Tamm-Horsfall protein excretion to predict the onset of renal insufficiency

María C. Romero, Noemí Zanaro, Liliana González, Pedro Trigo, Oscar Imventarza, Alcira Nesse

Abstract

Objective: Immunosuppressive therapy after liver transplantation may be a risk for kidney dysfunction. This work was designed to determine whether Tamm-Horsfall Protein (THP) could be considered as a marker for nephrotoxicity.

Design and Methods: THP was determined by an ELISA method in serial 24-h urine from liver transplant patients. Fourteen patients suffered renal insufficiency (LTr₁) and 20 showed no acute renal damage (LTr₂) after liver transplantation.

Results: No clear association could be seen between daily THP excretion and plasma creatinine levels by comparing serial samples collected at the same time. Nevertheless, significant differences were observed in pretransplant THP excretion between both groups of patients. The results (Median/Interquartile Range) were: Controls: 113.2/84.9 to 146.8 mg/24 h (p < 0.001 with respect to C and LTr₂); LTr₁: 36.9/18.3 to 54.5 mg/24 h; LTr₂: 90.8/61.5 to 139.7 mg/24 h.

Conclusions: The higher pretransplant synthesis and/or secretion of THP seem to have a protective role on the kidney during and after liver transplantation. © 2002 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Tamm-Horsfall Protein; Liver transplantation; Immunotherapy; Renal insufficiency; Nephrotoxicity; Biochemical markers

1. Introduction

Tamm-Horsfall Protein (THP) is a large glycoprotein with unknown physiologic function synthesized in the thick ascending limb of Henle’s loop and early distant convoluted tubules of the nephron. The physiologic role of THP has not been clearly established yet. Nevertheless, it seems very likely that this protein is involved in the development of cast nephropathy, in the formation of renal stones, in immunologic defense in the kidney, as well as in the modulation of systemic immunologic events. Urinary THP has also been postulated as a suitable parameter for determining the functional state of the kidney. In this context, THP excretion was found significantly lower in patients with renal disease and immediately after renal transplantation, reflecting the onset of acute renal failure in the early phase of transplantation. Besides, an association between glomerular filtration rate and THP excretion was reported for glomerular as well as for tubular diseases. In cases of delayed onset of renal graft function accompanied by extremely reduced urinary THP amounts, the functional recovery, whether spontaneous or brought about by treatment, was characterized by a continuous increase in urinary THP excretion. In addition, patients with diabetic nephropathy had a lower excretion rate of THP. After cardiac surgery, transient renal dysfunction often occurs and this has been related to low levels of THP excretion, among other pathologic biochemical values.

Immediately after liver transplantation, multiple factors related to either the surgical process or the immunosuppressive therapy might be associated with the development of early renal insufficiency. This renal complication increases the risk of morbidity and mortality in liver transplant patients. Nevertheless, it seems to be difficult to determine this risk in patients suffering severe liver dysfunction because neither cystatin C nor creatinine serum levels proved to
Table 1

<table>
<thead>
<tr>
<th>Individual populations</th>
<th>C</th>
<th>LTr1</th>
<th>LTr2</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>30</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Sex distribution</td>
<td>10 M, 20 W&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9 M, 5 W&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 M, 16 W&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (Median/Range)</td>
<td>41/25–60 NS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40/19–67 NS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31/16–60 NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pretransplant</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76 ± 8.1 µmol/L NS&lt;sup&gt;d&lt;/sup&gt;</td>
<td>61 ± 5.8 µmol/L NS&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma Creatinine</td>
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<sup>a</sup>M: males, W: women  
<sup>b</sup>ND: non-determined values  
<sup>c</sup>Expressed as Median/Range. NS: non-significant differences between groups  
<sup>d</sup>Expressed as Mean ± SEM. NS: non-significant differences between LTr<sub>1</sub> and LTr<sub>2</sub> levels of plasma creatinine

reliably predict exact glomerular filtration rate in these patients [12–14].

Taking into account all the above-mentioned information, the aim of this study was to find some early sensitive marker to predict and/or evaluate renal dysfunction in liver transplant patients. With this purpose, serial THP excretion was measured and its association with clinical and biochemical signs of nephrotoxicity investigated to evaluate whether this renal protein could be considered a useful marker for the onset of renal failure after surgery.

2. Materials and methods

2.1. Patients and sampling

Control subjects (C, n = 30) were hospitalized patients and laboratory staff without history of either liver or renal disease and no medical treatment (Table 1).

Thirty-four patients undergoing liver transplantation were included in this study. When analyzing the results, they were separated into two groups according to their condition after surgery. During the surgical procedure or in the immediate posttransplant period, 14 patients suffered a renal insufficiency complication (Table 1, LTr<sub>1</sub> Group), while the other 20 patients showed no acute renal damage (Table 1, LTr<sub>2</sub> Group). The clinical profile of LTr<sub>1</sub> group were: 6 patients with hepatitis C virus, 2 with familial amyloidosis, 2 with autoimmune hepatitis, 2 with alcoholic cirrhosis, 1 with hepatitis B virus and 1 with fulminant hepatitis. The clinical profile of LTr<sub>2</sub> group were: 6 patients with autoimmune hepatitis, 5 with primary biliary cirrhosis, 2 with primary sclerosis cholangitis, 3 with cryptogenic cirrhosis, 1 with hepatitis C virus, 1 with secondary biliary cirrhosis, 1 with Wilson disease and 1 with fulminant hepatitis. Although different etiologies were responsible for the liver failure, no patient with either pretransplant hepatorenal syndrome or any other illness that could affect the renal function was included in the study. No difference in renal function estimated by plasma creatinine was found between both groups of patients in the pretransplant condition (Table 1). Early renal insufficiency was defined as an increase in plasma creatinine concentration to values higher than 130 µmol/L. In general, patients in the LTr<sub>2</sub> group were younger than in the LTr<sub>1</sub> group but no significant differences in age were found between both groups.

Plasma samples were obtained from heparinized blood and serial 24-h urine samples collected from patients, before and after surgery. Urine samples from a control group were also studied. Aliquots of both plasma and urine samples were stored at −20°C until assayed.

Therapeutic management, such as immunosuppressive drugs and doses, dialytic treatment and transfusion events were registered.

2.2. Biochemical determinations

Urinary THP was quantitatively determined by an ELISA method developed in our laboratory [15]. Serial dilutions of urine samples and THP standard isolated in our laboratory [15] were applied to ELISA microplates (Corning R, NY, USA). Dilutions were prepared in buffer containing 15 mM Na<sub>2</sub>CO<sub>3</sub>, 25 mM NaHCO<sub>3</sub>, 0.02% (w/v) sodium azide, pH 9.6. After overnight incubation at 4°C, the plates were washed with PBS-Tween buffer, pH 7.4 (137 mM NaCl, 147 mM KNa<sub>2</sub>PO<sub>4</sub>, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.68 mM KCl, 0.02% (w/v) sodium azide, 0.05% (v/v) Tween). A blocking 1 h-incubation at room temperature with 0.1% (w/v) bovine serum albumin (Sigma Chemical Co., USA) was followed by repeated washing. Either experimental antihuman THP prepared in our laboratory [15] or commercial antihuman TH glycoprotein (Biomedical Technologies, USA) was added and left to stand for 4 h at 37°C. After washing, alkaline phosphatase conjugated antirabbit IgG (Dako, Denmark) was used as second antibody. An overnight incubation at room temperature and washing was followed by color development obtained by addition of 5.6 mM sodium p-nitrophenyl-phosphate in 10% (v/v) diethanolamine, 0.5% (w/v) MgCl<sub>2</sub>, pH 9.6. The color reaction was stopped by addition of 3 mol/L NaOH and the absorbance of the colored product immediately read at 405 nm in an ELISA reading equipment (Microwell System, Organon Teknika, Belgium). A standard curve ranging 16 to 250 µg THP/L was included in each plate. The interassay variabil-
ity of THP excretion determined by similar methodology proved to be 4.4% [15].

Creatinine concentrations were determined in plasma and urine samples obtained during the pre and posttransplant periods employing an autoanalyzer (Hitachi 704, Boehringer-Mannheim Diagnostics, USA).

Blood cyclosporine levels were assayed by means of a Fluorescence Polarization Monoclonal Immunoassay (FPIA), employing a TDX-ABBOTT Analyzer (Abbot laboratories, Argentina).

2.3. Statistical analysis

Results of THP excretion are expressed as Median and Interquartile Range, the latter including values between the 25th and 75th percentiles.

Comparisons between two groups were carried out by the nonparametric Mann-Whitney U test, while the Kruskal-Wallis one-way analysis of variance was applied to evaluate statistical differences among groups. Least significant differences with \( p < 0.01 \) were considered as criterion of statistical significance. Spearman’s correlation coefficients were calculated to measure the degree of association between variables.

3. Results

The amount of THP excretion was determined in samples collected before liver transplantation and during the hospitalized period after the surgical procedure (up to 15–150 days, depending on each patient’s postoperative hospital stay). The renal failure which appeared during either the surgical procedure or the posttransplant period let us divide the transplant patients into two subgroups, LTr1 and LTr2. Coincidentally, the values of daily THP excretion in both groups proved to be significantly different. The Figure 1 shows that the appearance of renal insufficiency seems to be restricted to patients who had shown low pretransplant THP excretion (LTr1), while patients in the LTr2 group excreted daily amounts of THP similar to those of the C group. Expressed as Median/Interquartile Range, data of pretransplant 24-h excretion of THP in the 3 groups were:

- C: 113.2/84.9 to 146.8 mg/24 h (n = 30)
- LTr1: 36.9/18.3 to 54.5 mg/24 h (n = 14, \( p < 0.001 \) with respect to C and LTr2)
- LTr2: 90.8/61.5 to 139.7 mg/24 h (n = 20)

In a previous work, no significant differences in THP excretion were observed between men and women and individual day-to-day variations were found between 10 and 20% [15]. Nevertheless, it seems unlikely that this variability could account for the significant differences observed between both groups of patients.

No patient in the LTr1 group showed THP excretion level higher than that of the 25th interquartile value corresponding to either the C or the LTr2 group. On the other hand, only one patient in the LTr2 group (N.G., woman, 43 yr) excreted THP as low as 25.6 mg/24 h before liver transplantation, although she never showed depressed kidney function throughout the study.

During the liver posttransplantation period, the amount of THP excretion showed high variability among patients and, generally, remained within the control range during the period studied (up to 15–150 days). Moreover, no significant differences could be found in the posttransplant THP excretion between both groups of patients, LTr1 and LTr2, and this was uninfluenced by pretransplant clinical conditions.

Tests to establish association between urinary THP and either clinical signs or biochemical parameters were carried out. To determine whether the amount of the excreted THP could be considered a reliable early biochemical sign of renal insufficiency, the urinary daily amount of THP and plasma creatinine concentration were compared in samples collected the same day before surgery and after the transplantation procedure took place. No evident changes in urinary THP were observed at the time of the onset of renal injury in patients of the LTr1 group. The finding of uniform levels of THP excretion while plasma creatinine increased threefold was proof of the lack of association between both variables. Even though some patients showed posttransplant THP amounts nearly fivefold as low as the daily THP excretion observed before the surgical procedure, this decrease did not precede any sign of renal insufficiency.

No relationship was found between THP urinary levels and either clinical or individual data (age, sex, aetiology of liver disease, rejection of the organ, immunosuppressive treatment and blood units for intrasurgical transfusion) or another biochemical parameter (urine output or plasma cyclosporine levels).
4. Discussion

This study shows a remarkable association between significant low pretransplant THP excretion and the onset of renal insufficiency in the immediate postsurgery period in liver transplant patients (Figure 1). In other words, the daily urinary THP amounts, determined just before surgery, could be considered as a predictive value for postsurgical acute renal dysfunction. To our knowledge this is the first report showing the importance of THP to keep kidney function intact. It can be assumed that a normal pretransplant synthesis and/or secretion of renal THP might have a protective role on the kidney, probably leading to more effective resistance of the organ to surgical stress. Mechanisms preventing tubular dehydration can be postulated, taking into account that THP may be involved in processes of urine dilution and concentration [1,16]. Nevertheless, a minor tubular dysfunction without any other expression of renal failure before liver transplantation cannot be discarded.

On the other hand, THP excretion seemed not to be a suitable parameter for renal disturbance diagnosis, at least for the patients included in this study. Several previous reports have underlined the influence of renal function upon the urinary THP content [3–6,8–11]. The excretion rate of this protein was found decreased in patients with diabetic nephropathy and this decrease was attributed to diminished renal function, as shown by a negative correlation between urinary THP and serum creatinine concentration [10]. Contrary to this finding, we were unable to detect a strong relationship between THP excretion and plasma creatinine levels. This lack of association between urinary THP and plasma creatinine, in disagreement with the above-mentioned results reported by other authors, could be due to differences in the characteristics of the kidney disease. Diabetic patients studied by Torffvit et al. [10] had clinical signs of nephropathy at the time of the study, suggesting deep disturbance of the renal medullar function, whereas the patients in the present work had severe liver illness without kidney dysfunction and the development of renal insufficiency was probably due to factors more related to the surgical procedure, such as hemodynamic disturbances.

In conclusion, the statement that arises shows that THP excretion could be determined as a useful predictive marker instead of an early diagnostic parameter of the onset of renal dysfunction after liver transplantation. Even though this topic should be studied in a larger population, it can be suggested, based on the present data, that quantitative determination of daily THP excretion should be performed before liver transplantation. This would allow establishing the likely risk of patients to develop renal dysfunction. Moreover, careful optimization of management of patients during surgical procedure and posttransplant evolution might be taken into account.

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