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Original article

Urinary nerve growth factor as an oncologic biomarker for prostate cancer aggressiveness

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Abstract

Objectives: We investigated urinary nerve growth factor (NGF) as a novel urinary biomarker for high-grade prostate cancer (PCa).

Methods and materials: After institutional review board approval for a prospective pilot study, we enrolled men at the preoperative visit before robotic-assisted radical prostatectomy. Demographics, urinary flow parameters, and urine samples were collected. Urinary NGF and urinary creatinine were obtained in the translational science laboratory. Pathologic and postoperative demographics were collected after surgery. NGF is the primary outcome variable (dependent variable). The pathologic Gleason score (ordinal variable ≤ 6, 7, and ≤ 8) served as an independent grouping variable. Multivariate analysis using a general linear model was conducted to investigate associations between independent variables and NGF (dependent variable) after adjusting for urinary concentration and volume.

Results: We enrolled and analyzed urine samples and pathologic data from 115 subjects. Patient pathology included 24% ($n = 28$) Gleason score 6 or less, 68% ($n = 78$) Gleason score 7, and 8% ($n = 9$) Gleason score 8 or greater. Perineural invasion was more prevalent in higher-grade disease ($P < 0.001$). The median NGF level was 24.1 pg/ml (range: 0.16–270.5 pg/ml) and was transformed to the log base 10 scale. Total bladder volume, urinary creatinine level, prostate-specific antigen level, and diabetes were correlated with the Log NGF. In a general linear model, adjusting for bladder volume and urinary creatinine, increasing Log_{10} NGF was associated with higher Gleason score (Gleason category ≤ 6, 7, and ≥ 8; $P = 0.003$).

Conclusions: Urinary NGF may be a biomarker for higher-grade PCa. Our pilot study suggests further investigation is warranted to determine whether urinary NGF could provide unique additional information in patients with PCa.

Keywords: Prostate cancer; Biomarker; Neurogenesis; Gleason score

1. Introduction

Prostate cancer (PCa) is considered a neurotropic cancer, a commonality with other epithelial tumors, such as pancreatic, bile duct, and head/neck cancers [1]. PCa "neurogenesis" is an evolving field of research and considered a dominant pathway of local invasion [2]. Perineural invasion (PNI) on prostate biopsy specimen may predict adverse outcomes after radical prostatectomy or radiation therapy; however, a consistent consensus has been hindered by variability in pathologic reporting and research study design [3–5].

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In vitro studies show that the perineural space provides an enhanced environment for PCa survival and growth through cell-cell interactions involving growth factors [3,6,7]. Subsequent, animal models involving hypogastric denervation have prevented PCa cell growth attributed to sympathetic and parasympathetic nerve alterations confirmed by increased nerve density in human PCa [8]. Moreover, a recent population-based study showed β-blocker use may be associated with a reduction in PCa-specific mortality suggesting that β-adrenergic receptor down-regulation may be the mechanism of action [9].

Nerve growth factor (NGF) is overexpressed in the prostate, and alterations in its receptors may cause PCa proliferation and metastasis [10,11]. Herein, we investigate the potential utility of urinary NGF as a urinary biomarker for PCa aggressiveness based on pathologic Gleason score.

2. Materials and methods

2.1. Study population and design

After internal review board approval, we prospectively recruited patients at the preoperative appointment before robotic-assisted radical prostatectomy. Urine samples are collected when the participants had a strong desire to void. Subjects voided volume was added to the postvoid residual to calculate total bladder volume (TV) to control for volume concentration. The postvoid residual volume was obtained by bladder scan ultrasound (Verathon BVI 3000 BladderScan, Bothell, WA) in all patients. To account for bladder volume and concentration, the total urinary volume was calculated at the time of the void as well as a urinary creatinine, which has been used with NGF in previous studies [12].

2.2. Sample collection

Voided urine was placed on ice immediately and transferred to the laboratory for preparation for NGF measurement. The urine samples were centrifuged at 3,000*g* for 10 minutes at 4°C. The supernatant was separated into aliquots in 1.5-ml tubes and preserved in a freezer at −80°C. At the same time, 3 ml of urine was taken to measure the urinary creatinine level.

2.3. Urinary NGF and Creatinine

Urinary NGF concentration was determined using an immunoassay system (Emax, Promega, Madison, WI) with a specific and highly sensitive enzyme-linked immunosorbent assay kit, which had a minimum sensitivity of 7.8 pg/ml. Assays were conducted according to the manufacturer's instructions. The detailed procedure was described previously. Generally, urine samples were not diluted in the enzyme-linked immunosorbent assay. When the urinary NGF concentration was higher than the upper detection limit (250 pg/ml), the urine samples were diluted to fit the detection limit. For urine samples with NGF concentrations lower than the detectable limit but above zero, they were concentrated using a column-protein concentrate kit (Amicon Ultra-15, Millipore) before measuring the NGF value. All samples were run in triplicate, and urinary NGF levels without a consistent value in 3 measures were repeated, and the values were averaged. The criterion for defining consistent values was that the coefficient of variation (standard deviation/mean) of the 3 absorbance values was <0.10. If the coefficient of variation of the samples was <0.10, data of the first run were discarded, and the samples were rerun in triplicate. The concentration of urinary creatinine (mg/dl) assay kit (Cayman Chemical Company, Ann Arbor, MI) was obtained and performed as per manufacturer's directions.

2.4. Study outcomes

The primary outcome was NGF in patients with higher-grade PCa compared with lower-grade PCa found on final pathology after prostatectomy. Other preoperative, pathologic, and postoperative outcomes were also collected prospectively.

Pathologic data consisted of pathologic Gleason score as is grouped into 3 categories (Gleason score 6 or less, Gleason score 7, or Gleason score 8 or greater). Tumor volume was determined as an overall percent of tumor in the radical prostatectomy specimen. If a range of percentages were given, the average percent tumor volume as calculated and used at the outcome. Additionally we documented positive surgical margins and upstaging defined as any increase of Gleason pattern from biopsy to pathology (includes 3 + 4 to 4 + 3). The pathologist as per standard at our institution documented PNI; however, no further quantification was performed. Final pathologic staging as per American Joint Committee on Cancer 2009 was used for analysis.

Postoperative oncologic follow-up consisted of 3-month prostate-specific antigen (PSA) levels and any PSA levels we could analyze thereafter for a goal of 12-month follow-up. Biochemical recurrence was defined as a PSA level >0.2 ng/ml, with a confirmatory PSA level or any patient who received adjuvant therapy.

2.5. Statistical analysis

We performed the statistical analysis with SPSSv.21 statistical program (IBM). Urinary NGF is not normally distributed; therefore, we transformed the variable with the log base 10 (Log NGF). Subsequently, we performed analysis of variance (ANOVA) and chi-square tests for associations of variables compared with pathologic Gleason score (3 groups: Gleason score 6 or less, Gleason score 7, or Gleason score 8 or greater). Other variables are tested with