Leptospirosis Kidney Disease

Subjects: Infectious Diseases

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Leptospirosis is a zoonotic and waterborne disease worldwide. Leptospirosis emerges as a leading cause of acute febrile illness along with hepatorenal injury in many countries, including Thailand. Although acute kidney injury in the spectrum of interstitial nephritis is a well-described characteristic in severe leptospirosis, chronic kidney disease from leptospirosis is widely discussed. Early recognition of severe leptospirosis leads to reduce morbidity and mortality.

acute kidney injury | immune response | interstitial nephritis | leptospirosis

1. Pathophysiology

1.1. Acute Kidney Injury in Leptospirosis

Acute kidney injury (AKI) is a common manifestation of leptospirosis. Renal involvement in leptospirosis varies from asymptomatic urinary abnormalities to severe AKI that requires supportive dialysis. The most common renal pathology in leptospirosis is acute tubulointerstitial nephritis (ATIN), whereas hypokalemia and sodium wasting are the common laboratory findings. Interestingly, AKI caused by leptospirosis is usually non-oliguric, and hypokalemia accounts for 45–50% of all AKI cases [1]. ATIN from leptospirosis is characterized by diffuse interstitial edema and mononuclear cell infiltration. Notably, glomerular involvement in leptospirosis is less common. Leptospirosis-induced vasculitis is rare, with unfavorable renal outcomes that can be attenuated by corticosteroid administration [2][3][4][5]. Different mechanisms have been proposed for non-oliguric, hypokalemic AKI in leptospirosis (discussed below).

Tubular Dysfunction and Related Electrolyte Disturbances

Several tubular defects have also been reported, such as bicarbonaturia, glucosuria, decreased proximal tubule sodium reabsorption, and high excretion of phosphate and uric acid, also known as Fanconi syndrome [6][7][8]. Reductions in sodium–hydrogen exchanger isoform 3 (NHE3), which is expressed along with aquaporin 1 (AQP1) in the apical membrane of the proximal tubule, and decrease in α-Na^+/K^+–ATPase [9] cause several complications. Hyponatremia in leptospirosis is attributed to several causes, including increased urinary sodium loss, cellular efflux of sodium from Na^+/K^+–ATPase defects, increased levels of antidiuretic hormone (ADH), and resetting of the osmoreceptors [10]. Accordingly, the combination of the clinical clues of hyponatremia, hypokalemia, and non-oliguric AKI (or polyuria) are unique characteristics of leptospirosis nephropathy.

In addition, downregulation of the sodium-potassium-2-chloride co-transporter (NKCC2) in the medullary thick ascending limb (mTAL) of the loop of Henle may also explain the loss of sodium and potassium in the urine [9][11][12]. Polyuria or non-oliguric AKI occurring during the first stage of leptospirosis might be another symptom related to the reduced expression of aquaporin 2 (AQP2) and a urinary concentration defect due to resistance of the inner medullary collecting duct to vasopressin. During the recovery phase of AKI, AQP2 expression increases as a compensatory mechanism [9].

Hypomagnesemia is also common in leptospirosis patients with AKI from magnesium wasting [13]. One experimental study also reported renal magnesium wasting secondary to decreased NKCC2 on the apical membrane of mTAL [12]. Accordingly, hypomagnesemia and hypophosphatemia caused by hypermagnesuria and hyperphosphaturia, respectively, should be closely monitored during the course of leptospirosis infection. Hypomagnesemia from tubular dysfunction in leptospirosis may cause dramatic changes in magnesium homeostasis, which needs substantial amounts of magnesium replacement, especially in patients with myalgia, lethargy, and arrhythmias. On the other hand, rapid correction of hypomagnesemia may cause unintentionally increase circulating magnesium levels because leptospirosis may contribute to AKI, which may decrease magnesium clearance. Likewise, severe hypophosphatemia (defined as serum phosphate <1.5 mg/dL) from proximal tubulopathy may contribute to metabolic encephalopathy and myopathy. Accordingly, as the authors' experience, serum magnesium and phosphate levels should be evaluated every 3–5 days and 1–2 days, respectively, particularly in severe leptospirosis.

Interestingly, leptospirosis-related AKI occasionally requires supportive renal replacement therapy in the acute phase of infection. The kidneys may fully recover after the complete course of early antimicrobial therapy. Effective treatment of leptospirosis reversed tubular dysfunction in an in vitro study [12]. Leptospirosis patients also have more favorable outcomes with non-oliguric than with oliguric AKI [1].

1.2. Leptospirosis with Systemic Inflammatory Response Syndrome (SIRS)

Severe leptospirosis is complicated by sepsis and septic shock [14][15]. In the early phase, leptospirosis is related to an overwhelming activation of inflammasomes and proinflammatory cytokines, causing kidney inflammation and subsequent damage. *Leptospira* can be found in the proximal tubular cells at day 10 of the infection, and it can subsequently be found in the tubular lumen at day 14 of the infection [16]. Its antigens are also found in the proximal tubular cells, macrophages, and the interstitium [17].

The outer membrane proteins (OMPs) of *Leptospira* contain antigenic and virulent compounds, including lipoproteins, lipopolysaccharides (LPS), and peptidoglycans, which determine the host responses. In animal models of sepsis, LPS or endotoxin cause detrimental effects to the host [18][19]. *Leptospira* LPS, located on the OMP, appear to be the major antigens that affect immunity to *Leptospira* and believe its functions relevant to host–pathogen interactions which determine virulence and pathogenesis. To elucidate the mechanisms of tubule-interstitial injury caused by *Leptospira*, the *Leptospira*l OMPs were extracted on cultured mouse renal epithelial cells, which showed the expression of a variety of genes related to tubular cell injury and inflammation [20]. The

*Leptospira*l OMPs activate nuclear transcription factor kappa B (NF-KB), activator protein-1, and several downstream genes expressed in the medullary thick ascending limb cells [20]. LipL32, a major pathogenic lipoprotein on the OMP, induces tubulointerstitial nephritis-mediated gene expression in mouse proximal tubular cells and is a prominent immunogen during leptospirosis infection in humans [21]. In addition, LipL32 is a hemolysin that causes hemolysis of erythrocytes during *Leptospira* infection [22], and it directly affects proximal tubular cells by substantially increasing the gene and protein expression of several pro-inflammatory cytokines, including inducible nitric oxide (iNOS), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α). Therefore, the identification of novel OMPs of the *Leptospira* should remain a primary focus for increasing knowledge of leptospirosis pathogenesis and treatment.

Toll-like receptors (TLRs) are proteins that recognize specific molecular patterns of pathogens and represent the first line of immune defense mechanisms in the innate immune response. The effects of TLRs were evaluated to determine whether TLRs could mediate the inflammatory response induced by *Leptospira*l OMP in renal proximal tubular cells. Interestingly, only TLR2 but not TLR4 increased the expression of iNOS and MCP-1. Accordingly, the findings indicate that the stimulation of iNOS and MCP-1 caused by pathogenic *Leptospira*l OMPs, in particular LipL32, in proximal tubular cells requires TLR2 (usually co-expressed with TLR1) for the early inflammatory response [23].

Then, a cascade of inflammation is activated in the renal tubular cells, as leptospirosis induces interleukin (IL)-1β and IL-18 secretion from human macrophage cells through reactive oxygen species and cathepsin B mediated-NLRP3 inflammasome activation [24]. Other circulatory cytokines and chemokines, including IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), and TNF-α [25], are also produced during leptospirosis infection. Acute cytokine and chemokine surges occurring in leptospirosis patients can cause a detrimental syndrome of sepsis and severe sepsis due to an imbalance between pro- and anti-inflammatory responses. Increased levels of MCP-1, IL-11, and small inducible cytokine A2 occur during leptospirosis with thrombocytopenia [26]. These findings infer a role of cytokine and chemokine production during the acute and subacute phases of leptospirosis infection. Conversely, chronic inflammasome activation may be the pathway that leads to renal parenchyma fibrosis or CKD [27].

AKI following leptospirosis may thus arise due to acute tubular necrosis (ATN) from ischemia and poor renal tissue perfusion as the result of sepsis and septic shock. In addition, sepsis-associated AKI (sepsis-AKI) in leptospirosis is also possible, especially in patients with a low blood pressure episode. Evidence from both experimental and clinical studies shows that septic shock develops into a sepsis-related immunosuppression state, leading to the death of the host because of innate and adaptive immunity disturbances [28]. Therefore, circulating cytokines and chemokines may interfere with the immune response in severe leptospirosis; for example, low circulating neutrophils levels may be associated with impaired antimicrobial activity [29][30][31].

1.3. Chronic Kidney Disease in Leptospirosis

The consequences of ATIN caused by leptospirosis infection include tubular atrophy and interstitial nephritis in the event of unsuccessful treatment or incomplete recovery. In an attempt to elucidate the causal association between

Leptospira and renal fibrosis, the effects of OMP from pathogenic *Leptospira* on the production and accumulation of extracellular matrix have been explored [32]. The binding of *Leptospira*l OMP to proximal tubular cells, HK-2 cells, led to an increase of type I and type IV collagens in a dose-dependent manner. Likewise, the active transforming growth factor (TGF)-β1 secretion was increased twice following the addition of *Leptospira* OMP, while anti-TGF-β1-neutralizing antibodies attenuated the increased production of type I and type IV collagen, indicating the participation of TGF-β1 in the cascade. This phenomenon was confirmed by the increased nuclear translocation of SMAD3 after the administration of *Leptospira*l OMP, and overexpression of the dominant-negative SMAD3 prevented the *Leptospira*l OMP-induced increase of type I and IV collagen production without any effects on metalloproteinase activity [32]. This clearly demonstrated the effects of *Leptospiral* OMP in terms of enhancing extracellular matrix synthesis mediated by the TGF-β1/SMAD pathway.

Although data from both in vitro and in vivo studies indicate the possibility of CKD and renal fibrosis due to leptospirosis infection [32][33][34], the results from both a meta-analysis [35] and a long-term, three-year follow up study are inconsistent, as no leptospirosis patients with dialysis dependence were reported [36]. Chronic tubulointerstitial nephritis (CTIN) is a common lesion associated with long-standing leptospirosis that may lead to CKD of unknown etiology and subsequent renal failure. Sustained tubulointerstitial lymphocyte infiltration might be a key factor resulting in the progression to CKD [36]. Perhaps, TLRs may be involved in the AKI–CKD continuum in leptospirosis because TLRs are known to be a key factor in the primary response to both innate and adaptive immunity [33] as well as ischemia-reperfusion injury, glomerulonephritis, and sepsis-AKI. Therefore, further studies should seek to identify the molecular factors that may act as a danger signal by triggering the inflammatory response to different exogenous and endogenous noxious stimuli.

2. Clinical Manifestations

The clinical manifestations of leptospirosis are diverse, ranging from mild, non-specific symptoms, such as flu-like symptoms (fever, myalgia, and headache), to severe symptoms along with end-stage organ injuries (e.g., AKI, acute hepatic failure, and bleeding diathesis, also known as Weil's syndrome), acute hemoptysis from pulmonary hemorrhage, acute confusion from aseptic meningoencephalitis, and acute heart failure from acute or subacute myocarditis [37].

Accordingly, the three indicators of suspected leptospirosis infection consist of (i) acute febrile illness, (ii) jaundice, and (iii) acute kidney injury [38]. The predictors of a severe form of leptospirosis are severe myalgia at onset, severe bleeding tendency, and marked jaundice. Interestingly, the presence of either pre-existing chronic kidney disease (small kidney size) or enlarged, congested kidneys with anuria is related to the worst outcome [38]. Thus, patients with leptospirosis-associated renal disease should be treated promptly.

3. Diagnosis

3.1. Clinical Diagnosis

A diagnosis of leptospirosis is based on the history of exposure, risk factors, and clinical manifestations. A high index of suspicion can circumvent later organ damage. However, symptoms of leptospirosis are often mistaken for other causes of acute febrile syndrome, such as dengue infection, malaria, hepatitis, and active autoimmune disease. The lack of pathognomonic signs of leptospirosis means that the diagnosis is tentatively based on the evaluation of fever and myalgia in patients from an endemic area. For this reason, laboratory diagnosis of leptospirosis is essential.

3.2. Laboratory Diagnosis

Leptospirosis is difficult to diagnose in the laboratory, especially during the acute phase. It can be performed by directly identifying spirochetes or their components in bodily fluids or tissues, isolating leptospires in culture, or detecting particular antibodies throughout different clinical phases. Leptospirosis is difficult to distinguish from illnesses, such as malaria, dengue fever, rickettsia, influenza, hepatitis, and yellow fever because of its vague clinical appearance. As a result, lab tests are needed to confirm the diagnosis. The detection of antibodies against leptospires, leptospires themselves, or their deoxyribonucleic acid is the basis for these tests (DNA). Current laboratory diagnosis includes both direct identification (the detection of *Leptospira* spirochetes or DNA in the samples or isolation of the organism from specimens) and indirect detection (serological diagnosis or serology for identifying *Leptospira*l infection, which is based on the detection of specific antibodies against various *Leptospiral* antigens).

3.2.1. Direct Microscopic Examination, Culture, and Antigen Detection

Leptospires are thin, bright, actively motile spirals with characteristically rapid spinning (twitching motility) under the conventional darkfield microscope (DFM) and approximately, 10 leptospires/ mL are necessary for the detection of one cell per field. With one week longer in the duration of the infection, there is approximately 10% deceased in yield of DFM (from 100 to 90%). To enhance the sensitivity, several special staining methods; Warthin–Starry silver staining [39] and immunostaining (immunohistochemistry, immunofluorescence, immunomagnetic antigen capture, and immunoperoxidase staining [40]) that requires the serovar specific primary antibodies (in isolation or in combination).

In parallel, *Leptospira* culture from blood, urine, cerebrospinal fluid, and biopsy tissue is possible during the first few days to 10 days post-onset of symptoms of illness (leptospiremic stage). Because they are slow-growing (6–8 h doubling time), fastidious and prone to contamination, the culture samples must be kept for at least 3–4 months before being discarded as negative) with only 23% sensitivity but it is necessary for the drug sensitivity test [41]. As such, EMJH (Ellinghausen–McCullough–Johnson–Harris) media (oleic albumin complex) consists of bovine serum albumin (fraction V), Tween 80, ammonium chloride thiamine, monopotassium phosphate, disodium phosphate, and various nutrients [42] is most commonly used, while the more specialized T80/40/LH media (polysorbate 40, lactalbumin hydrolysate, superoxide dismutase, and rabbit serum), is required for some serovars [43]. For the primary isolation of the large and diverse range of fastidious pathogenic leptospires, Hornsby–Alt–Nally (HAN), seems to be a good media [44].

Detection of *Leptospira*l antigens by immunoperoxidase, immunofluorescence, or an immunomagnetic antigen-capture system has been developed for specimens with low bacterial burdens or dark-field microscopy cannot be used but is not routinely performed due to the limitation on primary antibody [40]. In contrast, nucleic acid recognition with novel DNA amplification; polymerase chain reaction (PCR) (i.e., nested-PCR, quantitative PCR [qPCR]), loop-mediated isothermal amplification (LAMP), and next-generation sequencing (NGS) is valuable for an early and accurate laboratory diagnosis, especially with the *Leptospira* isolation using biological media inoculation and DNA hybridization (DNA probe) [45][46].

A variety of *Leptospira*l targets (such as 16S ribosomal RNA) or DNA can be amplified for diagnosis [47] and real-time PCR (RT-PCR) is more sensitive and specific than standard PCR [48]. Because some RT-PCR primers may bind to a non-specific site, leading to false positive results, most recent real-time multiplex PCR assays have been developed using two sets of primers [49]. Nested PCR also helps in detecting more specific and sensitive DNA sites with additional sets of primers [50][51]. However, current trends for leptospirosis diagnosis are the use of both serological and molecular techniques, such as PCR and ELISA (easier than the gold standard serological MAT), for resource-limited countries [52][53]. Additionally, the LAMP technique [54] to detect a 16S rRNA gene (rrs), is a cost-effective, rapid and high-yield for detecting the pathogenic leptospires in the urine. Currently, NGS is the most precisely-based culture-independent method on core genome analysis in body fluids (blood and urine) [55] and the future direction of leptospirosis tests would be to move towards the molecular classification of leptospires, which overcomes the limitation of culture isolation of leptospires from clinical samples. Hence, the value of PCR in the clinical diagnosis of leptospirosis is particularly good and several modern techniques are emerging.

3.2.2. Anti-Leptospira Antibody Detection

Several serological diagnoses are used; for example, genus-specific antibody tests (indirect hemagglutination [IHA], enzyme-linked immunosorbent assay [ELISA], Lepto Dri Dot [latex agglutination], microcapsule agglutination [MCAT]), and serovar-specific antibody tests (microscopic agglutination test [MAT]) [56][57]. As such, MAT criteria for diagnosis are (i) a fourfold titer rise in paired sera (seroconversion of the current infection) or (ii) a single high titer (≥1:400 or 1:800, higher in epidemic areas) (seropositivity) (antibodies may persist for some time after infection or the cross-react with other diseases). Additionally, MAT requires a live panel of all *Leptospira* serovars in the region, with a panel of locally standardized serovars [57][58] following the World Organization for Animal Health (19 antigens representative of 15 serogroups) [59]. Because antibodies are detectable at 5–7 days post-infection, the MAT test can be negative (titer < 1:50) during the first few days of infection. Diagnosis of leptospirosis can be performed by urine samples [60] using both serology and molecular detection (16S rRNA), particularly in patients with recent infection (MAT ≥1:800 or ELISA IgM-positive or both) [61].

With limited resources, a clinical prediction score [62] based on the relevant clinical history and related laboratory tests with scoring in seven aspects is as follows: hypotension (3), jaundice (2), muscle pain (2), AKI (1.5), low hemoglobin (3), hypokalemia with hyponatremia (3), and neutrophilia (1) is proposed (a cutoff summarized score of 4 has the area under the receiver operating characteristic curve 0.78 (95% CI 0.68–0.89) for leptospirosis

diagnosis [40]). This Thai-Lepto-on-admission probability score could be a diagnostic tool for early presumptive diagnosis of leptospirosis in patients presenting with severe clinical suspicion of the disease.

4. Treatments

4.1. Specific Treatments for Leptospirosis

The treatment of leptospirosis-related kidney disease usually depends on the clinical symptoms, particularly in the early phase of infection. Therefore, early recognition and diagnosis of leptospirosis kidney disease are the principal factors driving favorable outcomes. In severe leptospirosis cases, the recommended intravenous antibiotics must be promptly prescribed at the time of diagnosis; these include 0.5–1 g ampicillin every 6 h, 1 g ceftriaxone every 12 h, or 1 g cefotaxime every 6 h. Notably, a study from Thailand showed that administration of 1.5 million units of intravenous sodium penicillin G every 6 h is equally effective to ceftriaxone in patients with severe leptospirosis [63]. Once-daily dosing has the added benefit of intramuscular administration in an out-patient setting as an alternative to intravenous administration. However, adult outpatients with an early onset of infection should receive either 100 mg doxycycline twice a day or 500 mg azithromycin daily. Antibiotic treatment is effective within 7–10 days of injection, but the injection of 5 million units/day benzyl penicillin should be prescribed for only 5 days. Patients who are hypersensitive or allergic to the penicillin group may be given 250 mg erythromycin four times a day for 5 days or 100 mg doxycycline twice daily for 10 days. Tetracycline is contraindicated in children, pregnant women, and renal insufficiency patients [64].

4.2. Sepsis and Organ Failure in Leptospirosis

Sepsis in leptospirosis, with or without shock, can occur as an unusual presentation, primarily in urban areas [14]. Similar to general sepsis management, the treatment of sepsis in leptospirosis is based on rapid administration of the correct antibiotic and the best supportive care [65]. As such, fluid administration is the cornerstone of sepsis resuscitation. In patients with fluid responder (less than 40% of septic patients), the stroke volume increases by 10–15% after a fluid challenge (250–500 mL), following the Frank–Starling principle (as the preload increases, the stroke volume increases until the optimal preload is achieved) [66]. With the optimal preload, the further fluid administration does not increase the stroke volume but increases arterial pressure, venous pressure, pulmonary hydrostatic pressure, and natriuretic peptide (a fluid shifting inducer from the intravascular portion into the interstitial space). Increased venous pressure (and renal subcapsular pressure) decreases the glomerular filtration rate (GFR) of the kidney. According to the Acute Dialysis Quality Initiative (ADQI), fluid therapy in sepsis divides into rescue (high-volume resuscitation), optimization, stabilization, and de-escalation [67] depending on the individual patient. In the de-escalation phase, a reduction in total fluid administration, diuretics, and/or renal replacement therapy (RRT) might be necessary. For the fluid composition, normal saline (or 0.9% NaCl; a non-physiologic solution) might cause hyperchloremic metabolic acidosis that results in decreased renal blood flow [68]. Synthetic hydroxyethyl starch is potentially nephrotoxic [68]. Although normal saline is currently the main fluid replacement used in sepsis-AKI due to the availability with a reasonable price worldwide, a limited volume of normal saline with partial use of other fluid preparations might be beneficial.

Although acidosis is common in patients with sepsis, bicarbonate treatment is not recommended unless the blood pH is lower than 7.15 because sodium bicarbonate infusion leads to hypernatremia, hypervolemia, intracellular shifting of calcium-induced hypocalcemia, intracellular acidosis, and impaired oxygen delivery [69]. In contrast, the strategies for tissue perfusion improvement (proper respiratory support, and adjusted normal saline volume with other balance solutions) should be considered. Tris-hydroxy-methyl aminomethane (THAM), a weak base with intracellular diffusion that is excreted through the kidneys, is mentioned to reduce intracellular acidosis but causes hyperkalemia, hypoglycemia, pseudo hyponatremia, and an increased osmole gap, especially in patients with pre-existing renal dysfunction [70]. Because the reduced vascular tone is a major cause of hypotension and renal injury in sepsis, norepinephrine restores the normal capillary velocity, filtration pressure, mean arterial pressure, and increases renal medullary circulation without renal blood flow alteration, leading to improved renal function, using as the first-line drug for septic shock.

For the rapid reversal of AKI (due to direct toxins, hypotension and hypovolemia of leptospirosis), topics of renal replacement therapy (RRT); indications, timing, modality, and delivered dose should be applied. Accordingly, the common RRT indications, “A-E-I-O-U”; Acidosis, Electrolyte disturbance, Intoxication, fluid Overload, and Uremia should be used as severe metabolic acidosis, fluid overload, and uremia are the top three RRT indications in leptospirosis. For RRT modality, daily dialysis may provide superior outcomes to alternate-day dialysis in severe leptospirosis (Weil’s syndrome) [71] and extracorporeal blood purification (absorption therapy with polymyxin B or other cytokine absorbents) might be beneficial [72], especially for the hemodynamic improvement [73], but are still inconclusive. Therefore, the proper biomarkers for several aspects (i.e., stress, injury, functional loss, and recovery) for a proper selection of treatment methods are urgently needed. Among them, the base excess (BE) that is lower than −5 might be associated with the success of renal support discontinuation(unpublished data). On the other hand, in leptospirosis-related acute liver failure, extracorporeal support systems do not demonstrate any survival advantage in clinical studies and renal support is not recommended in AKI-superimposed chronic liver injury [74]. Nevertheless, renal support may be considered only in patients with reversible causes [75].

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