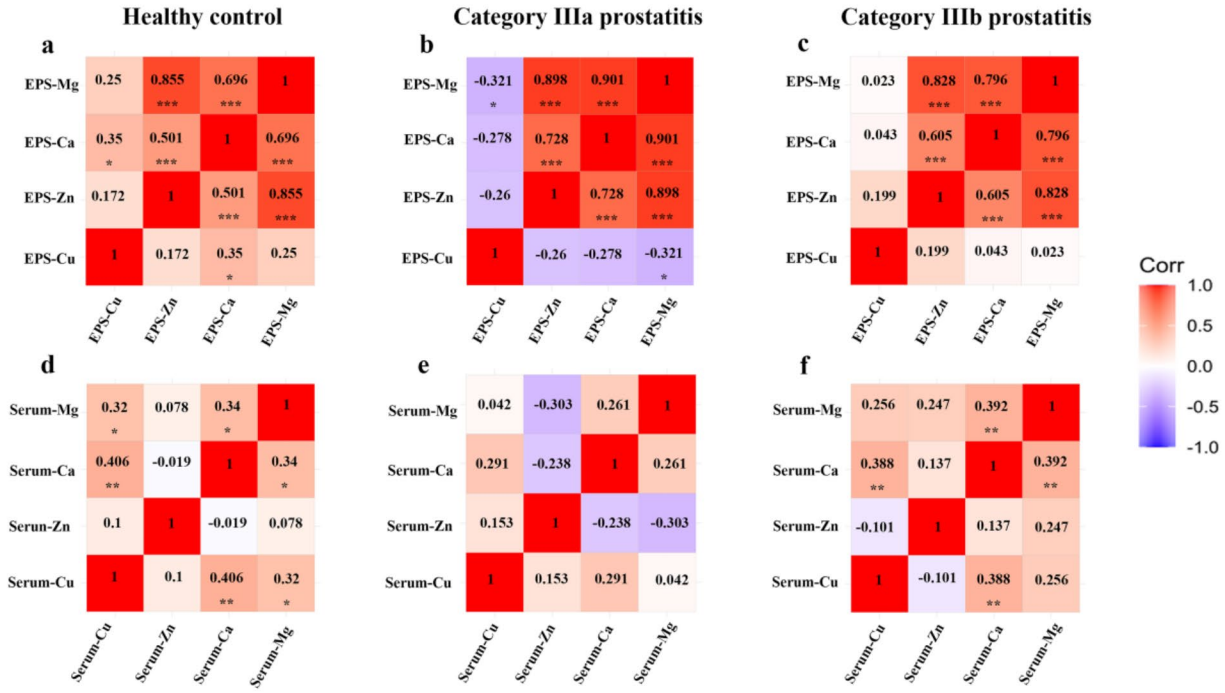

Fig. 1 Heatmap analysis of independent correlation of metals in patients with category III prostatitis and healthy controls. **a**, correlation of EPS metal levels in healthy controls. **b**, correlation of EPS metal levels in in patients with category IIIa prostatitis. **c**, correlation of EPS metal levels in patients with category IIIb prostatitis. **d**, correlation of serum metal levels in healthy controls. **e**, correlation of serum metal levels in in patients with category IIIa prostatitis. **f**, correlation of serum metal levels in patients with category IIIb prostatitis. EPS, expressed prostatic secretion. *, P-value < 0.05. **, P-value < 0.01, ***, P-value < 0.001

level has a negative correlation with EPS Mg (r value = -0.321, $P=0.046$). On the other hand, the level of serum Cu had statistically significant positive correlation with serum Ca and Mg in healthy control group, same as the relation between serum Ca and Mg (all $P<0.05$). And in the category IIIb prostatitis group, serum Cu correlated significantly with serum Ca ($P<0.01$), serum Ca was also positive related with serum Mg ($P<0.01$). However, there was no obvious relation among these four trace elements in serum samples of category IIIa prostatitis group.

Comparison of ratio in related elements between CP/CPPS and healthy controls

By comparing the ratio of notably relative trace metals, it was found that in EPS samples, the ratio of Cu/Ca, Zn/Ca, Zn/Mg in both category IIIa and IIIb prostatitis were significantly increased (all $P<0.05$). And in serum samples, the ratio of Cu/Ca and Cu/Mg improved prominently in category IIIa prostatitis group (all $P<0.01$). The results were listed in the Table 3.

ROC analysis in single element and element ratio with significant changes for the diagnosis in category IIIa and IIIb prostatitis

Based on the results obtained above, we constructed a ROC curve by plotting the relationship between sensitivity and specificity to evaluate the diagnostic significance of significantly changed metals (Cu, Zn, Ca, Mg in EPS and Zn, Ca in serum) and element ratios (Cu/Ca, Zn/Ca, Zn/Mg in EPS and Cu/Ca, Cu/Mg in serum) for category IIIa and category IIIb prostatitis. Calculated the Area Under the ROC Curve (AUC), which is a commonly used indicator for evaluating the diagnostic efficacy of potential biomarkers. To begin with, for the diagnosis of category IIIa prostatitis, the AUC of Zn, Ca, Mg, Cu/Ca, Zn/Ca, Zn/Mg in EPS were 0.764, 0.759, 0.796, 0.638, 0.700, 0.791, respectively, which predicts the sensitivity and specificity of category IIIa prostatitis were 76.19% and 73.33%, 76.19% and 68.89%, 85.71% and 71.11%, 78.72% and 81.08%, 88.10% and 42.22%, 90.48% and 62.22%, respectively (all $P<0.05$); and serum Zn and Ca also had a high AUC of 0.740 and 0.723 ($P<0.01$), followed by serum Cu/Ca and Cu/Mg, whose AUC were 0.663 and 0.636 ($P<0.05$). Afterwards, for the diagnostic efficacy of category IIIb prostatitis, EPS Cu and Cu/Ca appeared to have higher AUC of 0.880, 0.901 ($P<0.01$) respectively, which predicts the sensitivity and specificity of category

Table 3 The comparison of elements level between category III prostatitis and healthy controls

	Element ratio		Healthy control (*n* = 45)	Category IIIa prostatitis (*n* = 42)	Category IIIb prostatitis (*n* = 42)
EPS	Cu/Ca[b]	mean	0.000370	0.000925	0.001405
		median	0.000321	0.000517	0.001182
		IQR	0.0004	0.0006	0.0013
		P value	-	***0.038***	***0.000***
	Zn/Ca[b]	mean	0.00371	0.16152	0.13724
		median	0.1046	0.13361	0.12962
		IQR	0.0390	0.0896	0.0890
		P value	-	***0.001***	***0.048***
	Zn/Mg[b]	mean	0.15212	0.18774	0.17379
		median	0.13650	0.16086	0.14928
		IQR	0.0145	0.0618	0.0304
		P value	-	***0.000***	***0.009***
	Ca/Mg[b]	mean	1.3819	1.2531	1.3499
		median	1.3272	1.2566	1.3597
		IQR	0.2641	0.3231	0.5766
		P value	-	0.108	0.541
Serum	Cu/Ca[b]	mean	0.006962	0.007672	0.006862
		median	0.006758	0.007523	0.006716
		IQR	0.0022	0.0017	0.0019
		P value	-	***0.007***	0.842
	Cu/Mg[b]	mean	0.018456	0.20114	0.01863
		median	0.018697	0.01994	0.01863
		IQR	0.0066	0.0049	0.0061
		P value	-	***0.025***	0.733
	Ca/Mg[b]	mean	2.6561	2.6212	2.7157
		median	2.6448	2.6114	2.6711
		IQR	0.2690	0.2757	0.2944
		P value	-	0.402	0.269

b, Mann-Whitney U test, compared to control group; IQR: Interquartile range (IQR) calculated as the upper quartile (XU) – lower quartile (XL)

IIIb prostatitis were 70.27% and 100.00%, 75.00% and 97.06%; and the AUC of EPS Zn, Ca, Mg, Zn/Ca, Zn/Mg, serum Ca and Mg all were greater than 0.6 (all $P<0.05$). Table 4; Figs. 2 and 3 displayed detailed information about the diagnostic threshold of category III prostatitis.

Discussion

Category III prostatitis is the most common type of chronic prostatitis. Patients with Category III prostatitis always have chronic pelvic pain symptoms and possibly voiding symptoms in the absence of urogenital infections [23], which has a significant negative impact on the quality of life. The etiology of most CP/CPPS cases is unclear, and a debated cause is that CP/CPPS may be due to infections such as bacteria or viruses [23, 24]. K Wenninger et al., reported that the sickness of chronic prostatitis patients is similar to the patients suffering myocardial infarction, angina or Crohn's disease, according to their sickness impact profile mean total score, which is within the range of scores of those diseases reported in the literature [25]. And it was reported that the prevalence of sexual dysfunction in Chinese males with chronic prostatitis has a negative correlation with age, and the duration of chronic prostatitis (all $P<0.01$) [26], several studies also suggested that CP/CPPS might be related to the development of prostate cancer [27, 28]. Because of the accompanied calcification, the effective treatment of category III prostatitis is difficult. CP/CPPS has seriously affected the quality of males' life. Previous studies suggested that trace metals, such as Zn, play an important role in the development of prostatitis [29, 30]. Therefore, we studied the difference between the concentrations of Cu, Zn, Ca, Mg in healthy controls compared to the patients with CP/CPPS to provide insights of clinical diagnosis with trace metals in CP/CPPS.

In our study, Zn was significantly changed. It is an essential trace element, required for maintenance of structural integrity and DNA binding activity in over 2,000 transcription factors [31]. It has been reported that human seminal plasma contains high concentrations of Zn, primarily secreted by the prostate gland [32]. It plays a crucial physiological and pathological role in male health, as it is necessary for preserving the lining of the reproductive organs and may regulate the processes of capacitation and the acrosome reaction, the lack of Zn may weaken the immune and reproductive system [33]. In addition, Paola Bonaventura indicated that acute Zn deficiency could cause a decrease in innate and adaptive immunity, and chronic deficiency might increase inflammation [34]. In our previous study and present study, we found that the level of Zn in EPS was higher in the healthy controls than it in CP/CPPS [35], suggesting that the deficiency of Zn in EPS is closely related to the chronic prostatitis, but the specific mechanism still needs to be explored in depth. Nowadays, biologic Zn therapy is positively effective on sperm motility and the use of biologic Zn supplementation is an efficient way for the treatment of infertile males with chronic prostatitis [36]. In addition, we also found serum Zn level appears to be higher in category IIIa prostatitis, although no direct evidence showed that chronic prostatitis is related to prostate cancer, the level of serum Zn had been reported to be positively associated with prostate cancer risk [37]. Also, many researchers have confirmed that Zn^{2+} is significantly positively correlated with the risk of prostate cancer [37], when the concentration of Zn^{2+} and zinc transporter ZIP1 in the body is down-regulated, it is often accompanied by the oncogenic genetic transformation of prostate cells from a normal state to a tumor state with malignant potential [38]. Therefore, we think it is still valuable to further study the role of Zn level between chronic prostatitis and prostate cancer.

Table 4 The results of ROC analysis in elements with significant changes

		AUC	95%CI	Z	*P*	Cut off	Sensitivity	Specificity
Category IIIa prostatitis	**EPS**							
	Zn	0.764	0.661 to 0.848	5.179	***< 0.0001***	908.335 (μmol/l)	76.190	73.330
	Ca	0.759	0.655 to 0.844	5.061	***< 0.0001***	7.835 (mmol/l)	76.190	68.890
	Mg	0.796	0.696 to 0.874	5.991	***< 0.0001***	6.42 (mmol/l)	85.710	71.110
	Cu/Ca	0.638	0.556 to 0.786	2.743	***0.006***	0.0005	54.290	79.410
	Zn/Ca	0.700	0.592 to 0.794	3.583	***0.000***	0.0958	88.100	42.220
	Zn/Mg	0.791	0.691 to 0.871	5.795	***< 0.0001***	0.1383	90.480	62.220
	Serum							
	Zn	0.740	0.638 to 0.825	4.551	***< 0.0001***	15.880 (μmol/l)	85.710	60.000
	Ca	0.723	0.620 to 0.811	4.199	***< 0.0001***	2.350 (mmol/l)	88.100	52.000
	Cu/Ca	0.663	0.557 to 0.759	2.841	***0.0045***	0.0068	85.710	52.000
	Cu/Mg	0.636	0.529 to 0.734	2.334	***0.0196***	0.0168	90.480	40.000
Category IIIb prostatitis	**EPS**							
	Cu	0.880	0.784 to 0.944	8.496	***< 0.0001***	7.32 (μmol/l)	70.270	100.000
	Zn	0.628	0.518 to 0.729	2.119	***0.034***	866.85 (μmol/l)	42.860	82.220
	Ca	0.652	0.543 to 0.751	2.472	***0.013***	7.595 (mmol/l)	64.290	71.110
	Mg	0.681	0.572 to 0.777	3.072	***0.002***	6.35 (mmol/l)	69.050	71.110
	Cu/Ca	0.901	0.806 to 0.960	10.258	***< 0.0001***	0.0009	75.000	97.060
	Zn/Ca	0.623	0.513 to 0.725	1.977	***0.048***	0.1223	59.520	73.330
	Zn/Mg	0.662	0.553 to 0.760	2.679	***0.007***	0.1377	73.810	60.000
	Serum							
	Ca	0.755	0.656 to 0.838	5.063	***< 0.0001***	2.27 (mmol/l)	71.110	74.000
	Mg	0.740	0.640 to 0.824	4.611	***< 0.0001***	0.855 (mmol/l)	71.110	76.000

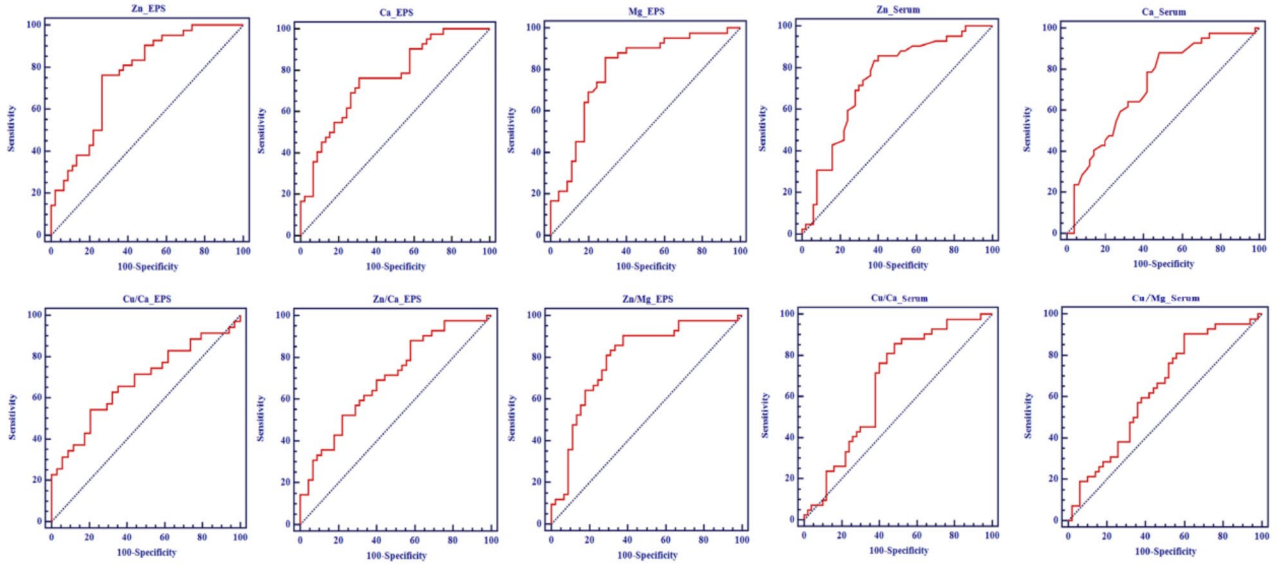

Fig. 2 ROC analysis of trace element for the diagnosis in category IIIa prostatitis. ROC analysis of Zn, Ca, Mg in EPS; Zn, Ca in serum and Cu/Ca, Zn/Ca, Zn/Mg ratio in EPS; Cu/Ca, Cu/Mg ratio in serum for the diagnosis in category IIIa prostatitis

The next interesting metal is Cu. Cu is an essential cofactor or a structural component in a number of meaningful enzymes, such as Cu/Zn superoxide dismutase (SOD1), which has functions in superoxide detoxification and signaling [39]. However, the over-loaded copper in a free state can trigger the production of a large number of free radicals, leading to the destruction of protein and DNA, and causing the inflammatory changes or even cancer in cells [40, 41]. Our previous study suggested that superoxide dismutase 3 (SOD3) containing catalytic copper and structural inc ions in their active sites is significantly higher in all types of prostatitis than in the control, and is a potential diagnostic marker of nonbacterial prostatitis [35, 39]. O A Adaramoye et al. also reported that

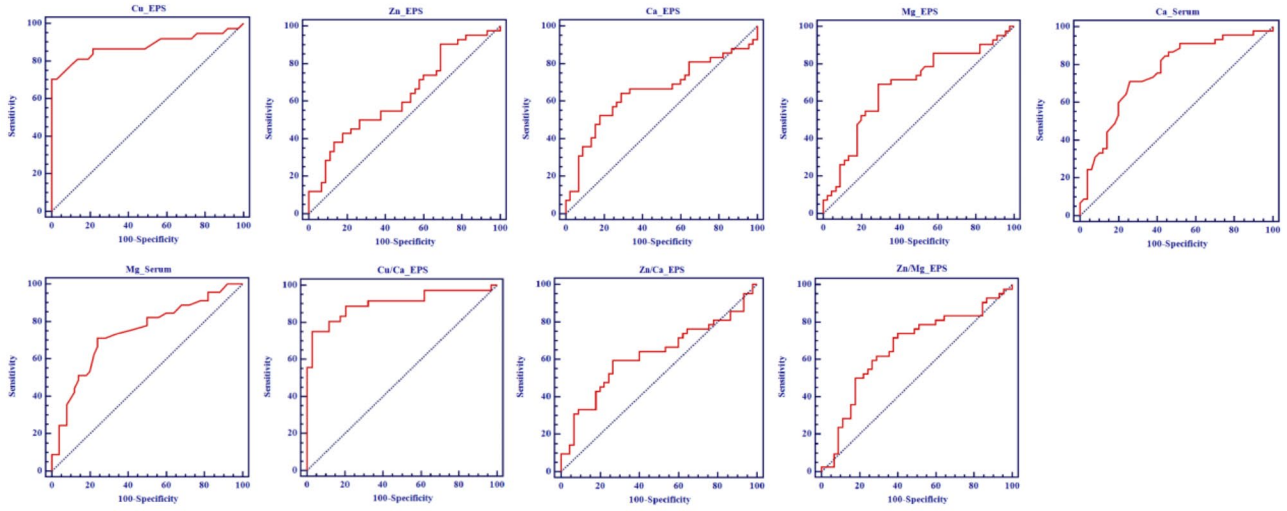

Fig. 3 ROC analysis of trace element for the diagnosis in category IIIb prostatitis. ROC analysis of Cu, Zn, Ca, Mg in EPS; Ca, Mg in serum and Cu/Ca, Zn/Ca, Zn/Mg ratio in EPS

the levels of whole blood superoxide dismutase (SOD) were significantly lower ($P<0.05$) in the PCa patients [42]. So we speculated that the increase of Cu may be one of the pathogenesis of category III prostatitis, and the prostatitis even cancer may be developed by effecting the expression of these Cu related proteins. In this data, the concentration of EPS Cu had a marked increase only in category IIIb prostatitis and the AUC of EPS Cu, Cu/Ca for predicting category IIIb prostatitis were greater than 0.88 ($P<0.0001$), indicating that Cu could be a useful factor in category IIIb prostatitis.

Ca and Mg were analyzed in this study as well. Their EPS concentrations were reduced sharply and positively correlated with EPS Zn level in both category IIIa and IIIb prostatitis. Chang-Sheng Zhan et al. found that calcium/calmodulin-dependent kinase III (CaMK4) was increased in the Th17 cells of experimental autoimmune prostatitis, which was activated by intracellular cytosolic Ca^{2+}, Ca^{2+} -CaMK4-Akt/mTOR-IL-17 A axis inhibition may serve as a promising therapeutic strategy for CP/CPPS [10]. Few studies explore the relationship between Ca, Mg level and CP/CPPS directly. C Y Yang etc. investigated the possible association between the increased risk of prostate cancer and the concentrations of Ca and Mg in drinking water from municipal supplies, and they found that the relationship between Ca levels in drinking water and prostate cancer was not statistically clear while Mg intake from drinking water and other dietary sources may has a significant protective effect against the risk of prostate cancer development [43]. Besides, both EPS and serum Ca, Mg had a higher AUC for predicting category IIIa and IIIb prostatitis. Although we have obtained some great discoveries through this study, some limitations are still obvious. The type of design of clinical trial was non-randomized, non-blinded. The total samples are small, and all samples were collected from patients who attended the healthy physical examination in the Guilin area in Guangxi, China, and the results could not be extrapolated to the whole population reliably, and in the future, it is necessary to use multicenter, randomized, double-blind clinical randomized controlled trials in further research to confirm our findings. And the trace metal elements may be also related to the patient's condition and psychological factors, which is also the direction of our future research. In future studies, we will further explore the relationship between trace metal elements and prostatitis by combining the relevant standards of UPOINT classification. Based on results above, further learning the function of these metals in category III prostatitis is important, and the specific role of these metals related proteins may also have potential research value.

Conclusion

Our study demonstrates that the concentration of Cu, Mg, Ca, Zn in EPS and serum samples with category III prostatitis have notable changes compared with those in healthy controls, the level of Mg, Zn/Mg in EPS might be promising to diagnose category IIIa prostatitis, and level of Cu and Cu/Ca in EPS could diagnose category IIIb prostatitis. It is recommended that these four elements may play important roles in the process of category III prostatitis, which provides a new strategy and further support for the diagnosis and treatment of this disease. And the specific functions of trace metals in category III prostatitis might need further investigation.

Acknowledgements

We would like to express our gratitude to Professor Jian Qin (Guangxi Medical University of Public Health) for his guidance and assistance in statistical analysis of the manuscript.

Author contributions
Zhidi Lin: Resources, Methodology, Data Curation. Mingjin Zhang: Validation and Resources. Muyan Li: Methodology. Zhidi Lin, Mingjin Zhang and Yimin Gong: Writing - Original Draft. Xiang Gan and Weiyuan Liang: Writing - Review & Editing. Yanjun Tan: Validation. Qian Gao: Investigation. Chong Zhang: Conceptualization. Xiaoli Yang: Supervision, Project administration.

Funding
This work was supported by the National Natural Science Foundation of China (Grant number: 22164007) and the Guangxi Nature Science Foundation (Grant number:2023GXNSFAA026307). Project for Enhancing Young and Middle-aged Teacher's Research Basis Ability in Colleges of Guangxi (Grant number: 2022KY0490, 2022KY0495). Guilin Science and Technology (Grant number: 20220139-6-1, 20210218-6).

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the the Guilin Medical University Ethics and Anthropology Committee (Number: GLMC202001011), and written informed consent was obtained from the patient.

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Scientific Research Center, Guilin Medical University, Guilin, Guangxi, China
[2]Guangxi Health Commission Key Laboratory of Disease Proteomics Research, Guilin, Guangxi, China
[3]Department of Urology, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China
[4]Pharmaceutical College, Guangxi Medical University, Nanning, Guangxi, China
[5]The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China
[6]Guangxi-ASEAN Collaborative Innovation Center for Major Disease Prevention and Treatment, Guangxi Medical University, Nanning, Guangxi, China
[7]Key Laboratory of Research and Development on Clinical Molecular Diagnosis for High-Incidence Disease of Baise, Baise, Guangxi, China

Received: 22 June 2024 / Accepted: 13 November 2024
Published online: 27 November 2024

References

1. Zhang J, Liang C, Shang X, Li H. Chronic Prostatitis/Chronic Pelvic Pain Syndrome: a Disease or Symptom? Current perspectives on diagnosis, treatment, and prognosis. Am J Men's Health. 2020;14(1):1557988320903200. https://doi.org/10.1177/1557988320903200.
2. Clemens JQ, Meenan RT, O'Keeffe Rosetti MC, Gao SY, Calhoun EA. Incidence and clinical characteristics of National institutes of Health type III prostatitis in the community. J Urol. 2005;174(6):2319–22. https://doi.org/10.1097/01.ju.0000182152.28519.e7.
3. Schaeffer AJ, Landis JR, Knauss JS, Propert KJ, Alexander RB, Litwin MS, Nickel JC, O'Leary MP, Nadler RB, Pontari MA, Shoskes DA, Zeitlin SI, Fowler JE Jr., Mazurick CA, Kisqhel L, Kusek JW. Nyberg. Demographic and clinical characteristics of men with chronic prostatitis: the national institutes of health chronic prostatitis cohort study. J Urol. 2002;168(2):593–8.
4. Liu Y, Mikrani R, Xie D, Wazir J, Shrestha S, Ullah R, Baig M, Ahmed A, Srivastava PK, Thapa KB, Zhou X. Chronic prostatitis/chronic pelvic pain syndrome and prostate cancer: study of immune cells and cytokines. Fundam Clin Pharmacol. 2020;34(2):160–72. https://doi.org/10.1111/fcp.12517.
5. Kim HK, Kim HJ, Hong JY, Park J, Lee HC, Lyu H, Cho SB. Interactive tissue reactions of 1064-nm focused picosecond-domain laser and dermal cohesive polydensified matrix hyaluronic acid treatment in in vivo rat skin. Skin research and technology: official journal of International Society for Bioengineering and the skin (ISBS) [and] International Society for Digital Imaging of skin (ISDIS) [and] International Society for skin imaging. (ISSI). 2020;26(5):683–9. https://doi.org/10.1111/srt.12853.
6. Morgia G, Mucciardi G, Galì A, Madonia M, Marchese F, Di Benedetto A, Romano G, Bonvissuto G, Castelli T, Macchione L, Magno C. Treatment of chronic prostatitis/chronic pelvic pain syndrome category IIIA with Serenoa repens plus selenium and lycopene (Profluss) versus S. repens alone: an Italian randomized multicenter-controlled study. Urol Int. 2010;84(4):400–6. https://doi.org/10.1159/000302716.
7. Magistro G, Wagenlehner FME, Pilatz A. Chronische Prostatitis/chronisches beckenschmerzsyndrom [Chronic prostatitis/chronic pelvic pain syndrome]. Urologie. 2023;62(6):590–6. https://doi.org/10.1007/s00120-023-02089-2.
8. Li J, Dong L, Yan X, Liu X, Li Y, Yu X, Chang D. Is acupuncture another good choice for Physicians in the treatment of Chronic Prostatitis/Chronic Pelvic Pain Syndrome? Review of the latest literature. Pain Res Manage. 2020;2020. https://doi.org/10.1155/2020/5921038.
9. Solakhan M, Cicek H, Orhan N, Yildirim M. Role of native Thiol, total Thiol and dynamic disulphide in diagnosis of patient with prostate cancer and prostatitis. Int Braz J Urol. 2019;45(3):495–502. https://doi.org/10.1590/s1677-5538.ibju.2018.0469.
10. Zhan CS, Chen J, Chen J, Zhang LG, Liu Y, Du HX, Wang H, Zheng MJ, Yu ZQ, Chen XG, Zhang L, Liang CZ. CaMK4-dependent phosphorylation of Akt/mTOR underlies Th17 excessive activation in experimental autoimmune prostatitis. FASEB Journal: Official Publication Federation Am Soc Experimental Biology. 2020;34(10):14006–23. https://doi.org/10.1096/fj.201902910RRR.
11. Chen P, Bornhorst J, Diana Neely M, Avila DS. Mechanisms and Disease Pathogenesis Underlying Metal-Induced oxidative stress. Oxidative medicine and cellular longevity 2018 (2018) 7612172, https://doi.org/10.1155/2018/7612172
12. Ozmen H, Erulas FA, Karatas F, Cukurovali A. Yalcin. Comparison of the concentration of trace metals (Ni, Zn, Co, Cu and Se), Fe, vitamins a, C and E, and lipid peroxidation in patients with prostate cancer. Clin Chem Lab Med. 2006;44(2):175–9. https://doi.org/10.1515/cclm.2006.032.
13. Li J, Wang Y. Golgi metal ion homeostasis in human health and diseases[J]. Cells. 2022;11(2):289. https://doi.org/10.3390/cells11020289.
14. Gaur S, Agnihotri R. Trace mineral micronutrients and chronic periodontitis—a review[J]. Biol Trace Elem Res. 2017;176:225–38. https://doi.org/10.1007/s12011-016-0832-y.
15. Lin SC, Singh K, Lin SC. Association between body levels of trace metals and glaucoma prevalence[J]. JAMA Ophthalmol. 2015;133(10):1144–50. https://doi.org/10.1001/jamaophthalmol.2015.2438.
16. Valko M, Jomova K, Rhodes CJ, Kuča K. Musílek. Redox- and non-redox-metal-induced formation of free radicals and their role in human disease. Arch Toxicol. 2016;90(1):1–37. https://doi.org/10.1007/s00204-015-1579-5.
17. Drozdz-Afelt JM, Koim-Puchowska B, Kaminski P. Concentration of trace elements in blood of Polish patients with prostate cancer. Environ Toxicol Pharmacol. 2024;107:104425. https://doi.org/10.1016/j.etap.2024.104425+.
18. Karunasinghe N. Zinc in prostate health and disease: a Mini Review. Biomedicines. 2022;10(12):3206. https://doi.org/10.3390/biomedicines10123206. Published 2022 Dec 10.
19. Gong Y, Lin Z, Gan X, et al. Diagnostic potential of trace metals concentration in expressed prostatic secretion and serum of patients with category IV prostatitis. J Trace Elem Med Biol. 2021;68:126819. https://doi.org/10.1016/j.jtemb.2021.126819.
20. Sarafanov AG, Todorov TI, Kajdacsy-Balla A, Gray MA, Macias V, Centeno JA. Analysis of iron, zinc, selenium and cadmium in paraffin-embedded prostate tissue specimens using inductively coupled plasma mass-spectrometry. J Trace Elem Med Biology: Organ Soc Minerals Trace Elem (GMS). 2008;22(4):305–14. https://doi.org/10.1016/j.jtemb.2008.03.010.
21. Franklin RB, Milon B, Feng P, Costello LC. Zinc and zinc transporters in normal prostate and the pathogenesis of prostate cancer. Front Bioscience: J Virtual Libr. 2005;10:2230–9. https://doi.org/10.2741/1692.
22. Gong Y, Lin Z, Gan X, Yang G, Li M, Tan Y, Zheng S, Wang X, Luo Z, Jiao Y, Teng R, Yang X. Diagnostic potential of trace metals concentration in expressed

prostatic secretion and serum of patients with category IV prostatitis. J Trace Elem Med Biology: Organ Soc Minerals Trace Elem (GMS). 2021;68:126819. https://doi.org/10.1016/j.jtemb.2021.126819.
23. Khan FU, Ihsan AU, Khan HU, Jana R, Wazir J, Khongorzul P, Waqar M. Zhou. Comprehensive overview of prostatitis. Biomed Pharmacotherapy = Biomedecine Pharmacotherapie. 2017;94:1064–76. https://doi.org/10.1016/j.biopha.2017.08.016.
24. Ihsan AU, Khan FU, Nawaz W, Khan MZ, Yang M, Zhou X. Establishment of a rat model of chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) induced by immunization with a novel peptide T2. Biomed Pharmacotherapy = Biomedecine Pharmacotherapie. 2017;91:687–92. https://doi.org/10.1016/j.biopha.2017.05.004.
25. Wenninger K, Heiman JR, Rothman I, Berghuis JP, Berger RE. Sickness impact of chronic nonbacterial prostatitis and its correlates. J Urol. 1996;155(3):965–8.
26. Liang CZ, Zhang XJ, Hao ZY, Shi HQ, Wang KX. Prevalence of sexual dysfunction in Chinese men with chronic prostatitis. BJU Int. 2004;93(4):568–70. https://doi.org/10.1111/j.1464-410x.2003.04662.x.
27. Jiang J, Li J, Yunxia Z, Zhu H, Liu J, Pumill C. The role of prostatitis in prostate cancer: meta-analysis. PLoS ONE. 2013;8(12):e85179. https://doi.org/10.1371/journal.pone.0085179.
28. Boehm K, Valdivieso R, Meskawi M, Larcher A, Schiffmann J, Sun M, Graefen M, Saad F, Parent M, Karakiewicz PI. Prostatitis, other genitourinary infections and prostate cancer: results from a population-based case-control study. World J Urol. 2016;34(3):425–30. https://doi.org/10.1007/s00345-015-1625-1.
29. Mo LJ, Chen X, Wang XM, Li GY, Zhang X, Huang S, Xie ZB. Mo. [Reduced zinc concentration in expressed prostatic secretion relates to the pain symptoms of types II and III prostatitis]. Zhonghua Nan Ke xue = National. J Androl. 2016;22(6):496–500.
30. Cui D, Han G, Shang Y, Mu L, Long Q, Du Y. The effect of chronic prostatitis on zinc concentration of prostatic fluid and seminal plasma: a systematic review and meta-analysis. Curr Med Res Opin. 2015;31(9):1763–9. https://doi.org/10.1185/03007995.2015.1072707.
31. Wang J, Zhao H, Xu Z, Cheng X. Zinc dysregulation in cancers and its potential as a therapeutic target. Cancer Biology Med. 2020;17(3):612–25. https://doi.org/10.20892/j.issn.2095-3941.2020.0106.
32. Marmar JL, Katz S, Praiss DE, DeBenedictis TJ. Semen zinc levels in infertile and postvasectomy patients and patients with prostatitis. Fertil Steril. 1975;26(11):1057–63. https://doi.org/10.1016/s0015-0282(16)41470-6.
33. Fallah A, Mohammad-Hasani A, Colagar AH. Zinc is an essential element for male fertility: a review of zn roles in men's health, germination, sperm quality, and fertilization. J Reprod Infertility. 2018;19(2):69–81.
34. Bonaventura P, Benedetti G, Albarède F, Miossec P. Zinc and its role in immunity and inflammation. Autoimmun rev. 2015;14(4):277–85. https://doi.org/10.1016/j.autrev.2014.11.008.
35. Yang X, Li H, Zhang C, Lin Z, Zhang X, Zhang Y, Yu Y, Liu K, Li M, Zhang Y, Lv W, Xie Y, Lu Z, Wu C, Teng R, Lu S, He M, Mo Z. Serum quantitative proteomic analysis reveals potential zinc-associated biomarkers for nonbacterial prostatitis. Prostate. 2015;75(14):1538–55. https://doi.org/10.1002/pros.23028.
36. Deng CH, Zheng B. She. [A clinical study of biological zinc for the treatment of male infertility with chronic prostatitis]. Zhonghua Nan Ke Xue = Natl J Androl. 2005;11(2):127–9.
37. Lim JT, Tan YQ, Valeri L, Lee J, Geok PP, Chia SE, Ong CN, Seow WJ. Association between serum heavy metals and prostate cancer risk - A multiple metal analysis. Environ Int. 2019;132:105109. https://doi.org/10.1016/j.envint.2019.105109.
38. Yuan Y, Wei Z, Chu C, Zhang J, Song X, Walczak P, Bulte JWM. Development of zinc-specific iCEST MRI as an imaging biomarker for prostate Cancer. Angew Chem Int Ed Engl. 2019;58(43):15512–7. https://doi.org/10.1002/anie.201909429.
39. Scheiber I, Dringen R, Mercer JF. Copper: effects of deficiency and overload. Metal Ions Life Sci. 2013;13:359–87. https://doi.org/10.1007/978-94-007-7500-8_11.
40. Ogórek M, Gąsior Ł, Pierzchała O, Daszkiewicz R, Lenartowicz M. Role of copper in the process of spermatogenesis. Postepy Hig Med Dosw(Online). 2017;71(0):663–83. https://doi.org/10.5604/01.3001.0010.3846.
41. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem. 2005;12(10):1161–208. https://doi.org/10.2174/0929867053764635.
42. Adaramoye OA, Akinloye O, Olatunji IK. Trace elements and vitamin E status in Nigerian patients with prostate cancer. Afr Health Sci. 2010;10(1):2–8.
43. Yang CY, Chiu HF, Tsai SS, Cheng MF, Lin MC, Sung FC. Calcium and magnesium in drinking water and risk of death from prostate cancer. J Toxicol Environ Health A. 2000;60(1):17–26. https://doi.org/10.1080/009841000156565.

Publisher's note