CHAPTER 3

INCREASED URINARY LEVELS OF TAMM-HORSFALL GLYCOPROTEIN SUGGEST A SYSTEMIC ETIOLOGY OF INTERSTITIAL CYSTITIS

BADE JJ, MARRINK J, KARRENBELD A, WEELE L VAN DER, MENSINK HJA.

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Abstract.

**Purpose.** To investigate the role of Tamm-Horsfall protein (THP) in interstitial cystitis (IC).

**Materials and Methods.** Analysis of urinary Tamm-Horsfall protein excretion in interstitial cystitis patients and control patients. Immunohistochemical staining of bladder biopsy specimens for THP.

**Results.** Urinary THP levels of female IC patients (n=28) were statistically significantly higher than those of female controls (n=25). No positive staining for THP could be demonstrated in the bladder tissue of the IC patients (n=10).

**Conclusions.** The results support the notion that the IC syndrome may have a systemic etiology. In addition, this type of assay might have clinical value in the diagnosis of IC.
INTRODUCTION
In 1950 two virologists, Tamm and Horsfall\(^1\), discovered a glycoprotein that now bears their names. Tamm-Horsfall Protein (THP) is the most abundant protein in normal urine and a major component of tubular and urinary casts. THP is synthesized exclusively in the kidney. It has a subunit size of approximately 100,000 Daltons but also a strong tendency to form macroaggregates of several million Daltons. The 24-hr quantity of THP excreted by human individuals has been reported to be 39 mg (±13 mg) and is not influenced by exercise, age or diuresis\(^2\). THP is essentially localized in the cells of the thick ascending limb of Henle’s loop. Two hypotheses have been put forward about its function: (1) THP could be a co-transporter of electrolytes and (2) THP could be responsible for the water impermeability of the nephron segment due to its ability to form gels\(^3\). Several pathophysiological actions have been suggested. For instance, THP was found to bind to E Coli bacteria which should prevent the adhesion of bacteria to the urinary epithelium\(^4\); THP seems to be a constant component of renal stones; and interstitial nephropathy was demonstrated in experimental rat and rabbit models after active immunisation with THP\(^5\).

Interstitial cystitis (IC) is a chronic benign disease of the bladder, that mainly affects women and has debilitating symptoms. The origin of interstitial cystitis is unknown. Currently, there is no compelling evidence to support any of the hypothetical causes. Studies that demonstrate intra-urothelial THP and elevated levels of antibody to THP in IC patients seem to confirm the theory of increased bladder permeability to urinary constituents as the etiological mechanism of IC and to offer a possible diagnostic marker\(^6,9\). However, more recent studies have contradicted previous reports\(^6,9\). A possible pathophysiological relation between Tamm-Horsfall protein and interstitial cystitis remains speculative and, as such, forms a challenge.

This study investigates the THP excretion of IC patients compared to controls. In addition, bladder biopsy specimens of IC patients have been examined for possible THP deposits.

METHODS AND PATIENTS
Patients. Multiple 24-hour urine samples were collected prospectively from 30 consecutive patients, diagnosed and treated for interstitial cystitis (IC-patients) and 46 controls (Control-patients). All of the interstitial cystitis patients were diagnosed according to the criteria published by the National Institutes of Health\(^10\) for the admittance of subjects into research studies and were suffering from symptoms typical of interstitial cystitis at the time of urine collection. The average functional bladder capacity of the IC-patients was 189 cc (range 70 cc to 385 cc) and their average disease
history 5.6 years (range 2 to 18 years). A functional bladder capacity of above 400 cc formed an exclusion criterion for the diagnosis of interstitial cystitis. The group of controls (Control-patients) comprised patients (n=46) with different urological diseases, shown in Table 1.

**ELISA assay.** Urine samples of 24-hour urine were kept frozen (-20 °C) until use for Tamm-Horsfall protein quantification. Tamm-Horsfall protein determinations were performed on all the urine samples according to a 'blind' experimental design using an in-house ELISA. Anti-THP antibodies and THP standard were purchased commercially. In short, polyclonal sheep-anti-human THP antibody (IgG fraction; The binding Site/Biomedical Diagnostics) was bound to the wells of microtitre plates. Standards (Calbiochem) and urine samples were incubated at 37°C for 45 minutes. Non-bound material was expelled and after washing, polyclonal sheep-anti-human THP-peroxidase conjugated antibody (the binding Site/BioMedical Diagnostics) was allowed to react at 37°C for 30 minutes. Reaction products were visualised with o-phenylenediamine as the substrate; absorbance was then measured at 492 nm. Reproducibility and the influence of prolonged frozen storage of the urine samples were tested.

**Immunohistochemical detection of Tamm-Horsfall protein.** Bladder tissue samples were obtained from all 30 IC patients during cystoscopy under anesthesia. The tissue samples were partly frozen (-20°C) and partly fixed in 10% buffered formalin. The latter samples were embedded in parafin and prepared according to routine light microscopic procedures. Sections (5 µ thick) from 10 interstitial cystitis and 3 control patients were incubated for 60 minutes with the murine monoclonal antibody against Tamm-Horsfall protein diluted 1:1000 in phosphate buffered saline (Sanbio - Uden, the Netherlands). The streptavidine-biotine immunoperoxidase technique was used to identify binding of the monoclonal antibody in the tissue sections. Specificity of the monoclonal antibody for Tamm-Horsfall protein was demonstrated by the immunohistochemical study of normal renal parenchyma using the aforementioned techniques.

**Data analysis.** Data analysis was performed with the commercial SPSS/PC Statistical Package. The chosen level of statistical significance was p = 0.05. Sensitivity was defined as the ability to detect the disease; specificity was defined as the ability to detect the absence of disease; and predictive diagnostic value was defined as the ability to detect patients with the disease (interstitial cystitis) in a given population.

**RESULTS**

We analysed 101 24-hour-urine collections from 30 interstitial cystitis patients (IC-patients) and 52
24-hour-urine collections from 46 control patients (Control-patients). All 24-hour-urine samples were collected between February 1993 and February 1995. Average storage time before analysis was 2.2 weeks at -20°C. The IC-patients were 28 females and 2 males with an average age of 55.2 years (24-79 yrs); the control-patients (Table 1) comprised 24 females and 22 males with an average age of 56.3 years (4-82 yrs). Creatinine clearance of all patients (based on serum creatinine, age and weight) was within the normal range.

Table 1.

<table>
<thead>
<tr>
<th>DIAGNOSIS</th>
<th>CONTROL-PATIENTS:</th>
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<tbody>
<tr>
<td>urinary tract infection</td>
<td>n=12</td>
</tr>
<tr>
<td>supravesicular obstruction</td>
<td>n=7</td>
</tr>
<tr>
<td>urinary diversion</td>
<td>n=6</td>
</tr>
<tr>
<td>infravesicular obstruction</td>
<td>n=4</td>
</tr>
<tr>
<td>urolithiasis</td>
<td>n=4</td>
</tr>
<tr>
<td>invasive bladder carcinoma</td>
<td>n=3</td>
</tr>
<tr>
<td>renal adenocarcinoma</td>
<td>n=3</td>
</tr>
<tr>
<td>superficial bladder carcinoma</td>
<td>n=3</td>
</tr>
<tr>
<td>vesico-ureteral reflux</td>
<td>n=2</td>
</tr>
<tr>
<td>pyeloureteral stenosis</td>
<td>n=2</td>
</tr>
<tr>
<td></td>
<td>n=46</td>
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</table>

To prevent selection bias we used the first urine sample test result of each patient for statistical analysis. Tamm-Horsfall concentrations in both groups showed a skewed distribution and a considerable range, reflected in high standard deviations. This violated the pre-assumptions for using Student’s t-test. Therefore, the non-parametric Mann-Whitney test was used to determine statistical significance. Analysis for age did not show a correlation between THP excretion and age. A similar analysis for gender showed a significantly higher THP excretion in the female patients than in the male patients, conform to the predominance of females among IC-patients. However, urinary THP
levels were also higher among female Control-patients (mean 25.2 mg/l and 42.7 mg/24 hrs) than those of the male patients (mean 14.8 mg/l and 26.4 mg/24 hrs). Although these differences were not statistically significant (p=0.208 for mg/l and p=0.496 for mg/24hrs, Mann-Whitney test), we decided to include only female patients in the further analyse.

The individual and mean values for urinary Tamm-Horsfall protein excretion, expressed in concentration (mg/l), total amount (mg/24-hrs) and related to creatinine (µg/mg creat) are shown in Figure 1, Figure 2 and Figure 3, respectively. Differences between IC-patients and Control-patients were statistically highly significant with p=0.006 (mg/l), p=0.003 (mg/24 hrs) and p=0.002 (µg/mg creat.). To evaluate the value of THP excretion as a diagnostic marker, we used ‘normal’ excretion values 40 mg/24 hrs or 40 µg/mg creat., as cut-off values. Distribution and percentages are presented in Table 2. Sensitivity was 67%, predictive diagnostic value was 67% and specificity was 78%.

Table 2.

Tamm-Horsfall protein excretion using cut-off values of 40 mg/24hrs and 40µg/mg creat., and statistical significance using the Chi-square test.

<table>
<thead>
<tr>
<th></th>
<th>IC-PATIENTS</th>
<th>CONTROLS</th>
<th>Chi-square test</th>
</tr>
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<tbody>
<tr>
<td>&gt; 40 MG/24 HRS</td>
<td>n=20 (71.4%)</td>
<td>n=6 (25.0%)</td>
<td></td>
</tr>
<tr>
<td>≤ 40 MG/24 HRS</td>
<td>n=8 (28.6%)</td>
<td>n=18 (75.0%)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>&gt; 40 µG/MG CREAT.</td>
<td>n=17 (60.7%)</td>
<td>n=6 (25.0%)</td>
<td></td>
</tr>
<tr>
<td>≤ 40 µG/MG CREAT.</td>
<td>n=11 (39.3%)</td>
<td>n=18 (75.0%)</td>
<td>p &lt; 0.0001</td>
</tr>
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</table>

 Differences between infection (positive urine cultures) and non-infection patients, and between stone and non-stone disease were not significant. In three patients with renal adenocarcinoma, THP excretion was 14 mg/24 hrs, 50 mg/24 hrs and 57 mg/24 hrs, respectively. In two control patients, THP excretion levels of over 100 mg/24 hrs were detected. One was diagnosed as having an obstructed ureter and was known to have SLE and rheumatism, while the second patient was diagnosed as having a pyobladder.
To rule out storage artefacts, 5 urine samples were stored at either -20°C or -80°C and analysed at 2, 4, 7, 9 and 12 weeks. Low THP concentrations (<25 mg/l) remained almost stable at different temperatures and after different storage intervals. High THP concentrations (>50 mg/l) fluctuated by up to 50% at -20°C, and by up to 40% at -80°C, but only after storage for more than 4 weeks. Values fluctuated between 0% and 22% after storage at -20°C for less than 4 weeks.

The bladder biopsy specimens of only one of the ten IC patients stained weakly positive for THP. Control staining of normal renal parenchyma was highly positive.

DISCUSSION

In a prospective study on urinary Tamm-Horsfall protein (THP) excretion, a significant difference was detected between (female) interstitial cystitis patients (n=28) and (female) controls (n=25). Despite the increased values, no Tamm-Horsfall protein bladder depositions could be demonstrated.

Urinary excretion of Tamm-Horsfall protein has previously been linked to uro-pathological conditions. Its potential role, stimulation or inhibition, in stone formation was studied in the late seventies but no increase in THP urinary excretion could be detected in individuals with bladder or renal urolithiasis when compared to healthy controls. More recently, the inhibitory effect of potassium citrate treatment on calcium oxalate monohydrate crystal agglomeration was associated with increased levels of THP. Reinhart et al. studied THP as a potential urinary defense mechanism against urinary tract infections. A decrease in THP urinary levels was reported in elderly patients especially during episodes of urinary tract infection. This could not be reproduced in young women with and without recurrent urinary tract infections. Urinary excretion of Tamm-Horsfall protein in interstitial cystitis patients was reported only once. Callahan et al. could not demonstrate a difference between IC patients and controls, but only random urine samples were analysed (µg THP per mg creatinine). In contrast to the latter study, our data, based on 24-hour-urine collections, demonstrated increased THP urinary levels in interstitial cystitis patients; in absolute values as well as in relation to creatinine excretion. These differences were statistically significant. A clear explanation for this increase is not readily at hand.

A number of factors are thought to influence the quantity of Tamm-Horsfall protein measured in urine. These include aggregation of the glycoprotein, alkalisation of urine, storage artefacts and difference between the sexes. Gender was also observed to have an influence in our Control-patients. As the IC-patients mainly comprised female patients, we excluded all the male patients.
from the statistical analyse to achieve a genuine comparison between controls and IC patients. IC-
patients and Control-patients were compatible with respect to age, renal function and urine pH. No positive correlation could be detected between urinary THP levels and urine culture results. Sixteen of the IC-patients underwent a detailed dietary interview which did not reveal any self-imposed specific dietary restrictions or avoidance of certain foods. A separate study was conducted to control for possible storage artefacts and showed that these could be excluded as possible causes of the difference in values between IC-patients and Control-patients. Presumably, if circumstances beyond our observation influenced the measurements, they did so in both groups. Thus, the difference in THP most probably reflects the difference in pathology between the two groups: interstitial cystitis.

Increased Tamm-Horsfall protein excretion in interstitial cystitis patients raises two questions. First, could the THP urine level function as an objective criterion or parameter in diagnosing IC? and second, how should one interpret the relation between THP urinary excretion and interstitial cystitis, as coincidental or causal?

At present, the diagnosis of interstitial cystitis is based on clinical criteria, without a reliable diagnostic marker. However, by using 40 mg/24 hrs and 40 µg/mg creat. as cut-off values, the sensitivity (67%) and predictive diagnostic value (67%) of THP failed to meet the ideal 100%. Although not (yet) suitable as a diagnostic marker, THP urine levels might be used as an additional positive indication for the diagnosis of interstitial cystitis.

The second question addresses the hypothetical explanation of the registered increment of urinary Tamm-Horsfall protein. Should it be regarded as being coincidental, or causative or reactively related to IC? The former seems unlikely in view of the statistical significance of the difference between the female patient groups. Only a speculative answer can be given to the second part of the question. Nevertheless, if increased THP excretion can be attributed to interstitial cystitis, it would suggest a causative relation because, according to present physiological knowledge, a bladder-confined disease has no feedback mechanism to the kidney and THP is solely produced in the kidney. THP levels might increase in combination with other, yet unknown, toxic urinary agents which cause or contribute to the symptoms of IC18. Or a systemic, i.e. an auto-immune disease may cause asymptomatic interstitial nephritis which is known to cause an increase in the THP level15. In animal experiments, an inflammatory reaction to THP has been observed, but a direct relation between an increased urinary THP level and bladder symptoms has never been reported. It has been suggested that increased permeability allows THP to infiltrate bladder tissue and may lead to
sensitisation to this protein. However, our data did not support this theory. In our patients, an increased urinary THP concentration was not associated with THP infiltration of bladder tissue. In addition, Thruong et al. reported on 18 cases with Tamm-Horsfall protein deposits in a series of 262 bladder specimens. Most deposits were related to carcinoma of the bladder, six to chronic cystitis and none to interstitial cystitis.

CONCLUSIONS

The increased urinary THP levels in interstitial cystitis patients support the etiological theory of a systemic disease and may offer new perspectives as diagnostic indicator for the diagnosis of the interstitial cystitis syndrome.

REFERENCES


Figure 1

Individual and mean urinary Tamm-Horsfall protein (THP) concentration. The (female) IC-patients excreted a mean 40.7 mg/l (S.D. 26.5) and the (female) Control-patients a mean 25.2 mg/l (S.D. 25.1). The difference was statistically significant (p=0.006) using the Mann-Whitney test.
<table>
<thead>
<tr>
<th>mg/24-hr</th>
<th>IC patients n=28</th>
<th>Control patients n=25</th>
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<tr>
<td>140</td>
<td></td>
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<tr>
<td>120</td>
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<td>100</td>
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<tr>
<td>80</td>
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<td>mean 69.8</td>
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<td>40</td>
<td>mean 42.7</td>
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Individual and mean 24-hour urinary Tamm-Horsfall protein (THP) excretion. The (female) IC-patients excreted a mean 69.8 mg/24 hrs (S.D. 35.5) and the (female) Control-patients a mean 42.7 mg/24 hrs (S.D. 49.0). The difference was statistically significant (p=0.003) using the Mann-Whitney test.
Figure 3

Individual and mean urinary Tamm-Horsfall protein (THP) excretion related to the creatinin concentration. The (female) IC-patients excreted a mean 65.9 µg/mg creat. (S.D. 46.9) and the (female) Control-patients a mean 43.0 µg/mg creat. (S.D. 61.4). The difference was statistically significant (p=0.002) using the Mann-Whitney test.