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ORIGINAL PAPER

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Tamm-Horsfall protein in patients with kidney damage and diabetes

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Abstract Tamm-Horsfall protein (THP) is a glycoprotein present abundantly in human urine. It is localized in the thick ascending limb of the loop of Henle (TAL) and the early distal convoluted tubule (DCT). The rate of urinary excretion of THP has been studied in various diabetic groups. It has been postulated that urinary THP may be a useful marker for renal damage. The aim of this study was to compare directly the immunogold localization of THP in diabetic and control kidney tissue specimens with or without kidney damage. Immunogold labeling was performed on archival tissue samples of 34 diabetic and 18 control human kidneys at the light microscope level. Slides were ranked as having a high, moderate or low degree of reaction. The majority of diabetic samples had a slightly lower degree of THP, while patients with known renal dysfunction had lowest THP. Previous studies have found a decreased excretion of urinary THP in diabetics. Our results show that decreased gold labeling is associated with known renal damage and may indicate damage to the thick ascending limb of the loop of Henle and the early distal convoluted tubule, irrespective of presence or absence of diabetes.

Keywords Tamm-Horsfall protein · Kidney · Diabetes · Immunogold labeling · Microscopy

Introduction

Tamm-Horsfall protein (THP) is a glycoprotein named after its discoverers Tamm and Horsfall [1]. It is the most abundant protein in human urine and a major constituent of urinary casts [2]. THP is present in the kidneys of all placental mammals, including humans [3]. THP is primarily localized to the thick ascending limb of the loop of Henle (TAL) and the early distal convoluted tubule (DCT) of the kidney. The DCT is thought to be embryologically and functionally similar to the TAL [4, 5]. Immunofluorescence studies and immunoultrastructural analysis have revealed that THP is localized within the cells of the TAL and early DCT, on both luminal and basolateral membranes outlining the infoldings [6]. Immunogold labeling of THP at the electron microscope level by Peach et al. [8] showed that all epithelial cells lining DCT had gold labeling only on their luminal plasmalemma. Cellular synthesis of THP can only be demonstrated in the cells of the TAL and early DCT [6]. Beyond the early DCT, THP is only detectable on the luminal pole of distal tubular cells [6, 7]. Rindler et al. [9] have presented data that THP is a glycosyl-phosphatidylinositol-linked plasma membrane-associated protein. Ontogenetic studies indicate that the appearance of THP coincides with the maturation of the TAL [10].

Several reports are available on the role of THP in patients with diabetes mellitus. All of these studies have compared urinary excretion rates of THP in various diabetic groups versus controls. Currently, no studies are available comparing the direct localization of THP at the light microscope level in these same groups of patients.

The purpose of this study was to compare directly the degree of localization of THP in diabetic and control human kidney specimens using the immunogold labeling technique to evaluate whether diabetes has an effect on THP secretion.

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Materials

Study materials

Archival tissue samples of human kidneys (34 diabetic and 18 control) and case histories were obtained from the Department of Pathology at the Medical College of Ohio, Toledo, OH, with the

approval of the project by the Institutional Review Board. Immunogold labeling was performed on 4–5 μm sections cut from these paraffin embedded blocks.

Immunogold labeling procedure

Briefly, tissue sections were heated at 60°C for 20 min. They were then placed directly in three changes of xylene for 6 min each. Following this, they were rehydrated in one change of each of the following for 6 min each: 100, 90 and 70% ethanol. The slides were placed in three changes of phosphate buffered saline (PBS: NaCl 7.2 g; Na_2HPO_4 1.48 g, NaH_2PO_4 0.43 g; KCL 0.2 g in 1 l dH_2O) for 3 min each. After removing excess PBS, they were placed in Lugol's solution (Sigma Chemical, St. Louis, MO, Cat. No. LG146) for 3 min and then thoroughly squirted with distilled water. The slides were placed in 5% sodium thiosulfate for 2 min. Next, they were placed in a Coplin jar of distilled water for 8 min. After the slides were blotted, serum blocking solution containing 1:10 normal goat serum (Dako, Carpinteria, CA, Cat. No. X0501) in PBS was added. Slides were incubated for 10 min at room temperature in a moist, covered box and then drained and blotted. Next, the primary antibody, anti-human THP monoclonal antibody (Accurate Chemical and Scientific, Westubury, NY, Cat. No. CL1032A), was added to the experimental slides in a 1:200 dilution of PBS containing 0.1%, Triton-X-100 (Sigma Chemical, St. Louis, MO), and 1% bovine serum albumin (Sigma Chemical, St. Louis, MO). Normal mouse serum (Dako, Carpinteria, CA, Cat. No. X0910) at a 1:10,000 dilution using bovine serum albumin-tris buffered saline (Trizmabase, 2.42 g; NaCl, 9 g; BSA, 1 g, sodium azide, 1.3 g in 1 l dH_2O) was added to the negative control slides as another means of accessing the specificity of the reaction. The primary antibody and normal mouse serum was left on the slides for 2 h at room temperature in a moist, covered incubation box. The primary antibody was drained off and the slides were rinsed well with PBS using a squirt bottle. Gold conjugate (Histogold-Mouse kit, Zymed, San Francisco, CA) was evenly applied to cover the tissue sections (~3–4 drops), and the slides were incubated for 30 min at room temperature in a moist, covered box. The slides were rinsed well with PBS using a squirt bottle. Then, they were placed in three consecutive changes of PBS for 3 min each. The slides were drained, and silverenhance solution (Histogold-Mouse kit, Zymed, San Francisco, CA) was applied to cover the tissue sections (~3–4 drops) for 4 min at room temperature. Next, they were drained and immersed in distilled water. Crystal mount was placed on the slides, and they were incubated for 10 min at 80°C. They were coverslipped with permount. Representative photomicrographs were taken using a Nikon-Optiphot light microscope at 100× magnification and enlarged to 4×.

Results

Table 1 summarizes the immunogold labeling results of the diabetic (total 34) and control (total 18) human kidney sections. Based on the amount of gold label observed using the light microscope, each patient biopsy

Table 1 Immunogold labeling results and patient information

# Biopsies	Age	Gender	Clinical manifestation	Degree of Rxn
Control (total 34)				
8	48–72	4f	Diffuse nephropathy; hypertension (1), infarction of kidney (1)	High (4+)
		3m	Nephrosclerosis (3)	
		1unk	Acute tubular necrosis; glomerulosclerosis; bilateral renal cortical adenoma (1)	
7	45–73	3f	End stage renal disease	Moderate (3+ to 2+)
		4m	Nodular glomerulosclerosis (1), polycystic kidney disease; renal tubular acidosis (1), IgA nephropathy (1), chronic pyelonephritis; hypertension (1), tubular necrosis; bilateral glomerulosclerosis; renal failure (1), nephrosclerosis (1), renal failure; liver, kidney, and splenic infarction (1)	
19	33–90	8f	Old renal infarct; vascular low	Low (1+ to 0)
		11m	Sclerosis; diffuse nodular nephropathy (1) acute renal failure; tubular necrosis; renal transplant (1), renal failure; glomerulosclerosis (2), rt. renal artery stenosis; rt. atrophic kidney; lymphocytic lymphoma; chronic pyelonephritis (1), chronic renal failure; acute tubular necrosis (1), old renal infarct; vascular sclerosis; hypertension (1), chronic renal failure; fibrosis (1), end stage renal disease; diffuse, scarring of glomeruli; arteriolar, nephrosclerosis; chronic, renal failure; hypertensive renal disease (1), nephrosclerosis; interstitial fibrosis (1), chronic renal failure; end stage renal disease (2), renal failure; renal transplant; arterionephrosclerosis (1), chronic renal failure; chronic pyelonephritis (1), congestive heart failure; arterionephrosclerosis (1), glomerulosclerosis; nephropathy; chronic pyelonephritis; arterionephrosclerosis; papillary necrosis (1), interstitial nephritis; early renal, failure; glomerulosclerosis (1), acute pyelonephritis; acute papillary necrosis; nodular glomerulosclerosis (1), nephrosclerosis (1)	
Control (total 18)				
9	48–89	4f	Arterionephrosclerosis (1)	High (4+)
		4m	Cortical cyst-lft. kidney (1	
		1unk	Chronic lymphocytic leukemia (1), severe atherosclerosis (1)	
5	45–93	2f	Mild arterionephrosclerosis (1)	Moderate (3+ to 2+)
		2m	Moderate nephrosclerosis; mild arteriosclerosis (1) arterionephrosclerosis; chronic pyelonephritis (1)	
4	53–79	3f	Hydronephrosis; bilateral	Low (1+ to 0)
		2m	Arterionephrosclerosis; retention cyst-kidney (1), acute tubular necrosis; acute renal failure (1)	

Number in parentheses of column 4 (Clinical manifestation) represents number of patients with this condition. *f* female, *m* male, *unk* unknown, *Rxn* reaction

slide was given a rank of 4+, 3+, 2+, 1+, or 0, with 4+ being the highest and 0 being the lowest (Table 1, *column 5*). Diabetic and control patient biopsies were grouped accordingly. It was not known whether the diabetics had non-insulin-dependent diabetes mellitus or insulin-dependent diabetes mellitus; therefore, they were grouped together. Table 1, *column 4* gives the clinical manifestations from the autopsy report. The numbers in parentheses under the clinical manifestation column give the number of patients with the condition indicated. These numbers do not add up to the total number of biopsies for each group because only information relevant to the renal system is included.

Figures 1, 2, and 3 are representative photomicrographs of the kidney sections stained for THP, using immunogold labeling. Figures 1a, 2a, and 3a are from control patients (without diabetes), and Figs. 1b, 2b, and 3b are from diabetic patients. A high degree of reaction (4+) was observed in both the control tissue specimen (Fig. 1a) as well as in the diabetic specimen

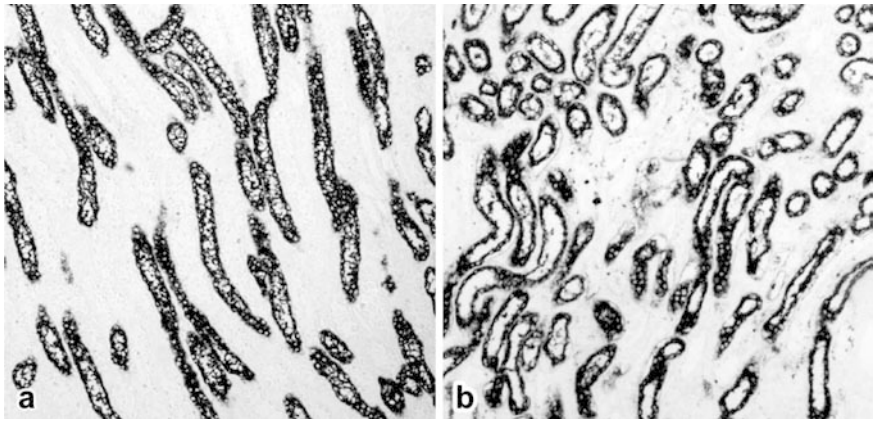

Fig. 1 a Immunogold labeling of control kidney section with high degree of reaction (4+). ×400. **b** Immunogold labeling of a diabetic kidney section with a high degree of reaction (4+). This patient did not have any other renal disease. ×400

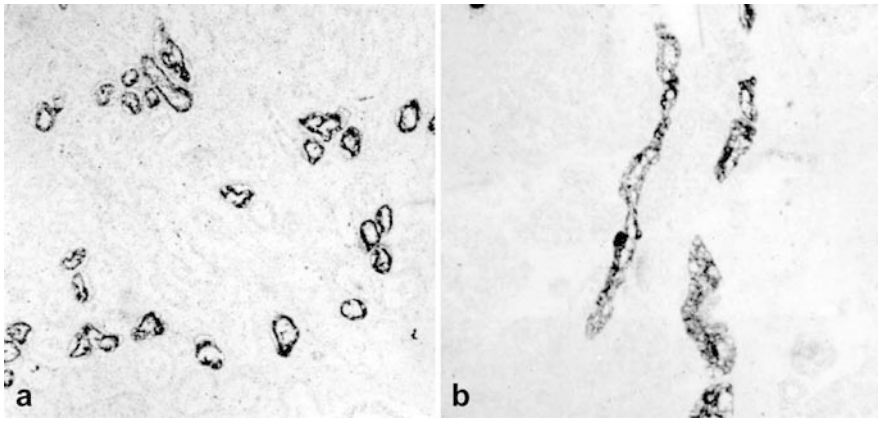

Fig. 2 a Control kidney section with moderate degree of reaction (3+) using the immunogold labeling technique. This individual had moderate, benign nephrosclerosis and mild arteriosclerosis. ×400. **b** This diabetic kidney section has a moderate degree of gold labeling (3+). This patient had polycystic kidney disease and renal tubular acidosis. ×400

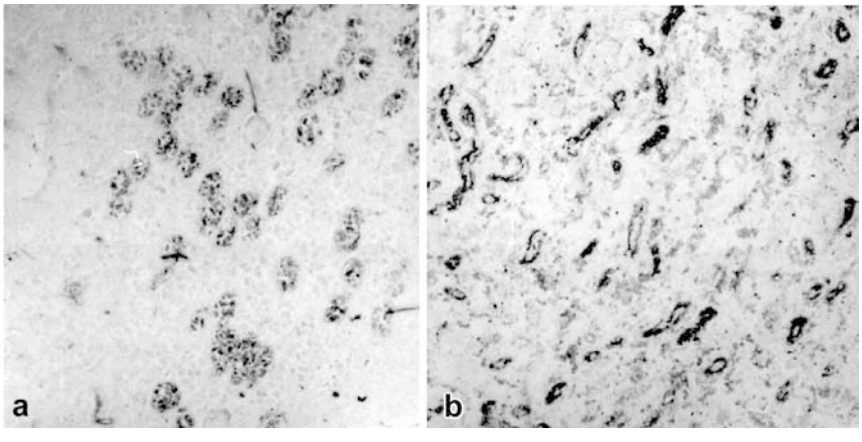

Fig. 3 a Immunogold labeling of control kidney section with a low degree of reaction (1+). This individual had hydronephrosis, bilateral arterionephrosclerosis, and a retention cyst in the kidney. ×400. **b** Immunogold labeling of diabetic kidney section showing a low degree of reaction (1+). This individual had arterionephrosclerosis. ×400

(Fig. 1b) with anti-THP antibody. The intense staining was most likely due to the lack of any renal problems at autopsy in both patients. However, a slight decrease in THP was noted in the diabetic tissue (Fig. 1b) in comparison to the control (Fig. 1a). Figure 2a shows a control slide with a moderate degree of labeling (3+). This control section was from a patient who had moderate, benign nephrosclerosis and mild arteriosclerosis at autopsy. Nephrosclerosis could be the reason for the slight decrease in the amount of labeling we observed in this section. Figure 2b shows a diabetic kidney section with a moderate degree of reaction (3+). This patient had polycystic kidney disease and renal tubular acidosis. The renal disease in this patient probably resulted in the decreased degree of staining. The control slide in Fig. 3a had a low degree of reaction (1+). This patient had hydronephrosis, bilateral arterionephrosclerosis, and a retention cyst in the kidney. Most likely, there was damage to the TAL resulting in a decreased amount of THP production. Fig. 3b is from a diabetic kidney section with a low amount of gold labeling (1+). This patient had arterionephrosclerosis.

Discussion

Tamm-Horsfall protein is primarily localized in the TAL and the early DCT of the kidney [4, 5]. Immunogold labeling of THP at the electron microscope level by Peach et al. [8] demonstrated gold labeling of the epithelial cells lining both the TAL and the DCT. Approximately 20% of TAL epithelial cells also had THP present throughout the cytoplasm at random. Golgi apparatus did not show any labeling, while endoplasmic reticulum very rarely showed positive labeling. Mitochondria and nuclei also were not positive for THP. It is not known whether cells containing cytoplasmic THP are actively synthesizing the glycoprotein or are involved in reabsorption [8]. The glomerulus, vascular bundles, interstitium, proximal tubules, thin limb of the loop of Henle, and collecting ducts were unlabeled [8]. The macula densa does not contain TH [8], which may allow for tubuloglomerular feedback to occur [11]. The abnormal release of THP from its intracellular and intraluminal locations into the renal interstitium is due to tubular rupture resulting from increased intratubular pressure or non-specific tubular damage [12]. In obstructive uropathy, there may be retrograde passage of THP into the proximal convoluted tubule and Bowman's capsule [13].This may explain some of the non-specific staining for THP we observed in our immunogold labeling results.

In general, we observed gold labeling in the TAL and early DCT. The most intense staining was found in control and diabetic kidney biopsies with the least amount of renal damage (Fig. 1a, b and Table 1). With increased renal damage in control and diabetic samples, immunogold labeling was decreased (Figs. 2a, b and 3a, b and Table 1). It can be seen from Table 1, that the majority of diabetic samples (19 out of 34) had a low degree of reaction to THP.

Several investigators have compared the urinary excretion rates of THP in various diabetic groups versus controls. Bernard et al. [14] demonstrated a pronounced decrease in the urinary excretion of THP in diabetics. In contrast, Jackle-Meyer et al. [15] and Torffvit et al. [16] did not find a significant difference between excretion rates of THP in diabetics and controls, suggesting that TAL function is not influenced by diabetes. It has been proposed that renal THP excretion may be used as an indicator of early TAL dysfunction in patients with diabetes mellitus type I [17, 18, 19, 20]. THP excretion was found to be elevated in patients with insulin-dependent diabetes mellitus duration greater than 10 years, while it was decreased in IDDM duration greater than 15 years [17]. Patients with diabetic nephropathy had THP levels significantly decreased compared with diabetics without nephropathy and controls [18]. Toffvit et al. [21] demonstrated that the urinary excretion of THP was correlated with renal function and distal tubular reabsorption of sodium in patients with chronic nephropathy. In our previous study, we observed a significant decrease in urinary THP concentration in patients with IDDM compared with patients with non-IDDM and controls by using the enzyme-linked immunosorbent technique [22]. These results indicate that the laboratory quantitation of urinary THP may be a useful indicator of cellular abnormalities such as reduced protein (THP) synthesis of the cells of the TAL and early DCT in some IDDM patients [22].

The results of our current direct immunogold labeling studies of human kidney tissues clearly indicate that there is a good correlation between renal dysfunction and the amount of THP in both diabetic and control kidney tissues. The decreased gold labeling indicates damage to the TAL and the early DCT, the area where THP is produced in the kidney. Further study on quantification of THP mRNA should be undertaken to determine the down-regulation of the gene. However, decreased level of THP is more related to renal damage than diabetes. Therefore, it may be beneficial to monitor excretion rates of urinary THP as an early indicator of renal damage to this portion of the kidney, irrespective of presence or absence of diabetes.

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