Introduction:

It is a zoonotic disease having worldwide distribution and caused by spirochetes of genus leptospira. It may be the most common zoonotic disease¹. The exact incidence of the disease is not known as it is greatly under-reported. It leads to chronic infection of the kidneys of various animals who excrete it in the urine and thereby contaminate the environment. It spreads to the human beings after they come in contact with the infected urine. The clinical spectrum may range from asymptomatic exposure to undifferentiated fever to multisystem disease with high mortality rate (5-40%). The disease was first described by Larrey in 1812 amongst Napoleon's troops at Cairo. The multisystem disease with jaundice and renal failure was first described by Adolf Weil in Heidelberg in 1886. The organism was first visualized from he autopsy specimens of a patient thought to have yellow fever². In 1918, Noguchi proposed the name leptospira (thin spirals) following microscopic observations³.

Microbiology:

Leptospira is derived from a Greek word leptos meaning thin and a Latin word spira meaning coiled. It is the most ancient spirochete that can live both in the animals and free in the environment. It is 0.1um in diameter and 6-20 um in length. Its both ends are bent into a hook and each end has a flagella. The flagellae provide motility to the organism. Leptospira are best visualized by dark field microscopy or phase contrast microscopy. They can be cultured in polysorbate-albumin medium. The cultures are maintained in the dark at 28-30°C for up to 3 months. In tissues organism can be visualized by silver impregnation (i.e. Warthin-Starry staining), immunohistochemistry or immunofluorescence microscopy.

Epidemiology:

It is endemic through out the world. It spreads during the rainy season in tropics and during late summers in temperate regions. The exact prevalence of the disease is not known due to the under reporting and under diagnosing of the disease. Men are affected more frequently than women. Its prevalence in rural and urban areas and amongst developing and developed countries is alike. However, the disease may be more commonly found in areas where people come in contact with water contaminated with urine of infected animals. Since 1980's the disease has been reported from various parts of India. It is endemic in Kerala, Tamil Nadu, Gujarat, Karnataka, Maharashtra and Andaman island⁴. However, in last decade there has been a rapid rise in incidence of leptospirosis in north India also⁵.

Transmission of the disease:

The reservoirs of infection include more than 100 mammals like rodents (most important), small mammals, dogs, livestock, pigs etc. they usually get infected during their infancy and

thereafter continue to excrete the organism in the urine lifelong. The leptospira traverse the interstitial spaces of the kidney, penetrate the basement membrane of the proximal renal tubules, cross through the proximal renal tubular epithelial cells and become adherent to the proximal tubular brush border and then are excreted in the urine. Human beings get infected by exposure to the infected urine (either by exposure to wet soil or water infected with urine). Human to human transmission does not occur.

Pathogenesis:

It enters the human body either through damaged skin, mucous membrane, conjunctiva or through inhalation of the droplets. Inside the body, they spread via bloodstream to various organs of the body including central nervous system and aqueous humor of the eye. Transendothelial migration is facilitated by systemic vasculitis leading to widespread clinical features. The vascular injury can lead to pulmonary hemorrhage, liver cell injury, renal cortical injury and tubular necrosis.

The mechanism of injury in leptospirosis is not clear. It may include immune mechanism, toxin production, adhesions or other surface proteins. Leptospiral polysaccharides (LPS) maybe poorly recognized by the innate immune system⁶. Even human toll like receptor (TLR) 4, which binds to very low concentration of gram negative LPS (endotoxin) is not able to bind to leptospiral LPS⁶. This is due to the presence of methylated phosphate residue of its lipid A. Furthermore, production of hemolytic toxins like sphingomyelinases, phospholipases or pore forming proteins lead to increased tissue damage. Human Leukocyte Antigen (HLA) DQ 6 polymorphism is thought to be an independent risk factor for this disease⁷. This polymorphism leads to production of superantigen by leptospira that leads to nonspecific T cell activation. Surface lipoproteins especially major surface lipoprotein LipL32 have been implicated in the pathogenesis of the disease (especially tubule-interstitial nephritis)⁸. Organism also resists the innate immune defenses like complement and proliferates in blood before spreading to other organs.

Clinical features:

The mean incubation period is 5-14 days (range 2-30 days). It can present clinically in one of these forms⁹:

- 1. Subclinical illness
- 2. Mild influenza like self limiting systemic illness- seen in 90% cases
- 3. Weil's syndrome: Severe, potentially fatal. Can lead to renal failure, liver failure or hemorrhagic diathesis.
- 4. Meningitis or meningo-encephalitis.
- 5. Pulmonary hemorrhage with respiratory failure.

The disease occurs in two phases:

- 1. Septicaemic phase : lasts 5-7 days
- 2. Immune phase : lasts 4-30 days

The *septicaemic phase* is characterized by high remittent fever with chills and rigors (100-104°F), headache, myalgias, conjunctival suffusion (dilated conjunctival blood vessels without any discharge), abdominal pain, anorexia, nausea, vomiting, diarrhea, cough and rarely pretibial macula-papular eruptions. Myalgias (involving the low back and calf regions) and conjunctival suffusion are the most characteristic findings. Examination findings are nonspecific and may reveal hepato-splenomegaly and lymphadenopathy. Other findings may include conjunctival suffusion, pharyngeal erythema without discharge, muscle tenderness, rales, rash (macular, maculo-papular, erythematous, ecchymotic or petechial), icterus, meningismus, hyporeflexia or areflexia. Laboratory investigations may be nonspecific. Leptospira maybe recovered from blood, urine or cerebrospinal fluid during this phase. There is spontaneous resolution in 7-10 days without any sequelae. Death is not common during this phase

Immune phase is characterized by disappearance of the leptospira from the blood and the cerebrospinal fluid and appearance of IgM antibodies in blood. However, the organism maybe found in brain, liver, lung, heart, kidney and urine. Apart from the above mentioned symptoms, this phase may have jaundice, renal failure, cardiac arrhythmias, respiratory symptoms, aseptic meningitis and photophobia (uveitis). Abdominal pain may indicate pancreatitis. Examination may show muscle tenderness, hepato-splenomegaly or lymphadenopathy. Aseptic meningitis may occur in up to 80% of the patients¹⁰. Some may have lymphocytic pleocytosis (up to 500 cells/ mm³)² in the CSF along with marginal rise in proteins (up to 100 mg/dl) and normal sugar level. Rarely, it maybe associated with meningo-encephalitis, coma, transverse myelitis, hemiplegia or Guillian Barre syndrome¹¹. Weil's disease is the most severe form of the illness characterized by hepatic and kidney failure, hemorrhagic pneumonitis, cardiac arrhythmias, circulatory collapse or hemorrhage in gastrointestinal tract, retroperitoneum, pericardium and brain. The mortality rate may range from 5-40%¹¹.

Poor prognostic markers include¹²:

- 1. Age > 40 years
- 2. Encephalopathy
- 3. Acute renal failure with creatinine > 3.0 mg/dl
- 4. Adult respiratory distress syndrome
- 5. Hypotension
- 6. Cardiac arrhythmias

In a jaundiced patient, serum bilirubin may rise upto 80 mg/dl with modest transaminitis (upto $200 \text{ IU/l})^{13}$. However, it never leads to increased mortality in absence of renal failure. Histologically, hepatocyte degeneration with kupffer cell hypertrophy, cholestasis,

erythrophagocytosis and mononuclear cell infiltration is seen. There is no evidence of hepatocellular necrosis¹⁴.

Renal failure is initially non-oliguric with associated impaired sodium reabsorption and increased potassium loss. Impaired sodium reabsorption results from loss of ENaC sodium channel in the epithelial cells of the proximal convoluted tubule. Blood urea nitrogen is below 100 mg/dl and serum creatinine between 2-8 mg/dl¹⁵. This maybe associated with thrombocytopenia without DIC. Histologically, kidney shows features of acute interstitial nephritis (at times immune complex glomerulo-nephritis). The non-oliguric renal failure may progress to oliguric renal failure if not managed appropriately with fluid and electrolyte replacement. In ARF, oliguria is a significant predictor of death. Postmortem analysis may show yellow swollen kidneys with prominent cortical blood vessels. Histologically, tubulo-interstitial infiltration by lymphocytes, neutrophils, plasma cells and macrophages is noted alongwith focal areas of tubular necrosis¹⁴.

Apart from the renal and hepatic involvement, it can involve the lungs and present with cough without purulent expectoration, hemoptysis and respiratory distress. Chest X-ray may show small nodular densities (snowflake like) or alveolar infiltrates in the lower lobes (confluent consolidation is uncommon). Patients subsequently develop acute respiratory distress syndrome and may develop shock. Radiologically, it is difficult to distinguish alveolar hemorrhage from ARDS. Autopsy shows congested lungs with focal areas of hemorrhage¹⁴. Histological findings include capillary endothelial damage leading to congestion and areas of interstitial and alveolar hemorrhage.al However inflammatory infiltrates are not present and there is paucity of organisms.

Rarely, leptospirosis may lead to congestive cardiac failure. Cardiac arrhythmias including atrial fibrillation, flutter or tachycardia, ventricular ectopics and ventricular tachycardia may occur¹⁶. Autopsy shows myocarditis with inflammatory involvement of the conduction system, coronary arteritis or aortitis, pericardial or endocardial hemorrhage alongwith dilatation of both the ventricles. Prothrombin time and activated partial thromboplastin time are usually normal and fibrinogen levels are elevated. Thrombocytopenia results from platelet consumption in the activated endothelial surface.

Investigations:

Biochemical, hematological and urinalysis findings are non-specific in acute leptospirosis. Weil's disease is characterized by elevated levels of blood urea, creatinine, hyperbilirubinemia and transaminitis (upto 5 times the normal). Urine may show leucocytes, erythrocytes and hyaline and granular casts. CBC shows mild to moderate anemia, leucocytosis or leucopenia and thrombocytopenia. CXR may show alveolar infiltrates, diffuse interstitial infiltrates (ARDS), small nodular infiltrates and pleural based densities representing hemorrhage. Definitive diagnosis rests on:

Direct Detection Methods: Direct visualization of the leptospira by darkfield microscopic examination in the blood or urine has low sensitivity (40.2%) and specificity (61.5%)¹⁷.

Polymerase chain reaction (PCR) assay may be helpful in confirming the diagnosis during acute phase (before appearance of IgM antibodies)¹⁸. PCR maybe done on serum, urine, aqueous humor or other body tissues. Leptospira can be cultured from blood, urine, CSF and peritoneal dialysate fluids during first ten days of the illness. Specimen should be collected before starting the antibiotic therapy. 1-2 drops of blood are inoculated directly into the culture bottle at the bedside. Urine is collected after 1st week of illness. It should be collected in a sterile container without any preservatives and processed within one hour. Cultures are put in albumin polysorbate media like Ellinghausen-McCullough-Johnson-Harris media (EMJH). They are incubated at 30°C for several weeks (upto 6 months) as initial growth may be very slow. Urine culture may stay positive for months or years despite treatment. Isolated leptospires are subjected to serologic or molecular methods to identify the serovar. These may include multilocus sequence typing (MLST) and multiple locus variable number tandem repeat analysis (MLVA). However, they have been used in epidemiologic analysis only and yet to be widely used.

Indirect Detection Methods: Serological methods are mostly used for diagnosis of leptospirosis. The reference standard assay is microscopic agglutination test (MAT). MAT is negative in first 7-10 days. In this live antigens of leptospires are reacted with serum samples and then examined for agglutination by darkfield microscopy. However, this is a complex test which is being used in few reference laboratories. Positive test is defined as four fold rise in MAT titre between acute and convalescent phase serum samples. Also a titre of 1:800 in presence of compatible symptoms indicates recent infection¹⁹. Titre of 1:200 in presence of symptoms is suggestive of recent infection. In 10% of the patients, sero-conversion may take more than a month. Cross reactive antibodies maybe associated with syphilis, relapsing fever, lyme disease, viral hepatitis, HIV, legionellosis and auto-immune disorders. Other serological tests include Enzyme linked Immuno Sorbent Assay, Indirect Haemagglutination, Dot Blot and Lateral Flow.

Differential diagnosis:

- 1. Influenza especially when fever and myalgias predominate
- 2. Malaria
- 3. Rickettsial diseases
- 4. Arbovirus infections like Dengue and Chikungunya
- 5. Enteric fever
- 6. Hantavirus infection
- 7. Viral hepatitis

Treatment:

Antibiotic therapy probably shortens the course and progression of mild disease. However, the role of antibiotics is controversial after fifth day of illness. For mild disease, the drugs which can be given are:

1. I	Doxycycline	100 mg BD
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- 2. Ampicillin 500 mg Q6H
- 3. Amoxycillin 500 mg Q6H

For moderate to severe disease, drugs are:

- 2. Ceftriaxone 1 gm Q12 H
- 3. Cefotaxime 1 gm Q6H
- 4. Ampicillin 0.5 1 gm Q6H

All drugs are given for one week.

For chemoprophylaxis, Doxycycline 200 mg once a week is given

Role of steroids in ARDS due to leptospirosis is controversial but pulse therapy with high dose methyl prednisolone is useful in early ARDS²⁰. Even Cyclophosphamide has been used successfully in severe pulmonary haemorrhage²¹.

Penicillin maybe associated with Jarisch Herxheimer reaction which can be associated with increased morbidity and mortality. Patients with early renal failure should receive aggressive volume repletion to avoid severe dehydration and acute tubular necrosis. With the onset of oliguric renal failure, hemodialysis is the procedure of choice. Renal failure is completely reversible.

Patients having pulmonary hemorrhage or ARDS may need intubation and ventilation. These patients benefit most from low tidal volume ventilation using less than 6 ml/Kg.

Prognosis: Chronic alcoholism is associated with severe disease. Amongst hospitalized patients the mortality rate may vary from 5=20%.

Prevention: This can be achieved by:

- 1. Avoiding high risk exposures like swimming pools or direct contact with infected animals
- 2. Immunization of the animals with killed vaccines (limited utility)
- 3. Human vaccination is not widely practiced. Vaccine containing serovar icterohaemorrhagiae is available for workers in high risk occupations
- 4. Chemoprophylaxis with Doxycycline 200 mg once a week is recommended for those who have had exposure to leptospira. Azithromycin 250 mg once or twice a week is being investigated as an alternative to doxycycline (esp. in pregnancy)²²

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