Leptospirosis renal disease

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Abstract
Leptospirosis is a re-emerging infectious disease, affecting both animals and humans worldwide. Multiple organ involvement may be encountered in leptospirosis, and early renal involvement is very common, characterized by tubulo-interstitial nephritis and tubular dysfunction. All 12 patients diagnosed in Chang Gung Memorial Hospital (Taiwan) between 1997 and 1999 had acute renal failure, with five patients requiring dialysis. Leptospira shermanii is the main serovar encountered in Taiwan, and penicillin may dramatically rescue patients from multiple organ failure provided it is given early. To understand the mechanism behind tubular injuries by leptospirosis infection, outer membrane proteins (OMPs) extracted from pathogenic leptospiro were given to tubular cells in culture. Our in vitro experiment showed that OMPs of pathogenic leptospiro activate nuclear NFκB binding and stimulate downstream inducible nitric oxide (iNOS), monocyte chemotactant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) expression. These results indicate that leptospirosis infection may induce tubulo-interstitial nephritis through a toxic component in the outer membrane followed by expression of inflammatory genes.

Keywords: acute renal failure; leptospirosis; tubulo-interstitial nephritis

Introduction
Leptospirosis, a spirochaetal infection, is considered the most widespread zoonosis, particularly in warm and humid climates [1]. Infected animals shed urine in water or soil, infecting humans via abraded wounds, mucosa, or swallowing of contaminated water. A broad spectrum of clinical manifestations may occur in humans when encountering pathogenic leptospiro from animals. Risk factors of the infection include occupational exposure, recreational activities and households with close contact to animals.

The clinical syndromes of leptospirosis vary from subclinical infection and self-limited anicteric febrile illness to severe and potentially fatal disease [1,2]. Five to 10 per cent of leptospirosis infections induce multiple organ damage, including kidney, liver and lung lesions. Weil’s syndrome is a most severe form of the infection presented by febrile illness with haemorrhagic tendencies, hepatic dysfunction and acute renal failure, leading to fatality in a short time if not treated.

As a worldwide infection, endemic and epidemic spread of leptospirosis has caused morbidity and mortality, and is considered a re-emerging infectious disease throughout the world, although the disease tends to be ignored in many cases [3,4]. In Taiwan, leptospirosis has not been thought of as common infectious disease for the past 20 years and appeared to have been underestimated as a cause of acute renal failure until we reported on it at the Chang Gung Memorial Hospital in 1997 [5]. Since then an increasing number of leptospirosis cases have been diagnosed with the aid of the veterinary diagnostic facilities at the Graduate Institute of Veterinary Medicine, National Taiwan University [6].

Tubulo-interstitial nephritis is the main cause of acute renal injury in leptospirosis. The pathogenic mechanism became clear when an in vitro model was employed to dissect the role of the leptospiro infection in tubulo-interstitial nephritis. In this review, we report the findings of a collection of 12 patients with severe leptospirosis and the possible pathogenic mechanism of renal injury.

Clinical experience of leptospirosis renal disease

Patients with leptospirosis

Between May 1996 and August 1999, a series of 12 patients in Chang Gung Memorial Hospital with a mean age of 56.3 ± 13.3 years (range 28–77 years) were
diagnosed to have severe leptospirosis. Among these patients, six were farmers, one was an employee of a beef slaughterhouse, and two patients raised dogs as pets at home. There was a preference towards male gender (nine males, three females).

**Clinical presentations**

Multiple organs were involved in these severe leptospirosis patients. The major presenting symptoms of leptospirosis were fever (10/12), jaundice (10/12) and acute renal failure (12/12). Abdominal pain (8/12) and myalgia (7/12) were early symptoms. Splenomegaly occurred in six patients and hepatomegaly in three. Other associated presentations in these patients were acute respiratory failure (9/12), disturbed consciousness (6/12), haemorrhagic tendency (4/12), rhabdomyolysis (3/12) and haemophagocytic syndrome (1/12), indicating a wide variety of leptospirosis injuries in multiple organs. Acute pancreatitis occurred in three patients who presented with elevated serum amylase and lipase. Analysis of the biochemical laboratory data showed that the mean highest bilirubin level was 15.2 ± 16.1 (0.4–42.2) mg/dl, the mean highest BUN level was 112.2 ± 26.9 (74–144) mg/dl, and the mean highest creatinine level was 7.3 ± 1.6 (4.8–9.5) mg/dl.

**Diagnosis method: the most common pathogen in Taiwan—Leptospira shermani**

Diagnosis of leptospirosis depends on serological methods via a microscopic agglutination test (MAT) to detect antibodies to leptosira and/or DNA detection by polymerase chain reaction (PCR). Serological diagnosis was made based on a single MAT titre of >1:400, or a 2-week, 4-fold increase in titres against leptospiira serovar tested. Among 240 serovars, *Leptospira shermani* was the main infecting serovar (9/12), followed by one case each of *Leptospira bratislava*, *Leptospira balum* and *Leptospira copenhageni*.

**Acute renal failure and thrombocytopenia**

Acute renal failure was presented in all patients. Five patients had non-oliguric acute renal failure while seven patients presented with oliguric acute renal failure. Hypokalemia was found on more than one occasion in nine patients during admission. Among these patients, four required temporary intermittent haemodialysis and one received continuous venous-venous haemodialysis (CVVHD) therapy. Acute tubulo-interstitial nephritis was diagnosed in two patients by renal biopsy. Although leukocytosis was found in eight patients during the course of leptospirosis, two patients were leukopenic. As previously described by others, thrombocytopenia may be associated with severe endotoxin injury due to leptospirosis, and may appear in association with acute renal failure. In our series, thrombocytopenia occurred in eight patients and, in particular, in all five patients who required haemodialysis. Three patients that required haemodialysis had severe thrombocytopenia (8000, 12,000, 41,000 cm).

**Characteristic renal sonographic findings**

All patients received renal sonography studies at the acute renal failure stage. A characteristic renal sonographic finding of acute renal involvement of leptospirosis was swollen kidneys (mean ± SD of 12.3 ± 1.2 cm in the left kidney and 12.2 ± 1.3 cm in right) and relatively normal parenchymal echogenicity. This finding may indicate tubulo-interstitial oedema by the invasion of leptospiira [5].

**Renal tubular clearance test**

To localize the renal tubular defect, tubular clearance tests were performed in four patients at the recovery phase of acute renal failure. These tests included: the bicarbonate infusion test, the furosemide test and the thiazide test, as described previously [5]. Proximal tubular defects and incomplete renal tubular acidosis were found in one patient infected with *Leptospira bratislava*. A medullary thick ascending limb dysfunction was found in two patients infected with *Leptospira shermani* and normal tubular function was found in one patient recovering from infection by *Leptospira shermani*.

**Penicillin treatment rescues patients from mortality**

Penicillin and tetracycline are the drugs of choice for leptospirosis treatment and may significantly improve multiple organ failure (Table 1). Several lines of evidence have shown that early treatment may rescue patients from multiple organ failure caused by leptospirosis [7]. In our experience, the treatment outcome for leptospirosis was very favourable if intravenous penicillin was initiated early. Among 12 patients, eight survived because treatment was given early when leptospirosis was suspected. Penicillin treatment (mean 22.9 ± 24.3 days) dramatically saved seven patients from severe multiple organ failure. One of

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<th>Table 1. Clinical outcome of leptospirosis</th>
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<td>Patients (n)</td>
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<tr>
<td>Age</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Animal exposure</td>
</tr>
<tr>
<td>Multiple organ involvement (n)</td>
</tr>
<tr>
<td>Need for dialysis (n)</td>
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<td>Most common early presentation</td>
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<td>Total outcome</td>
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<td>Penicillin treatment</td>
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(tetracycline)/three died
the surviving patients received tetracycline treatment because of the relatively mild leptospirosis. One penicillin-treated patient died because of irreversible severe multiple organ failure. Because of late recognition of the disease, three patients died without treatment. Besides the multiple nature of the organ involvement, the common presentations in deceased patients were progressive jaundice, hepatic failure, severe renal failure followed by hypotensive shock, and coma. The Jarisch-Herximer reaction, a condition described for a temporary worsening condition after effective treatment due to lysis of organisms, occurred in one patient. However, supportive treatment rescued the patient.

**Understanding the pathogenic mechanism of leptospirosis renal disease**

**Tubulo-interstitial nephritis is the main manifestation of renal injury**

Although leptospirosis is an important cause of acute renal failure worldwide, mechanisms of renal dysfunction have not been fully studied and understood. Tubulo-interstitial nephritis is the main manifestation of acute renal failure with the characteristic renal lesions, including interstitial oedema and cellular infiltrates in the tubulo-interstitial area [8,9]. Previous reports have shown that the main factors in the pathogenesis of renal lesions are related to the presence of organisms elicited by their migration and elaboration of their virulent toxins, including products released by lysis of the microorganism [10]. Tubulo-interstitial changes may be reversible if treatment is initiated early. However, if left untreated, the illness may lead to a chronic carrier state of the disease, in which leptospirosis localizes and remains viable in the renal tubules despite the presence of humoral or cellular immunity of the host [11].

Similar renal injuries could be reproduced in animal models of leptospirosis. The kidneys of guinea pigs with leptospirosis showed tubular cell injury, interstitial nephritis and associated microvascular injury, particularly at the corticomedullary junction [12,13]. Ultrastructural study of the kidney after inoculation of Leptospira pomona in mice demonstrated the route of entry of leptospiroa by penetrating capillary lumen at day 2 followed by entering the interstitial tissue elaborating oedema and cellular infiltration at days 4–8. By day 10, leptospiroa can be found in the proximal tubular cells, and by day 14 are invading the tubular lumen [14].

**Renal tubular dysfunction is caused by leptospiroa endotoxin**

Tubular dysfunction is commonly seen in infected individuals. Clinically, acute renal injury in leptospirosis is usually associated with a special form of tubular dysfunction. Polyuria and hypokalemia appear frequently with an elevated urinary fractional excretion of potassium [15]. The cause of hypokalemia is thought to be due to proximal tubular lesions. Animal studies of guinea pigs suggested that proximal tubular injury leading to the decrease in sodium and water reabsorption might be responsible for the hypokalemia. The increase in sodium and water delivery to the distal tubule may enhance potassium secretion [16].

The general effect of leptospiral renal dysfunction has been thought to originate from leptospiroa endotoxin. Administration of leptospiroa endotoxin to human mononuclear cells induces an increase of tumor necrosis factor-α (TNF-α) [17]. Younes-Ibrahim et al. [18] suggested that the Na⁺–K⁺ ATPase along the nephron is a molecular target for the Leptospira interrogans endotoxin and the glycolipoprotein fraction extracted contains a potent inhibitor of renal Na⁺–K⁺ ATPase. The inhibition of Na⁺–K⁺ ATPase may subsequently affect the apical located Na⁺–K⁺–Cl⁻ co-transporter and accounts for the observed potassium wasting. However, a direct inhibitory effect on Na⁺–K⁺–Cl⁻ co-transporter, abundantly located on the apical membrane of the thick ascending limb segment, was found in Leptospira shermani infected patients who presented with polyuria and hypokalemia [19]. It is thus possible that the leptospiroa endotoxin attacks miscellaneous nephron segments, depending on different serotypes and various concentrations.

**Leptospiroa outer membrane protein and endotoxin**

Spirochaetes and Gram-negative bacteria contain an outer membrane, which serves as a permeability barrier. The outer membrane of leptospiroa contains antigenic and virulent components, including lipopolysaccharide, peptidoglycan and polysaccharide. By virtue of their location on the cell surface, leptospiroa outer membrane proteins are likely to be relevant to host–pathogen interactions determining virulence and pathogenesis. Clinical and pathological observations suggest that outer membrane toxins may play a role in the pathogenesis of leptospirosis, result in immunity to leptospiroa and be responsible for renal dysfunction [20]. Therefore, identification of outer membrane proteins has become an important step in current leptospiroa research. By immuno-electron microscopy, gold-labelled leptospiroa antigens were found adjacent to cell membranes of hepatocytes, kidney tubular cells and endothelial cells of the interstitial capillary in animal studies [21]. Leptospiroa glycolipoprotein expression was also detected in renal tubules and vascular lumen of interstitium, and paralleled tubulo-interstitial nephritic changes [13]. Recently, several outer membrane proteins of pathogenic leptospiroa have been identified and localized to proximal tubules and the interstitium in infected animals [22]. Therefore, a disturbed tubular function may be elicited by leptospiroa outer membrane protein components.
The NF-κB associated pathway is involved in tubulo-interstitial injury

In order to elucidate the mechanism of tubulo-interstitial injury caused by leptospirosis infection, we have analysed the effect of the leptospiral outer membrane protein extract on cultured mouse renal tubular epithelial cells on the expression of a variety of genes related to tubular injury and inflammation [23].

Outer membrane protein extract of virulent leptospiira was extracted and administered to a model of cultured thick ascending limb cells derived from a normal mouse. Administration of outer membrane extract from pathogenic Leptospira shermanii to these cultured medullary thick ascending limb cells (mTAL) induced a significant nuclear DNA binding of the NF-κB transcription factor, a major transcriptional factor responsible for injury and inflammation. Forty-eight hours after adding the outer membrane protein (0.2 μg/ml) to cultured cells, the expression of inducible nitric oxide (iNOS) mRNA increased by 4.2-fold, monocyte chemotactant protein-1 (MCP-1) by 3-fold, and TNF-α by 2.4-fold as compared with untreated cells. Supernatant nitrite, MCP-1 and TNF-α protein levels similarly increased by 1.8-, 7.1- and 5.0-fold, respectively. The outer membrane protein is heat labile and digestible by proteinase K, and an antiserum raised against Leptospira shermanii prevented these effects. Outer membrane protein extracts from another pathogenic Leptospira bratislava caused similar but lesser effects than Leptospira shermanii in the mTAL cells.

The increase in nitric oxide by mTAL cells may suggest a haemodynamic adaptation for a protective vasodilatation mechanism was elicited; on the other hand, it may induce injurious conditions because of peroxy nitrate and free oxygen radical production. MCP-1 is a chemokine involved in interstitial nephritis, which is one of the most important initiating factors of infiltrating monocyctic cells in interstitial nephritis. The increase of MCP-1 may indicate a role of the outer membrane protein in tubulo-interstitial nephritis. TNF-α is an immune and inflammatory cytokine, involved as a mediator of endotoxemia, which may play a role in inflammation in leptospirosis renal disease (Figure 1).

Contrary to this, outer membrane protein extracts from the avirulent non-pathogenic serovar Leptospira patoc did not induce any changes in the gene expression. These findings are compatible with the notion that the saprophytic, avirulent Leptospira biflexa does not induce clinical manifestation, as demonstrated by its lack of characteristic leptospiral endotoxin, a different pattern of outer membrane antigens expression by immunoblotting assay [24], and the fact that it is readily phagocytosed by human monocyte and polymorphonuclear cells [25,26]. The fact that only pathogenic leptospirosis, not the avirulent non-pathogenic serovar Leptospira biflexa, adhere to renal epithelial cells in culture [27] indicates that the wide variety of gene expression may be induced by outer membrane proteins from virulent leptospirosis.

Conclusion and new perspectives

Leptospirosis is a re-emerging infectious disease worldwide. Increased alertness may help to identify affected patients from those with other causes of multiple organ involvement. Increased surveillance in the public health system may also help identify patients from areas where leptospirosis is not considered a

![Diagram](https://example.com/diagram.png)

**Fig. 1.** Schematic drawing of the induction and signalling through NF-κB occurring in leptospirosis tubulo-interstitial nephritis. The pathogenic leptospirosis outer membrane protein interacts with a still unknown (?) cellular receptor and activates translocation of the transcriptional factor NFκB, further inducing the downstream gene expression of iNOS, MCP-1 and TNF-α in the renal tubular cells. The consequence of the gene expression may further enhance cellular injury, cell recruitment and the inflammatory process, leading to tubulo-interstitial nephritis.
frequent cause of infectious disease. Early treatment with penicillin may rescue patients dramatically. Pathogenic leptospiral infection may induce renal tubular cell injury and inflammation through NF-κB-associated pathways by leptoaspilral outer membrane protein components. Findings of this study may be important in understanding the pathogenesis of tubulo-interstitial nephritis caused by these organisms.

References