Zinc in normal and pathological human prostate gland

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ABSTRACT

Objectives: This study is conducted to detect quantitatively zinc in the nuclei and cytoplasm of epithelial cells of the prostate from normal, acute prostatitis, benign prostatic hyperplasia and adenocarcinoma.

Methods: Prostatic tissues from normal, acute prostatitis, benign prostatic hyperplasia and adenocarcinoma were obtained from patients and processed for zinc detection using x-ray microanalysis technique. The samples were collected over a period of 2-3 years and were processed at Jordan University of Science and Technology, Irbid, Jordan.

Results: Zinc was increased and decreased both in the nuclei and the cytoplasm of the glandular epithelium of benign prostatic hyperplasia and adenocarcinoma of the prostate. Although zinc was increased in the nuclei and cytoplasm of epithelial cells of acute prostatitis, it was not significant.

Conclusion: These findings might be caused by factors affecting the zinc metabolic pathway directly or through the zinc bound protein metallothionein. In addition, these findings could be used in diagnosing different prostatic pathological conditions and advancing prostatic tumors and those with similar histopathological profiles.

Keywords: X-ray micro analysis, metallothionein.

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Prostate cancer is the leading cause of death after the age of 70, 2nd to lung cancer.[1] The male reproductive tract contains a high concentration of zinc. The role of zinc in prostate gland has been studied extensively by many investigators in an attempt to correlate zinc with varoius physiological functions of the prostate.[2] Others have reported that zinc-binding proteins were involved in the regulation of genes, which play an important role in cell differentiation and proliferation.[2-4] Zinc is a key structure of a variety of zinc finger proteins. Metallopanstimulin, a zinc finger protein, has been expressed in high levels in prostatic cancers[5] and basonuclin, another zinc finger protein, which was found in the germ cells of the testis.[6] Metallothioncin (MT), a zinc-bound protein, has been detected in the rat and human prostate gland.[7,8] It was found that zinc within the cytosol of cultured (human prostatic adenocarcinoma) PC-3 cells and in the prostatic fluid was bound to MT.[9,10] Variations in metal content were reported in different pathological conditions.[11-13] Zinc levels were increased and decreased in benign protastatic hyperplasia (BPH) and adenocarcinoma of the prostate.[14,15] Decreased zinc level in prostate was implicated in the development of prostatic cancer, while exposure of human malignant tumor cell line or PC-3 cells to zinc results in necrosis of these cells.[16,17] The intra cellular localization of zinc in normal, hyperplastic, and neoplastic human prostate was reported using x-ray microanalysis.[18] The x-ray microanalysis[18] and atomic absorption spectrophotometry techniques have been used to detect intracellular distribution of zinc in normal, hyperplastic and neoplastic human prostate gland.[15]

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Neither of these studies have demonstrated the zinc concentration in the nuclei. In this study x-ray microanalysis was used to determine quantitatively zinc concentration in the cytosol and nuclei of different pathological conditions of human prostate. It is anticipated that this study will lead to a better understanding of the role of zinc in the etiology and diagnosis of prostatic diseases.

Methods. Prostatic tissues were taken from patients with BPH (21 cases), and prostatic adenocarcinoma (Gleason grade IV) (7 cases) through transurethral or suprapubic resection, over a period of 2-3 years, and were processed at Jordan University of Science and Technology, Irbid, Jordan. Three cases of BPH showed signs of acute prostatitis in addition to hyperplasia. Four specimens with no pathological signs were used as control. All cases were confirmed histopathologically. Tissues from the prostate gland were excised, diced into 1mm cubes and immersion fixed in 1% osmium tetroxide in 2% potassium pyroantimonate to precipitate zinc. Tissues were transferred to fresh fixative at room temperature for 90 minutes and rapidly dehydrated in absolute alcohol (3 times 10 minutes each) before immersion in 50/50 absolute alcohol and propylene oxide for 10 minutes. The tissues were washed twice in propylene oxide for 10 minutes each followed by 50/50 propylene oxide and Spurr's resin for 10 minutes. Two changes of resin, 30 minutes each preceded final resin embedding of tissues in Beem plastic capsules and polymerization at 70°C overnight. One hundred to 120 nm ultrathin sections were cut and collected on 150-mesh aluminum grids. The grids were coated with a thin layer of carbon in vacuo to stabilize the sections against the electron beam. X-ray microanalysis for zinc was performed using Hitachi H-600 analytical microscope, coupled with a kevex multichannel analyzer. The principles, techniques and instrumentation and calculations have been discussed by many investigators.[19,20] At least 20 randomly selected areas in the nuclei and the cytoplasm of the glandular epithelium of the prostate gland from all groups were analyzed. Grids with standard zinc concentration of 10, 25 and 50μg% were used to calculate the final concentration of zinc in the areas studied.

Results. Normal and pathological specimens of the prostate showed variations in zinc concentration in the nuclei and cytoplasm of the specimens studied. **Table 1** shows values of zinc concentration in the nuclei and cytoplasm of human prostatic cells from normal, acute prostatitis, BPH and adenocarcinoma. Values represent mean ± standard deviation. Benign prostatic hyperplasia has the highest zinc concentration both in the nuclei and the cytoplasm while the adenocarcinoma of the prostate has the lowest zinc concentration both in the nuclei and the cytoplasm. These values are significant when compared to normal. Although zinc concentration in acute prostatitis has increased both in the nuclei and the cytoplasm, this increase was insignificant. There is no correlation between the nuclear and cytoplasmic concentration among the 3 pathological groups compared to normal.

Table 1 - Nuclear and cytoplasmic zinc concentration. Values are expressed as mean ± SD.

Zinc location	Normal ng%	Prostatitis ng%	BPH ng%	CA ng%
Nuclear Zinc	16.42 ± 6.14	18.63 ± 6.26	38.28 ± 16.12	7.66 ± 3.52
Cytoplasmic Zinc	19.21 ± 7.70	22.54 ± 8.34	32.61 ± 14.68	13.47 ± 5.75

BPH=Benign prostatic hypertrophy, CA=Adenocarcinoma of the prostate, SD=standard deviation

Discussion. X-ray microanalysis has the advantage in determining the subcellular concentration of zinc while other techniques are unable to detect nuclear zinc.[15] The present study shows increased and decreased zinc levels in the nuclei and cytoplasm of BPH and adenocarcinoma of the prostate. These findings were consistent, to some extent, with other findings using other means.[14,15] These sudies have shown increased and decreased total zinc levels in BPH and adenocarcinoma of the prostate. The variation in zinc concentration in the nuclei and the cytoplasm of acute prostatitis, BPH and adenocarcinoma of the prostate could be used in diagnosing the different pathological states of the prostate. The consistent finding of increased and decreased zinc levels in the nuclei of BPH and adenocarcinoma of the prostate may give additional criteria in diagnosing these conditions. It was found that all hepatic cirrhoses had a very low zinc concentration, whereas zinc concentration was increased in liver tumors and invasive metastases.[21] These finding show that tumors vary in their abilities to retain zinc irrespective to their site of origin and malignancies. In addition, x-ray microanalysis can detect differences in zinc concentration in cells exhibiting almost the same histological features. In histological profiles, not all nuclei show the same significant difference for diagnosing pathological conditions. It was demonstrated, by histopathological means that metastatic foci of the skin, liver and skin of prostatic adenocarcinoma R-3327H cell line of Copenhagen rats exhibited essentially the same

histopathological features,[22] but show differences in nuclear zinc concentration.[23]

Nuclear zinc concentration per se was used as an estimating factor for chromatin stability. It was found that low chromatin stability was associated with low zinc content in human sperm.[24] So, low zinc in the nuclei of prostatic adenocarcinoma may be associated with low chromatin stability. In addition, the magnitude of reduction of zinc levels in the nuclei of the prostatic adenocarcinoma may be associated with advancing stage of the prostatic adenocarcinoma. In colorectal cancers, an increased zinc level has been shown with advanced tumor stage.[25] Zinc levels detected by x-ray microanalysis could lend some support to quantitative nuclear features used as a new method in grading the malignancy of prostate carcinoma.[26]

Regulation of zinc metabolism in prostate is crucial for the structure and function of the prostate. It is well evident that the loss of the unique capability to retain, by the prostate, a high level of zinc is an important factor for the development and progression of malignant prostatic cells.[27] It was found that normal prostate and BPH contain high amount of zinc which is responsible for the inhibition of citrate oxidation.[16] Oxidation of citrate is a crucial step for adenosine triphosphate production which is needed for progression of malignancy.[16] In contrast, it has been suggested that zinc inhibits human prostatic carcinoma cell growth, possibly due to induction of cell cycle arrest and apoptosis.[27] Zinc is found in free and bound forms.[28] Whether these findings are attributed to the zinc-bound form or free zinc needed to be elucidated.

In conclusion, the findings could be used as a working hypothesis to establish a correlation between zinc status of human prostate and the development and advancement of pathological conditions.

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