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ORIGINAL ARTICLE

Association of ferritin with prostate cancer

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Summary

Purpose: *Prostate specific antigen (PSA) has been widely used as the unique serum biomarker for the diagnosis and/or pre-diagnosis of prostate cancer (PCa). However, the diagnostic value of PSA is a subject of ongoing debate owing to its lack of specificity, especially when the PSA level is moderately increased (e.g., 4-10 ng/ml). Thus, we suggest the need for identification of a new biomarker to discriminate PCa cases from benign prostatic hyperplasia (BPH) and normal controls (N).*

Methods: *Urine or tissue samples of PCa patients, BPH patients, and normal controls were systematically collected. The expression of ferritin light chain (FTL) and ferritin heavy chain (FTH) was verified by immunohistochemistry in the tissue. The concentration of urinary ferritin was measured by Access Immunoassay. The level of creatinine in the urine was detected on a HITACHI 7080 system to calculate the ferritin-creatinine ratio (FCR). The data were statistically analyzed using the rank sum test.*

Results: *Immunohistochemical characterization of tissues in patients with PCa and BPH was conducted. We found representative immunohistochemical expression of FTL and FTH, with strong staining intensity in PCa and weak staining intensity in BPH. Furthermore, there were differences in urinary FCR among the three groups, with significant differences in the PCa group (134.46±47.01) compared to both the BPH (24.18±3.17, p = 0.009) and control (6.42±0.82, p = 0.003) groups. In contrast, there was no significant difference between the BPH and N groups (p = 0.649).*

Conclusions: *Ferritin is a potential urinary biomarker to discriminate between PCa and BPH patients.*

Key words: *benign prostatic hyperplasia, ferritin, ferritin-creatinine ratio, prostate cancer, urinary proteomics*

Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer in males worldwide [1]. In 2008, 10% of all patients that died of PCa were located in East Asia [2]. Prostate specific antigen (PSA) has been widely used as the unique serum biomarker for the diagnosis and/or pre-diagnosis of PCa. However, the value of this marker is a topic of active and ongoing debate owing to the lack of specificity, especially when PSA is moderately increased (e.g., 4-10 ng/ml) [3,4].

Na et al. [5] reported that only 4.7% to 14.8% of men with aPSA levelfrom 4 ng/ml to10 ng/ml were ultimately diagnosed with PCa, which is a much lower rate than that reported in Western countries. As a result, many researchers have been seeking to identify new potential biomarkers and to better understand the molecular pathogenesis of PCa.

Human urine has become increasingly important for clinical diagnostics by virtue of its non-invasiveness and availability, especially in the diagnosis of urinary system cancers. As the terminal metabolic product of the blood, the changes of the composition, quantity, and quality of the urine can provide information reflecting the generation, development, and prognosis of

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urinary diseases. Compared with the collection of plasma and other body fluid samples, the collection of urine is more convenient and less invasive. Recently, an increasing number of scientists have begun to pay more attention to urinary proteomics, and this field has seen steady progress alongside the developments of high-throughput proteomic techniques. Therefore, we aimed to find potential tumor markers in the urine for identification and discrimination of PCa and BPH.

Serum ferritin is the storage form of iron, which is elevated in many diseases. Some recent reports have shown that serum ferritin expression was up-regulated in many tumor-associated diseases such as breast cancer, liver cancer, and lung cancer [6-9]. Other reports have also suggested an association of serum ferritin with urinary tract tumors. However, the relationship between urinary ferritin and PCa has not been evaluated.

In the present study, we collected urine samples from patients with PCa and BPH, and from healthy volunteers. Using an immunohistochemical approach, we found that ferritin was differentially expressed in tissues from the patients with PCa and BPH. Furthermore, to eliminate the influence of water content on urine protein analysis, we evaluated the differences in the ferritin levels in the urine of PCa and BPH patients using both ferritin and the ferritin-creatinine ratio (FCR) as indicators. Ultimately, we found that urine ferritin shows potential as a useful biomarker for PCa diagnosis.

Methods

Study population

This study was approved by the local research ethics boards. This study included 9 patients with PCa, 9 patients with BPH, and 9 sex- and ethnicity-matched healthy donors who visited Beijing Shijitan Hospital between 2012 and 2015. All the patients with PCa and BPH had histopathologically confirmed tumors, and none had received chemotherapy or radiation before enrollment. Pathologic staging was done according to the 2002 (AJCC) TNM staging system, and the Gleason Score classification was used to grade the tumors. The control subjects were healthy volunteers with no history of cancer who were recruited from the medical examination center of Beijing Shijitan Hospital. We also excluded control subjects with chronic urinary tract diseases.

Immunohistochemistry

The tissue blocks from patients with PCa and BPH were cut into 5-μm-thick sections. Tissue sections were kept overnight at 55°C on super frost slides. Paraffin sections (5 μm) were deparaffinized and rehydrated in a series of xylene and ethanol baths of decreasing concentrations. Endogenous peroxidase was inhibited by incubation of the sections in 3% hydrogen peroxide in phosphate buffered saline (PBS) for 10 min. Antigen retrieval was performed by heating the sections in 0.01 M citrate buffer in a microwave oven. Non-specific binding was blocked by incubating the tissue sections with 10% bovine serum albumin in PBS for 60 min. Slides were then incubated overnight at 4°C with monoclonal mouse anti-ferritin light chain (FTL) or anti-ferritin heavy chain (FTH) antibody (Abcam, USA). After washing, a visualizing system containing the secondary antibody, streptavidin–biotin, and DAB was used. These steps were carried out in a wet chamber. The immunohistochemical staining level was scored based on the percentage of immune-positive staining as follows: negative staining, –; weak positive staining, +; positive strong staining, ++.

Analysis of urine ferritin and creatinine levels

Ten milliliters of clean midstream urine samples from PCa and BPH patients, and from the normal controls were centrifuged at 2000 *g*, 4°C for 15 min within 2 h of collection. The supernatant was used for further analysis. Each group included 9 patients or volunteers. The levels of ferritin protein were tested with Access Immunoassay System (Abbott, USA). The quantity of creatinine was measured with the HITACHI 7080 system (Hitachi, Japan). To eliminate the influence of urine volume due to differences in water intake [10-13], the urinary FCR (ferritin [ng/ml]/creatinine [mg/ml]) was used for standardization.

Statistics

Statistical analysis was performed using SPSS 17.0 and the data were assessed by the rank sum test. P values < 0.05 were deemed statistically significant.

Results

Validation of FTL and FTH

For immunohistochemistry, we detected FTL and FTH expression in the tissues of PCa and BPH patients. Representative immunohistochemical images for FTL and FTH expression are presented in Figure 1. Both FTL and FTH showed strong staining intensity (++) in PCa patients and weak staining intensity (+) in BPH patients.

FCR in crude urine

To eliminate the influence of variation in water content on the detection of ferritin expression and verify the differential expression of ferritin

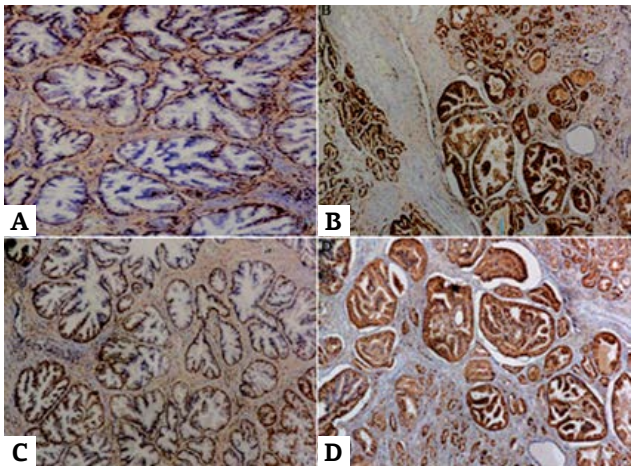

Figure 1. Representative immunohistochemical expression for FTL and FTH. FTH and FTL expression were verified in the tissue of BPH and PCa patients by immunohistochemistry . FTL showed weak staining intensity (+) in a BPH patient **(A)** and strong staining intensity (++) in PCa **(B)**. FTH showed weak staining intensity (+) in a BPH patient **(C)** and strong staining intensity (++) in PCa **(D)**. BPH: benign prostatic hyperplasia, PCa: prostate cancer. Magnification of all photomicrographs x40.

Table 1. Specific information of patients and statistical analysis of ferritin

	PCa	*BPH*	*Normal*
Number	9	9	9
Median age, years	77.1	73.9	26
Stage	class 3,4	PIN I 2	_
	class 4,5	PIN I-II 7	_
Mean±SE (ng/mg)	134.46±47.01*#	24.18±3.17△	

*Comparison with normal group showed significant difference (p=0.003), #Comparison with BPH group showed significant difference (p=0.009), △Comparison with normal group showed no statistical difference (p=0.649).
PCa: prostate cancer, BPH: benign prostatic hyperplasia, PIN: one grade prostate intraepithelial neoplasia

among PCa patients, BPH patients, and normal controls, we examined the levels of ferritin and creatinine respectively for every sample, and compared the urinary FCR among groups based on the rank sum test. Table 1 shows the mean and standard deviation values of FCR in the three groups. Significant differences were observed among the three groups; specifically the FCR in the PCa group was significantly higher than that in the BPH (p=0.009) and control (p=0.003) groups. In contrast, there was no significant difference between the FCR of the BPH patients and normal controls (p=0.649). Figure 2 provides a box chart of the FCR among the three groups.

Discussion

We evaluated the value of ferritin for discriminating between PCa and BPH. Using immunohistochemistry, we verified the FTL and FTH expression in tissue samples. As expected, compared to tissue samples from BPH patients, FTL and FTH were both up-regulated in PCa patients.

Ferritin is the primary iron storage protein form, and is critical for iron homeostasis [14]. Ferritin is composed of two types of subunits, including the light chain and heavy chain. It is increasingly recognized that ferritin also plays an important role in other conditions, including inflammatory [15], neurodegenerative [16], and chronic renal diseases [17,18]; cardiovascular diseases [19-21]; and malignant diseases [22,23]. It has been confirmed that the ratio of these subunits depends on tissue types, and can be modified in inflammatory and infectious conditions [24-26]. The ratio of the two subunits in the urine of PCa patients was unknown, and further evidence was required to prove this association.

To eliminate the influence of potential interference factors such as water intake, urine volume, and others, the FCR was used to compare the difference of ferritin contents among the groups of PCa, BPH, and normal controls. The results of FCR analysis showed that the expression of ferritin in PCa tissues was higher than that in BPH and control tissues. Therefore, ferritin can help to distinguish PCa patients from BPH patients. The major limitation of our study was the small quantity of samples analyzed. Therefore, large-scale studies and more samples are needed for further validation.

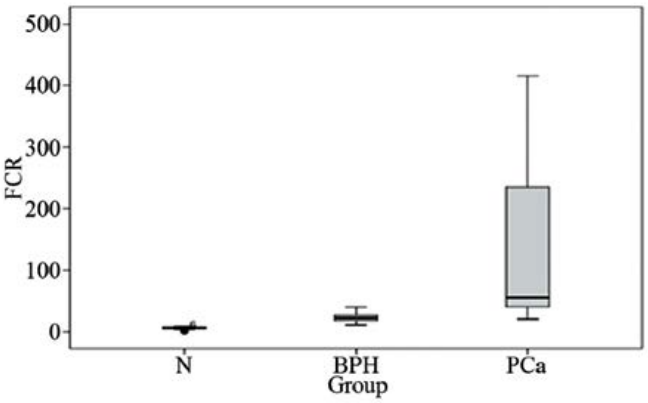

Figure 2. Box chart of urinary FCR in three groups. FCR was evaluated by ferritin-creatinine ratio (FCR) and was significantly highly expressed in PCa group compared to the BPH (p=0.009) and control groups (p=0.003). There was no significant difference between BPH and normal controls (p=0.649).

Conclusions

The expression of FTL and FTH in PCa pa-

tients, BPH patients, and normal controls was validated by immunohistochemistry. The levels of ferritin were verified to be significantly higher in the urine of PCa patients than in that of BPH patients and controls. We concluded that ferritin can be used to distinguish PCa patients from BPH patients.

Authors' contributions

Qiang Su participated in the acquisition of data, analysis and interpretation of data and performed the statistical analysis. Ting Lei participated in the manuscript draft and statistical analysis. Man Zhang designed the research, paid all of the ground, conceived of the study, helped to draft the manuscript and was supervisor of the whole process of research.

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Conflict of interests

The authors declare no confict of interests.

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