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SERUM CONCENTRATION OF CU, ZN AND SE IN MALIGNANT AND BENIGN PROSTATIC LESIONS

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ABSTRACT

Prostate cancer (PCa) is the second leading cause of death among cancer in men. Trace elements are involved in many vital functions. Fluctuations in serum levels of these elements may reflect the occurrence and/or severity of PCa.This prospective case/control study aimed to assess the serum concentration of Cu, Zn and Se in patients with PCa (newly diagnosed and longstanding) and with benign prostatic hyperplasia (BPH).

The study recruited 60 patients with PCa (30 newly diagnosed and 30 longstanding patients) and 30 patients with BPH. Other 30 healthy men were randomly selected to be a control group. Sera were separated from blood samples which were collected from each participant. Atomic absorption spectrophotometry was used to measure serum concentration of the studied trace elements.

The newly diagnosed patients with PCa showed significantly ($P<0.05$) higher serum concentration of Cu (3.14±1.1 µg/ml) than each of longstanding group (1.48±0.91 µg/ml), BPH (0.96± 0.17µg/ml), and controls (1.1±0.23 µg/ml), while there were no significant differences between different groups regarding Zn concentration. In newly diagnosed patients, Se concentration was 58.2± 6.12 ng/ml) which is significantly lower than other groups ($P<0.05$). In longstanding patients, the concentration was relatively high (98.4± 12.38 ng/ml) and differed significantly from patients with BPH (72.4± 8.11 ng/ml) and non-significantly from controls (108.1±15.34 ng/ml)

These results indicate that serum levels of Cu and Se undergo a marked change in newly diagnosed patients with PCa, and thus could be used as additional marker for this malignancy.

Keywords: Prostate cancer, benign prostatic hyperplasia, copper, zinc, selenium

INTRODUCTION

Prostate cancer (PCa) is typically a neoplasm of men with old ages. It is the second leading cause of death among cancers in men, after lung cancer (Jemal et al., 2007). The exact causes of this malignancy are not clear; however, studies showed that ethnic, eating habits, recurrent urinary tract infections, environmental factors, and lifestyle are important risk factors (Zaichick, 2004, Al-Mayah et al., 2013). Benign prostatic hyperplasia is a non-malignant enlargement of the prostate affecting large percentage of men according their ages. For example, about 50% of men over 50-year experience this disease, and this percentage reaches nearly 90% after the ninth decade of life (Leitao et al., 2009).

Essential trace elements have a vital role in the body. Yaman et al., (2005) proposed four major function for these elements: they act as stabilizers, elements of structure, necessary for hormonal function, and cofactors in several enzymes. Thus monitoring levels of these elements may provide a critical biomarker for large number of pathologies. Not only do deficiency of these element harm the body, but also excess amount of them can be toxic. In fact, both cases are associated with many metabolic disorders as well as cellular growth disturbance such as mutation and cancer development (Eken et al., 2016). Of these elements, Se, Cu and Zn are considered to have prior importance due to their involvement in obvious body activities. For example, Zn is an important constituent of prostatic fluid and is known to have a critical role in the development and normal function of prostate (Yaman et al. 2005; Christudoss et al., 2011). Clark et al. (1996) conducted 4.5-year study to investigate the role of Se in PCa. They found that men supplied with 200µg Se/day had two-third lower incidence of PCa compared with placebo group. Although cupper is very essential as coenzyme, it is excess may predispose to cancer through DNA damage via toxic free radicals (Theophanides and Anastassopoulou, 2002).

This study aimed to investigate serum levels of Cu, Zn and Se in PCa patients (treated and newly diagnosed) and BPH patients for better understanding of biochemical interaction inside the prostate with different pathologies and the possible use of these elements as marker for prostate pathologies.

MATERIALS AND METHODS

Study Population: This prospective case-control study recruited 60 patients with PCa (30 newly diagnosed and 30 longstanding patients) and 30 patients with BPH who were attending Oncology

Clinic in Al-Imamain Al-Kadhumain Medical City during the period from August 2016 to November 2017. In addition, 30 healthy men were randomly selected as controls.

Exclusion criteria were the presence of any other malignancy (for patients) diabetes, hormonal and/or steroid therapy, history of taking antioxidant or vitamin supplements for 10 days before sampling.

Sample and Data: Five mL of venous blood were collected from each participant. Sera were separated by centrifugation and kept at -20 ^{0}C until be used. Data regarding age, weight, height and residence were obtained through informed consent from. Serum levels of prostate specific antigen were obtained from patients' records. This study was approved by Institutional Review Board/ College of Medicine/ Al-Nahrain University

Measurement of Trace Elements: Atomic absorption spectrophotometer (Unicam Ltd, Cambridge, UK) was used to measure the concentration of trace element in serum samples. Briefly, 2 mL of nitric oxide was added to 0.7 gram of serum and incubated at 70^{0}C for 30 min before adding 1 mL of the same acid. The solution was transferred into a Teflon vessel bomb which was incubated in microwave at 450W for 3 min. Again, 0.5ml of HNO_3 were added and the same incubation in microwave was repeated. Two mL of 0.1mol/L HNO3 were added and the mixture was placed in pyrex vessel which underwent cent-rifugation. The supernatant was used to measure Zn, Cu and Se. standard stock solutions of these metals were prepared using the corresponding high purity standard substances. The resultant standard curves are shown in figure 1.

Statistical Analysis: Numeric data were expressed as mean± standard deviation (SD) and analyzed with one-way analysis of variance (ANOVA). Categorical variables were expressed as frequency and percentage and analyzed with Chi-square test. All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 20. A p value of less than 0.05 was considered significant.

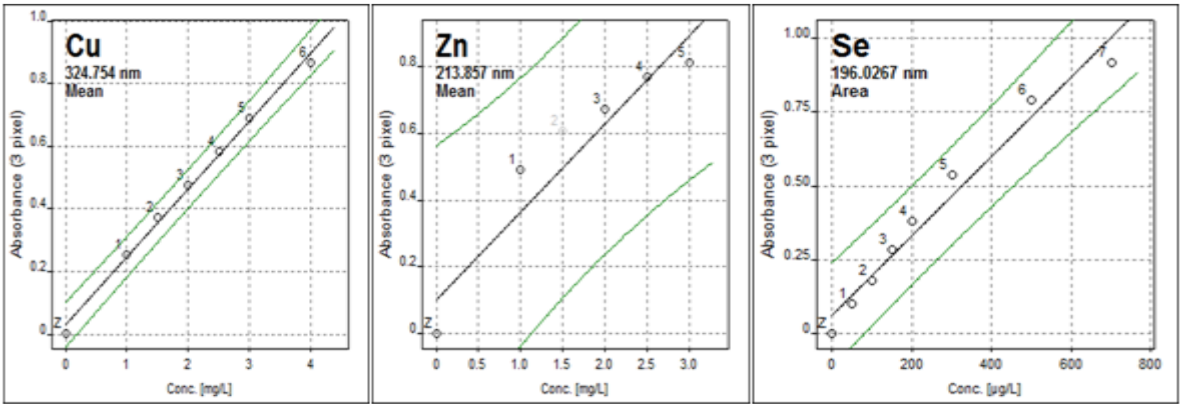

Figure 1: standard curves for trace elements (Se, Zn, Cu)

RESULTS AND DISCUSSION

Demographic and Clinical Data: Table 1 shows the baseline characteristics of the study population. No significant differences were observed in age, residence and body mass index (BMI) between the four groups. However, PCa patients (newly diagnosed and longstanding) showed significantly higher serum levels of total PSA than BPH patients.

Table 1: Demographic and clinical data of the study population

Characteristic	Newly diagnosed PCa (30)	Longstanding PCa (30)	BPH (30)	Healthy controls (30)	P-value
Age, years (mean±SD)	64.91±8.12	67.54±12.1	66.34±10.2	62.6±9.92	0.534
BMI (kg/m^2)	26.5±2.3	26.18±4.1	26.±3.7	25.7±3.3	0.421
Residence **Rural** **Urban**	 18(60%) 12(40%)	 16(53.33%) 14(46.67%)	 18(60%) 12(40%)	 11(36.67%) 19(63.33%)	0.308
Total PSA	14.28±11.4*	13.86±8.2*	6.8±4.1	NA	<0.001

SD: standard deviation, PCa: prostate cancer, BPH: benign prostatic hyperplasia, BMI: body mass index, PSA: prostate specific antigen, NA: not available, *significant difference from BPH group.

Serum Levels of Cu, Zn and Se: Serum concentration of copper and zinc in the different groups are shown in figure 2. For copper, the newly diagnosed patients with PCa showed significantly (P<0.05) higher concentration (3.14±1.1 µg/ml) than each of langstanding group (1.48±0.91µg/ml), BPH (0.96± 0.17µg/ml), and controls (1.1± 0.23 µg/ml), while there were no significant differences among the last three groups (P>0.05).

On the other hand, serum concentration of zinc in newly diagnosed, longstanding, BPH and controls were 0.48±0.14 µg/ml, 1.28±0.71 µg/ml, 0.89±0.21 µg/ml and 1.24±0.82 µg/ml respectively. Although PCa patients who were receiving regular doses of chemotherapy exhibited higher concentration of zinc than the other groups, the differences were not significant (P>0.05).

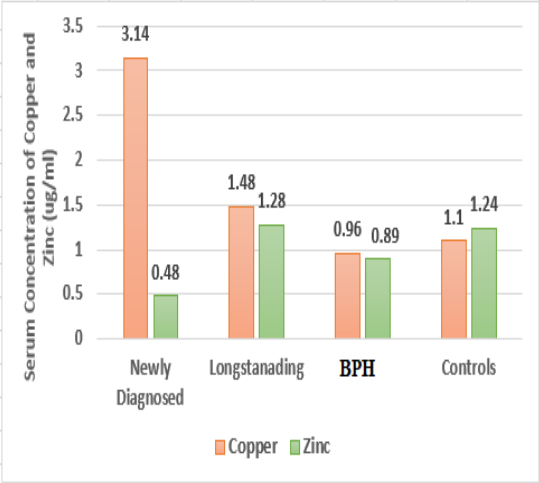

Figure 2: serum concentration of copper and zinc in patients and controls

BPH: Benign prostatic hyperplasia: Generally, serum concentrations of selenium decreased in patients compared with controls (Figure 3). In newly diagnosed patients, this concentration was 58.2±6.12ng/ml) which is significantly lower than other groups (P<0.05). In treated patients, the concentration was relatively high (98.4± 12.38 ng/ml) and differed significantly from patients with BPH (72.4±8.11ng/ml) and non-significantly from controls (108.1±15.34 ng/ml).

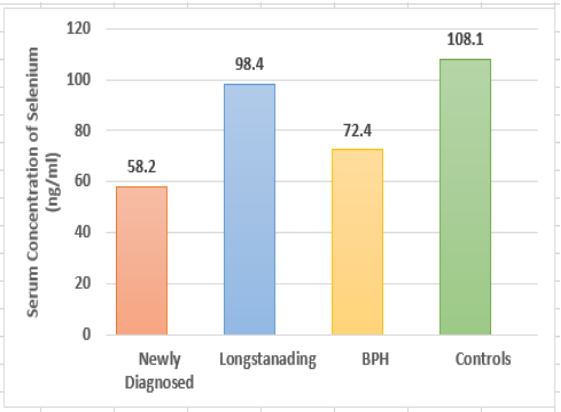

Figure 3: serum concentration of selenium in patients and controls

BPH: Benign prostatic hyperplasia: This study aimed to investigate the serum levels of three important trace elements in patients with PCa (treated and newly diagnosed) and BPH and compare these levels with a control group. The present study is unique because it involved both newly diagnosed and longstanding PCa patients.

The study population was carefully selected to be matched in all demographic factors in order to get a sense results regarding the levels of trace elements. However, it was not possible to match PSA concentrations between the different groups as it is well known that this concentration is fluctuated according to the disease progression.

The study revealed an increase in the serum concentration of cupper in newly diagnosed PCa patients compared to treated patients, BPH patients and healthy controls. Almost similar result was obtained by (Nayak et al., 2003; Obiageli et al., 2015). In a more recent study, Eken et al. (2016) investigated 10 trace elements in PCa, BPH and chronic prostatitis in Turkish patients. They reported a significant increase in Cu in PCa patients compared to other groups.

In another recent study, Denoyer et al. (2015) assessed copper concentration in prostate tissue samples from patients with PCa. The authors grouped those patients according to the Gleason score, and found that cancerous tissues had 1.6-fold more Cu than age-matched normal tissues (P<0.05). Furthermore, patients with more aggressive disease had 1.8-fold more Cu than patients with less aggressive disease. The most reasonable explanation for this result is that high concentration of Cu in plasma usually reflects a relatively high concentration of this element in prostate tissue. Studies showed that excess Cu may induce growth proliferation with toxic free hydroxyl radicals (Theophanides and Anastassopoulou, 2002). Such radicals could cause a damage for cell DNA and predispose the cell for can-

cer. It seems that the treatment protocol had significant effect on copper serum levels may be through interaction with plasma Cu.

The current study showed no significant differences in Zn concentration between different groups. In fact, there are highly conflicting results regarding Zn concentration either in sera or tissue specimens form PCa patients or BPH patients. (Singh et al., 2016) reported a significantly lower Zn levels in malignant prostatic tissues than age-matched healthy or BPH tissues. Goel and Sankhwar (2006) found that plasma levels of Zn in PCa, BPH patients and healthy controls were 59.6 ng/ml, 172.7 ng/ml and 94.5 ng/ml respectively and suggested using Zn levels as a diagnostic tool for PCa. This finding is almost identical with that obtained in several previous studies (Guntupalli et al., 2007; Sapota et al., 2009; Christudoss et al., 2011). In contrast, other studies either reported a reverse result (Yao et al., 1977) or no significant differences (Leitao etal., 2009; Eken et al., 2016; Mahmoud et al., 2016). This variation in the results, even among studies which measured Zn in the prostate tissue, may be attributed to several factors, the most important of which are the dietary habits and Zn/Cu ratio in the plasma.

In healthy individual, Zn is involved in the regulation of the growth an apoptosis of prostate epithelial cells (Ku et al., 2012). Several studies in this regard demonstrated that zinc inhibits prostate carcinoma may be through the induction of cell-cycle arrest and apoptosis (Makhov et al., 2011). These observations implied that newly diagnosed PCa patients should have a lower serum levels of Zn than another group. That is exactly the real results even though the differences did not rise to significant levels because the estimation was in serum instead of prostate tissue.

One of the most prominent results of the current study is the significant differences in Se levels among the different groups. The relatively low level of Se in newly diagnosed patients is in accord with the result obtained by (Singh et al., 2016) who showed that both Zn and Se levels were significantly lower in PCa tissues as compared to BPH or normal tissue. Furthermore, Guntupalli et al. (2007) revealed significantly lower Se in both PCa and BPH versus normal prostatic tissues. Almost similar result was obtained by Ozmen et al (2006) who found that Se levels were significantly lower in PCa than controls. In a recent study using serum samples, Eken et al. (2016) reported significant decrease in Se levels in PCa, BPH and CP patients compared to control. In contrast, Zachara et al. (2005) reported higher Se level in prostate compared with either heathy or BPH tissues with a significant difference.

Based on these data, the high concentration of Se in treated patients in this study may be attributed to the effectiveness of the chemotherapy protocol. Another observation is that Se is not restricted to the cancerous process itself. Rather, different prostatic pathologies, such as BPH in this study are associated with decrease serum levels of Se. Of note, even with proper treatment regime, patients with BPH still have enlarged prostate gland. For this reason, they have relatively decreased levels of Se.

Collectively, these data indicate the importance of Cu and Se as markers for PCa. In newly diagnosed patients there is high serum value of Cu and low value of Se, while in treated patients both elements tend to resemble their corresponding values in healthy individuals.

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