

Anicteric Leptospirosis: An Unusual Cause of Acute Pancreatitis

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Abstract

Leptospirosis is one of the most common zoonotic diseases, that is potentially fatal but it is quite underdiagnosed and under reported. However, acute necrotizing pancreatitis, which is usually associated with high mortality rate, is a rare complication of leptospirosis. This is a report of leptospirosis case presenting with acute pancreatitis. A previously healthy 35 year old male Indian farmer presented to the emergency department with chief complaints of high grade fever, chills and rigors but no rash for the last 3 days. There was no history of cough, breathlessness, pain abdomen, vomiting, hemoptysis or haematemesis, altered sensorium or burning micturition. There was no history of any addiction or previous hospitalization. Respiratory, cardiovascular and neurological systems examination was normal. Leptospira IgM Elisa was undertaken on the 7th day of admission, which was found to be positive. IgG negative and a Leptospira microagglutination test was also positive (at 1/200, *L. icterohemorrhagica*). Severe leptospirosis may be fatal before IgM antibody is reliably produced; furthermore, leptospiremia may be difficult to detect due to negative serologic results and blood cultures. Therefore, repeat serology after the first week of illness and empirical treatment prior to serologic results may be essential for improving outcome in patients with severe leptospirosis.

Keywords: Leptospirosis; Anicteric; Thrombocytopenia; Pancreatitis; Necrosis; Renal failure

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Introduction

Leptospirosis was first mentioned in 1812 by Larrey and is commonly known as the "yellow fever". It is the most frequent zoonosis in the world caused by the pathogenic spirochetes from leptospira family and is characterized by a broad-spectrum of clinical manifestation varying from inapparent infection to fulminant fatal disease [1,2].

It affects both humans and animals especially, rodents. This disease is seen frequently in certain occupational groups like veterinarians, agriculture workers, slaughterhouse employees and sewage workers. Leptospirosis mainly affects liver and kidney. Rarely, other organs such as lung, heart, gallbladder, brain, and ophthalmic tissues are involved, mainly due to vasculitis [3,4].

Association of necrotizing pancreatitis with leptospirosis has been rarely reported [5-7].

Case Report

A previously healthy 35 year old male, farmer by occupation, presented to the emergency department with chief complaints of high grade fever with chills and rigors but no rash for the last 3 days. There was no history of cough, breathlessness, pain abdomen, vomiting, hemoptysis or haematemesis, altered sensorium or burning micturition. There was no history of any addiction or previous hospitalization. On examination, he was conscious, oriented, febrile with temperature of 101.6 Vol. 6 No. 3:4, blood pressure of 124/76 mm Hg, pulse rate of 100/min low in volume, regular, respiratory rate 18/min with no pallor, icterus, cyanosis or clubbing. Respiratory, cardiovascular and neurological systems examination was normal. Arterial blood gas analysis was also normal. The investigations revealed hemoglobin 10.8 g/dL (reference range: 12-16 g/dL), total leukocyte count 14,600/mm³(reference range: 4,000-11,000/mm³), detailed leukocyte count: neutrophils 74% (reference range: 40-56%), lymphocytes

20% (reference range: 20-40%) and eosinophils 1% (reference range: 0-5%), and platelet count 78,000/mm³ (reference range: 150,000-450,000/mm³). Blood urea was 48mg/dL (reference range: 14-50 mg/dL), serum creatinine 1.4 mg/dL (reference range: 0.5-1.4 mg/dL), serum Na⁺ 140 mEq/L (reference range: 135-145 mEq/L), K⁺ 4.6 mEq/L (reference range: 3.5-5.0 mEq/L), serum Ca²⁺ 8.2 mg/dL (reference range: 8.5-10.2 mg/dL) and blood glucose was 83 mg/dL (reference range: 70-110 mg/dL). Serum bilirubin was 1.9 mg/dL (reference range: 0.3-1.3 mg/dL) with direct 1.2 mg/dL (reference range: 0.1-0.4 mg/dL), transaminases were SGPT 46 U/L and SGOT 50 U/L (reference range: 8-40 U/L and 10-38 U/L, respectively), alkaline phosphatase was 133U/L (reference range: 13-100 U/L), serum triglyceride 124 mg/dL (reference range: 70-140 mg/dL) and serum albumin was 2.8 g/dL (reference range: 3.5-5.5 g/dL). Malarial parasite quantitative buffy coat (MPQBC) test for *Plasmodium vivax* malaria, histidine-rich, protein based immunochromatographic card test for *Plasmodium falciparum* malaria as well as peripheral blood smear examination were negative. Typhidot was negative for *Salmonella typhi*. Serological tests for the herpes simplex virus (HSV), dengue and NS1 antigen assay, hepatitis A, B, E, and the human immunodeficiency virus (HIV) were also negative. Chest X-ray and electrocardiography were normal. In view of the persistently thrombocytopenia, *Leptospira* IgM Elisa was undertaken on the second day of admission which was found to be negative (5 Panbio units). IgG negative and a *Leptospira* microagglutination test (MAT) was negative (at 1/20, *L. Icterohemorrhagiae*).

Microscopic Agglutination Test

Microscopic agglutination test (MAT) was performed with a panel of 20 serovars as shown in the **Table 1**. Two fold serial dilutions of serum sample were made with 0.01M phosphate buffered saline (pH 7.2) starting from 1:25. The diluted serum samples were incubated with equal volume of live cultures for 2 h at room temperature with suspensions of live leptospires. As per standard protocol, the end point was determined as highest dilution of serum showing 50% reduction in the number of free moving leptospires [8].

The cut off titre was considered as 1:100, with 50% reduction in free moving leptospires as established earlier improving outcome in patients with severe leptospirosis [9].

Leptospira IgM ELISA

Detection of IgM antibodies to *Leptospira* species was determined using a commercially available *Leptospira* IgM ELISA (Panbio Pty., Ltd., Queensland, Australia). The assay was performed according to the manufacturer's instructions. Briefly, test sera, cutoff calibrator, and positive and negative control sera were diluted 1:100 in serum diluent, and 100 µL added to *Leptospira* antigen-coated microwells and incubated for 30 minutes at 37°C. After washing with phosphate-buffered saline containing 0.05% Tween 20, 100 µL of HRP conjugated anti-human IgM was added and incubated for another 30 minutes at 37°C. After further washing, 100 µL of tetramethylbenzidine substrate was added and incubated at room temperature for 10 minutes, after which the reaction was stopped with 100 µL of 1 M phosphoric acid. The absorbance of each well was read at a wavelength of 450

nm with a Bio-Tek ELX 808 plate reader (Bio-Tek Instruments, Winooski, VT). The results were expressed as Panbio units calculated by the ratio of sample absorbance to the mean cut off absorbance multiplied by 10. The recommended cut-off for a positive result is a value of ≥ 11 Panbio units, and is interpreted by the manufacturer to indicate recent infection of leptospirosis. The sensitivity and specificity of IgM ELISA on paired sera was reported to be ranging from 90.8- 100% and 55.1- 98% [10,11].

Patient was started on injection ceftriaxone 1 g 12 hourly, antipyretics and intravenous fluids. On 3rd day of hospital stay, he complained acute onset pain in abdomen localized to epigastrium and umbilical region and decrease in urine output was noticed (700 ml/24 hours) with a systolic blood pressure of 82 mmHg. On examination, abdomen was soft, but tenderness was present in the epigastrium. Bowels sounds were 4-5/min, no free fluid or organomegaly was noted. Ionotropic support was started and an erect x-ray abdomen was done which was normal. The serum amylase was 1040 U/L (reference range: 10-200 U/L) and serum lipase was 302 U/L (reference range: 10-80 U/L), platelet count 74,000/mm³ (reference range: 150,000-450,000/mm³), blood urea was 54 mg/dL (reference range: 14-50 mg/dL), serum creatinine 1.5 mg/dL which rose to 5.6 mg/dl on day 4 of admission (reference range: 0.5-1.4 mg/dL) alongwith with persistent oliguria. Ultrasonography of abdomen revealed a bulky pancreas, no gallstones, ascites or splenomegaly was seen. A contrast-enhanced computed tomography (CT) scan of the abdomen was done which was suggestive of diffuse pancreatitis with modified CT severity index of 6 as shown in **Figure 1**.

In view of the persistently thrombocytopenia, *Leptospira* IgM Elisa was undertaken on the 7th day of admission which was found to be positive. IgG negative and a *Leptospira* MAT was positive (at 1/200, *L. icterohemorrhagiae*).

A diagnosis of *Leptospira* associated acute pancreatitis was made.

Table 1: List of Serovars used for microscopic agglutination test.

Serogroup	Serovar	Strain
Australis	lora	Lora
Autumnalis	autumnalis	Akiyami
Icterohaemorrhagiae	copenhageni	Wijnberg
Louisiana	lanka	Le740
Semarang	patoc	Patoc 1
Icterohaemorrhagiae	lai	Lai
Djasiman	djasiman	Djasiman
Australis	bratislava	Jez Bratislava
Sejroe	wolfii	3705
Sejroe	hardjo	Hardjoprajitno
Pomona	pomona	Pomona
Javanica	menoni	Kerala
Celledoni	celledoni	Celledoni
Louisiana	louisiana	LSU1945
Pyrogenes	alexis	HS616
Autumnalis	bulgarica	Nikolaevo
Cynopteri	cynopteri	3522C
Grippotyphosa	ratnapura	Wumalaseena
Andamana	Andaman	Ch11
Australis	australis	Ballico

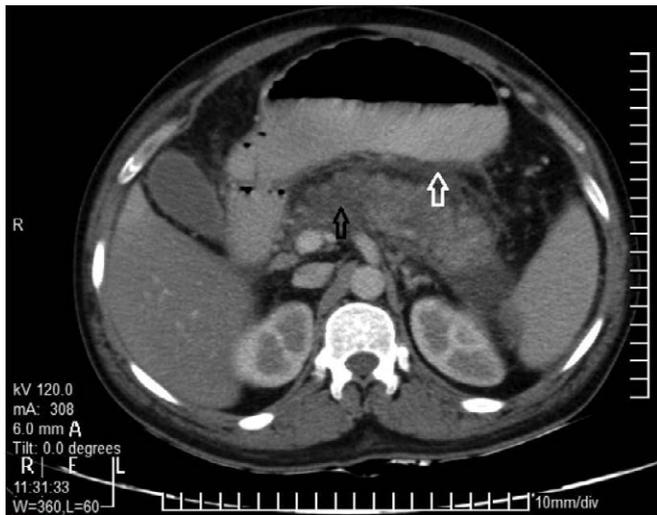


Figure 1 CECT axial section reveals nonenhancing pancreatic parenchyma (black arrow) in the region of head as well as tail of pancreas suggestive of necrosis. Concomitant peripancreatic inflammatory changes co-exist (white arrow).

Patient was managed conservatively with intravenous fluids, analgesics and imipenem-cilastin 500mg thrice daily which was continued for 10 days along with supportive therapy. Inotropic support was gradually tapered off after 36 hours. Sustained low-efficiency dialysis (SLED) was initiated on day 4 of admission with a blood flow rate of 100 ml/min and 5 sessions of SLED were given subsequently. Nasojejunal feeding tube was inserted under endoscopic guidance for adequate caloric intake. Five days after the treatment, body temperature decreased to 99.6°C, amylase levels decreased to normal value 142 U/L and platelet count increased to 160,000/mm³. Bilirubin levels, liver function tests, and creatinine level slowly returned to normal within 11 days of hospital stay. Patient recovered completely and was discharged on 15th day of admission in a stable condition (laboratory tests at the discharge time revealed WBC: 7 600/mm³, Hb: 11 g/L, total bilirubin: 0.9mg/dL, Blood urea: 38 mg/dL, Cr: 1.2 mg/dL, AST: 25 U/L, ALT: 46 U/L, LDH: 366 U/L).

Discussion

This disease occurs as two clinically recognizable syndromes: the anicteric leptospirosis (80-90% of all cases) and the remainder icteric leptospirosis [3,4].

Icteric leptospirosis is known as Weil's disease, which is characterized by hemorrhage, renal failure, and jaundice. Icteric leptospirosis is a much more severe disease than anicteric form. The clinical course is often rapidly progressing. The source of infection in humans is usually either direct or indirect contact with the urine of infected animals. These bacteria infect humans by entering through abraded skin, mucous membrane, conjunctivae. Direct transmission between humans is rare [3,4]. Leptospirosis is a common disease in rice-field workers due to prevalence of wild rats [4]. Rice field with stagnant water and humid condition is an ideal environment for *leptospira*. Our patient was also a farmer by occupation.

The incubation period is usually 1 to 2 weeks. Laboratory findings reveal thrombocytopenia as a common finding occurring in 40–85% of cases. ESR and leucocyte are also elevated.

Pathogenesis of organ dysfunction is yet to be fully understood. It is thought to be related to leptospira burden, associated cytotoxic factors in the tissue especially in liver and kidney and host immune mechanism especially in lungs [12-14]. An immunological basis for pathogenesis of leptospirosis including Toll Like Receptor (TLR) 2 activation is described recently [13]. TLR 2 plays a major role in the development of pulmonary and renal manifestations of leptospirosis [15]. *Leptospira* lipoprotein LipL32 triggers an inflammatory response in renal proximal tubule cells by activation of TLR 2 and hence nuclear factor-kappa B and mitogen-activated protein kinases [13,15]. Though it is not yet described in relation to leptospirosis, TLR may contribute to myocarditis in sepsis and may involve in the pathogenesis of acute pancreatitis [16,17]. Bacterial peptidoglycan associated lipoprotein uses the TLR2 signaling pathway to induce cardiomyocyte dysfunction and inflammatory response in mice [16]. In acute pancreatitis, increased expression and activation of TLR 2/4 has been recognized and their role in multi-organ involvement was identified [17]. Thus a similar mechanism involving TLR may explain the presentation of our patient.

A rapid, accurate method for the diagnosis of leptospirosis is important in order to start appropriate treatment. Although leptospirosis is one of the common causes of acute febrile illness with multiorgan failure in developing countries, it remains under diagnosed mainly because of protean manifestations and lack of proper diagnostic technique [18].

MAT is considered as the gold standard for serodiagnosis of leptospirosis which was also positive in our patient, but in previous studies its sensitivity was found to vary from 30% to 76%, with a specificity of 97%. [12,19]. But in combination, the sensitivity of IgM ELISA and MAT was found to increase to 70% as reported earlier by Shekathkar et al. [20]. Several recombinant proteins (rLipL32, rLipL41) have been identified to be specific to pathogenic leptospires, but with varied sensitivity and specificity in serodiagnosis. One such recombinant protein LipL32 IgG ELISA, the sensitivity and specificity was 96.2% and 90% respectively, which was comparable with Pan Bio IgM ELISA [21]. Senthilkumar et al. have used rLipL 41, the sensitivity and specificity was found to be 89.7% and 90.45% with reference to MAT [22].

Several IgM based, commercial kits are available for the diagnosis of systemic leptospirosis using broadly reactive leptospiral antigen [18]. Winslow et al., have reported Panbio kit to be highly sensitive for diagnosis of systemic leptospirosis, the sensitivity and specificity was 100% and 98% [11]. But recently Desakorn et al. [10] using the cutoff value recommended by the manufacturer (11 Panbio units), sensitivity and specificity of IgM ELISA on paired sera was reported to be 90.8% and 55.1%. A receiver operating characteristic curve was used to determine the optimal cut-off value. This was 20 Panbio units, which gave a sensitivity and specificity of 76.1% and 82.6%, respectively, on paired sera.

The diagnosis of pancreatitis was based on biochemical and radiological evidence (Figure 1). Increased serum lipase more

than 3 times the upper normal value is highly specific for pancreatitis in this patient especially at a time of normal renal functions [23,24]. The other endemic pathogens which lead to multisystem involvement such as dengue hemorrhagic fever, hepatitis virus, malaria were excluded.

Though the reported incidence of pancreatitis in leptospirosis is infrequent, in reality pancreatic involvement may be more common. Under-recognition could be due to several reasons. Pancreatic involvement could be subclinical or clinically unrecognized when dramatic and rapidly dynamic alterations of clinical and biochemical parameters take place in multi-organ dysfunction in leptospirosis. Thus a clinician may find it difficult to identify each and every complication such as pancreatitis, acalculous cholecystitis, cerebral venous thrombosis and myositis [25,26].

Additionally in a clinical setup, when a patient presents with acute pancreatitis alone as in this case, leptospirosis might not be considered as an aetiology in the initial work-up because of the rarity. Later the patient may develop multi-organ failure due to leptospirosis, yet that might be attributed to the multi-organ involvement of acute pancreatitis. Ultimately, recognition of leptospirosis might get delayed compromising the optimum management.

In disease-endemic areas, acute pancreatitis should be suspected even in anicteric leptospirosis patients with appropriate epidemiologic and clinical findings and abdominal pain; conversely, leptospirosis should be considered as a possible cause of pancreatitis. It is important to note that in our patient, acute serologic results obtained on day 5 of symptoms (hospital day 2) were negative, and the diagnosis was only obtained upon repeat testing 5 days later.

Severe leptospirosis may be fatal before IgM antibody is reliably

produced, and leptospiremia may be difficult to detect; negative serologic results and blood cultures (even placed into specific growth media) do not exclude the diagnosis [4,12]. Repeat serology after the first week of illness and empirical treatment prior to serologic results may be essential for improving outcome in patients with severe leptospirosis.

Hyperamylasemia can be present in leptospirosis infection due to renal impairment, so serum lipase should be preferred [27,28]. So the diagnosis of leptospira associated pancreatitis should be made on proper clinical, biochemical and radiological findings.

In management, most of the patients of leptospirosis show spontaneous recovery and do not require any specific therapy. Although, the use of antibiotics is not well proven in leptospirosis, its early initiation can shorten the course of severity and prevent the progression of mild disease [29]. Penicillin, tetracycline, ceftriaxone and doxycycline are the preferred antibiotics. A Cochrane systematic review failed to find sufficient evidence to provide clear guidelines for use of antibiotics [30]. Patients with severe leptospirosis require correction of hypovolemia, hypotension and electrolyte abnormalities.

Conclusion

This case illustrates diagnostic difficulties especially in low resource settings with farming being one of the principle occupations in our country, where leptospirosis is more common. Although Weil's syndrome has been seen associated with majority of the complications, our case highlights the fact that anicteric leptospirosis can be delirious too. High index of suspicion can be lifesaving in such cases which are often labelled as idiopathic pancreatitis or even antibiotic induced gastritis, especially in low resource settings.

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