Association of Urine Oncofetal Fibronectin Levels with Urology’s Most Common Disorders

Alejandro Alías-Melgar¹, Ernesto Neave-Sánchez¹, Juan Antonio Suárez-Cuenca², and Jesús Morales-Covarrubias¹

Departamentos de ¹Urología e ²Investigación Clínica, Centro Médico Nacional “20 de Noviembre”, I.S.S.S.T.E., Mexico City, Mexico.

Abstract. Urine oncofetal fibronectin (OnfFN) has proven useful in the assessment of malignant diseases such as transitional cell carcinoma (TCC) of the bladder. This study aimed to explore whether OnfFN may identify benign and common urinary diseases.

Methods. The urine OnfFN concentrations from patients who had bladder TCC (8 patients), benign urinary diseases (10 benign prostatic enlargement [BPE] patients, 10 urolithiasis patients), or controls (10 healthy individuals) were determined by ELISA and compared.

Results. The urine OnfFN concentration was significantly higher in patients with bladder TCC and lithiasis (mean±SE 0.43±0.18 and 0.45±0.23 μg/mL) than in patients with BPE and in healthy individuals (0.15±0.06 and 0.10±0.02 μg/mL, p<0.05). The urine OnfFN level (cutoff value 0.038 μg/mL), was able to identify 75% of patients with bladder TCC, 60% of patients with BPE and 80% of patients with urolithiasis, achieving a sensitivity of 0.75 for the recognition of either cancer or a urinary disorder. The OnfFN level had a high sensitivity (0.9) for the identification of urolithiasis. Conclusion. The urine OnfFN level proved helpful in the identification of bladder TCC patients. However, it had a better performance for the identification of urolithiasis, highlighting the potential usefulness of OnfFN as a biomarker for urothelial inflammation and repair.

Key words: oncofetal fibronectin, diagnosis, bladder cancer, urology, urothelial inflammation, urothelial repair.

Introduction

Advanced transitional cell carcinoma (TCC) of the bladder, a diagnosis that implies the invasion of the superficial layer, is already present in up to 80% of patients at the time of diagnosis. Typically, there is also a lack of suggestive history or symptoms that would prompt timely identification [1]. Novel molecular markers have been used in the development of screening methods and diagnostic tools for malignant diseases of the urinary tract, such as bladder TCC and prostate cancer [2-4].

The fibronectin family includes several protein isoforms located in the extracellular matrix that have organ-specific distributions. The generic term “fetal” or “oncofetal” fibronectin (OnfFN) refers to any isoform that is almost exclusively expressed by fetal tissue, tumor cells, or immortalized hepatoma and sarcoma cell lines; this protein can also be detected in samples of the placenta, amniotic liquid, or fetal tissue [5].

Studies on pre-term delivery and some types of cancer have suggested that OnfFN may be a useful clinical biomarker. The diagnostic performance of OnfFN is comparable to that of other urine tests, and it is possible that the OnfFN assay could be optimized. Therefore, screening methods based on OnfFN may represent alternatives to invasive cystoscopy, providing potential benefits for both the early diagnosis and the more effective treatment of bladder TCC [6].

The current diagnostic performance of OnfFN is limited by its association with benign abnormalities of the urological tract [7,8] and by the lack of cutoff values to identify patients with advanced tumors. Consequently, this study assessed the expression of...
OnfFN in patients with different malignant and non-malignant urinary tract diseases of both the bladder and extra-bladder organs.

**Materials and Methods**

**Patients.** The urine OnfFN concentrations of patients with biopsy-confirmed diagnoses of bladder TCC, biopsy/ultrasound-evidenced prostate enlargement (PE), or urography-proven urinary lithiasis as well as the urine OnfFN concentrations of healthy volunteers were determined using a cross-sectional study design. Patients with chronic urinary abnormalities were excluded, as were pregnant women.

Early-morning urine samples were collected at the Urology Department, Centro Médico Nacional “20 de Noviembre”, ISSSTE, from May to December 2004, after informed consent was obtained. The study was authorized by the Local Ethical Committee and Institutional Review Board, in accordance with the principles of medical research established by the Helsinki Declaration [9] and the currently applicable Mexican Local Policies “Reglamento de la Ley General de Salud en Materia de Investigación para la Salud”, version 2011.

**OnfFN analysis.** Thirty to 200 mL of urine was collected in tubes and processed within 3 hours of collection; small aliquots were then stored at −70°C. Analysis was performed using a solid-phase enzyme-linked immunosorbent assay (ELISA) with the monoclonal FDC-6 antibody, which targets the III-CS segment of the variable region of the OnfFN protein [10]. Standards and samples were assessed in duplicate and incubated in microtiter wells coated with FDC-6. The resulting antibody-antigen complex was washed to remove non-specifically bound material and then reacted with an enzyme-labeled antibody. Following formation of the antigen-antibody “sandwich,” the microtiter wells were washed and incubated with an enzyme substrate. The levels of OnfFN in the specimens were determined spectrophotometrically at a wavelength of 550 nanometers. The assay was performed in collaboration with Adeza Biomedical Corporation, California, USA.

**Statistical analysis.** Fisher’s exact test and odds ratios were used to analyze categorical variables and associations, and an unpaired T-test was used to analyze the differences in the mean OnfFN level between groups. The results were considered statistically significant when \( p \leq 0.05 \).

**Results**

Twenty-six male patients and twelve female patients, aged 53±12.5 years old [mean ± standard deviation] were recruited. The study group consisted of patients with biopsy-confirmed bladder TCC (n=8). The additional groups included patients with non-malignant urinary diseases such as PE (n=10) and urinary lithiasis (n=10). The control group consisted of healthy individuals (n=10). The subjects’ characteristics are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Population Characteristics.</th>
<th>Bladder TCC n=8</th>
<th>NMUD controls n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years old [m ± SD])</strong></td>
<td>55±12.7</td>
<td>51±16.9</td>
</tr>
<tr>
<td><strong>Gender (male / female)</strong></td>
<td>5 / 3</td>
<td>21 / 9</td>
</tr>
<tr>
<td><strong>Pathology n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- bladder TCC</td>
<td>8 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>- prostate enlargement</td>
<td>-</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>- urinary lithiasis</td>
<td>-</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td>- healthy subjects</td>
<td>-</td>
<td>10 (33.3%)</td>
</tr>
<tr>
<td><strong>OnfFN (ug/mL)</strong></td>
<td>0.28±0.338</td>
<td>0.21±0.260</td>
</tr>
</tbody>
</table>

TCC, Transitional cell carcinoma; NMUD, non-malignant urinary diseases; m, mean; SD, standard deviation.
patients with PE and urinary lithiasis, respectively. When the results were distributed in a 2x2 table (Table 2), the urine OnfFN levels exhibited a weak association with urinary malignancy (Fisher’s exact test = 0.19 and OR=3, CI95% 0.5-17.3). In contrast, it had a sensitivity of 0.75 for the discrimination of bladder TCC patients from both patients with urinary abnormalities and healthy individuals. It is noteworthy that better sensitivity was achieved in the identification of urinary lithiasis (0.9). The specificity was less than 0.5 for all groups.

Discussion

Urine tests based on molecular markers, such as nuclear matrix protein 22 (NMP22) or fibrin-fibrinogen degradation products, are diagnostic tools that are already commercially available, though they are not broadly recommended as routine tests because of the small number of scientific studies that have evaluated their utility and potential benefits [11,12]. The urine OnfFN concentration has been proposed as a biomarker of bladder TCC in the early stages and/or in malignant invasion because the production of OnfFN is associated with tumor development, extracellular matrix remodeling, and neo-angiogenesis. In addition, it is easy to identify this protein in urine samples.

The diagnostic performance for the discrimination between malignant and healthy tissue is higher than 95%, according to studies using small cohorts of patients [13-15]. However, OnfFN is also synthesized during inflammatory or repair processes [16,17], and there is therefore potential for misinterpretation.

Whether urine OnfFN concentrations can be used to distinguish between bladder TCC and benign, common pathologies of the urological tract is not known. To our knowledge, this is the first comparative study of the urine OnfFN concentrations of patients with malignant and benign pathologies of the urinary tract. In general, we found comparatively higher urine levels of OnfFN in the bladder TCC group (Table 1), in accordance with the results presented in the literature, but lower sensitivity and specificity than reported previously, possibly due to the specific features of our study, including the design, the comparators, the small sample of patients, the method of measuring the OnfFN concentration and/or the cutoff value used. Of note is that the urine OnfFN concentration was highly sensitive for the detection of urolithiasis, but not for PE, suggesting that there is a close association with the former. It is likely that lithiasis-induced urothelial inflammation and repair are responsible for the nonmalignant increase in the urine OnfFN concentrations in our cohort [16,18]. The production of OnfFN by patients with non-malignant disease might represent a limitation in the ability to identify patients with bladder TCC but highlights the potential use of urine OnfFN levels in the clinical assessment of tumor progression, when linked to invasion or metastasis, instead of other early diagnostic screening strategies.

In conclusion, the urine OnfFN concentration proved to be an acceptable indicator of bladder TCC. This method showed good performance for the identification of urolithiasis but not for the identification of PE. This result suggests that OnfFN has the potential to be used as marker of urothelial inflammation, tissue repair, and advanced stages of malignancy characterized by tissue invasion or metastasis.
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Table 2. Distribution of Patients according to Urine OnfFN Cutoff:

<table>
<thead>
<tr>
<th>Urine OnfFN</th>
<th>Bladder TCC</th>
<th>NMUD controls</th>
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</thead>
<tbody>
<tr>
<td>&gt; 0.038 ug/mL</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>≤ 0.038 ug/mL</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

TCC, Transitional cell carcinoma; NMUD, non-malignant urinary diseases, including prostate enlargement, urinary lithiasis and healthy individuals.

Acknowledgement

The authors acknowledge the support provided by Adeza Biomedical Corporation, California, USA, regarding onfFN determination.

References