Determination of a liver biochemical profile and liver ultrasonographic findings in patients with Leptospirosis in a tertiary hospital

Carlos Rolando G. Cuaño, MD[1,a], Patricia Maria Gregoria M. Cuaño MD[1], John Mark K. Torres MD[1], Janus P. Ong, MD, MPH[1,a]

[1] University of the Philippines Manila, Department of Medicine

[a] Division of Gastroenterology

ABSTRACT

Rationale: Leptospirosis is an important zoonotic disease commonly found in tropical or sub-tropical countries. The most severe form is Weil Syndrome which presents with jaundice and renal failure. Although jaundice is associated with severe leptospirosis, no studies on the liver profile of these patients have been done before.

Objective: To describe the liver biochemical profile and liver ultrasonographic findings in adult patients with leptospirosis.

Methods: This retrospective correlational study reviewed all available cases of adult patients with leptospirosis admitted in the Philippine General Hospital from January 2008 to December 2019. The clinical feature, liver biochemical profiles, and ultrasound findings were recorded and analyzed.

Results: Serum aminotransferases were 2 to 3x elevated. Direct hyperbilirubinemia were at least 9x elevated. Alkaline phosphatase and coagulation parameters are mild elevated. Leptospirosis ultrasounds were typically unenlarged livers with normal parenchymal echogenicity and non dilated biliary trees.

Conclusion: To date, no local studies yet have fully explored the liver profile of patients with leptospirosis. Our study is compatible with previous ones showing that leptospirosis presents with predominantly elevated direct bilirubin from cholestasis and systemic infection. The AST is elevated to a lesser degree with an AST:ALT ratio greater than 1 and mildly elevated alkaline phosphatase and coagulation parameter. Results also showed that an elevated ALT is associated with mortality suggestive of hepatocyte injury and apoptosis. Liver ultrasonography showed normal echogenicity. Contrary to literature, our study found no association between jaundice and mortality.

Keywords: Leptospirosis, Liver profile, Liver ultrasound

INTRODUCTION

Leptospirosis is an important zoonotic disease worldwide. It was first described in 1886 by Adolf Weil with a presentation consisting of splenomegaly, jaundice and nephritis[1]. Leptospirosis can present with great clinical variability, from a mild flu-like illness to life threatening shock and hemorrhage[1].

Epidemiology: In developed countires, infection is through exposure to contaminated water (via agriculture, outdoor recreational activites, and travel to endemic areas)[2]. Whereas, in developing countries, outbreaks are related to poor sanitation, overcrowding, climatic conditions and normal daily activities[3].

This disease is endemic to many countries in the Asia Pacific region such as Bangladesh, Cambodia, India, Indonesia, Laos, Nepal, Sri Lanka, Thailand, Vietnam, and the Philippines[3]. Acute epidemics have been documented after flooding in Orrisa, Jakarta, Mumbai, and the Philippines[3, 4].

In the Philippines, there were a total of 5,232 cases reported in 2018, showing an increase of 70.59% compared to 2017. Most cases were from the National Capital Region (37.98%), Region VI (12.63%), and Region I (11.16%). Males comprised the majority of cases (84%), with most (14%) belonging to the 20-24 years old age group. There were a total of 505 deaths from Leptospirosis—a steep increase from the 317 deaths documented in 2017. Though there was an increase in sheer numbers, the case fatality rate decreased to 9.65% from 10.33%[5].

Review of Related Literature:

Pathogenesis: The genus *Leptospira* consists of 22 species; 10 of these are pathogenic, while five are intermediately pathogenic[6]. Humans are infected by direct or indirect exposure to the urine of animals carrying spirochetes of *Leptospira.* Leptospires

enter the blood stream via cuts, abrasions, or through mucous membranes. Rodents, cattle, pigs, and dogs are the major reservoir hosts, but all mammalian species can serve as carriers[2].

Clinical Manifestations: The clinical presentation of leptospirosis ranges from a subclinical infection to fulminant fatal disease. The incubation period ranges from one to 30 days but is commonly one to two weeks. Mild leptospirosis presents with nonspecific symptoms such as fever, myalgia, headache, conjunctival suffusion, non-purulent pharyngeal congestion, crackles, rash, and meningismus. Severe leptospirosis, also called Weil Syndrome, presents with jaundice, renal dysfunction, and hemorrhagic diathesis.

The most common causes of death from leptospirosis is septic shock with multiorgan failure, and severe bleeding complications commonly involving the lungs, gastrointestinal tract, urogenital tract, skin, and bleeding from venipuncture sites[1]. In a study conducted in nine tertiary hospitals in the Philippines on the clinical profile of leptospirosis after a heavy rainfall typhoon, jaundice occurred in 38.0%, and elevated AST (>37 SI units) occurred in 33.3%[4].

Locally, a retrospective study conducted among presumptive cases of leptospirosis admitted at the Jose Reyes Memorial Hospital found the folllowing patient characteristics to be associated with poorer outcomes: leukocytosis (WBC >10,000), thrombocytopenia (platelet count <100,000/ul), evidence of bleeding, oliguria, and more than 6 days from time of disease onset to time of consult. [9]

Definitions

Suspected Leptospirosis is defined as a history of exposure to floodwaters plus any of the following signs and symptoms: fever, headache, myalgia, conjunctival

suffusion, diarrhea and abdominal pain, jaundice, decreased urine output and changes in sensorium or meningismus.

Confirmed Leptospirosis is defined as laboratory confirmation of the diagnosis via any of the following tests (WHO, 2003): a) positive leptospiral culture of the blood and/or urine; b) high positive single micro-agglutination test (MAT) titer of greater than or equal to 1:1,600; c) seroconversion from an initial negative to a positive antibody titer by MAT; d) fourfold rise in antibody titers by MAT from the acute to convalescent phase.

Severe Leptosirosis, according to the 2010 Philippine College of Physicians Clinical Practice Guidelines, is indicated by any of the following laboratory tests: leukocytosis (WBC >12,000 cells/mm3) with neutrophilia, thrombocytopenia (platelets <100,000 cells/mm3), elevated serum creatinine (>3 mg/dl), elevated liver function tests (AST/ALT ratio >4, bilirubin >190 umol/L), prolonged prothrombin time (PT %<85%), serum potassium >4 mmol/L, severe metabolic acidosis (ph <7.2, serum bicarbonate <10), hypoxemia (oai2 <60 mmhg), chest radiograph with extensive alveolar infiltrates, and ECG findings of myocarditis, repolarization abnormalities, and heart block.[10] Aside from this, patients presenting with hemodynamic instability, an altered mental status, renal failure, oliguria, abdominal pain, frank jaundice, meningismus, dyspnea, and pulmonary hemorrhage are classified as having severe leptospirosis.[11]

Leptospirosis and the Liver

Jaundice occurs in 5-10% of patients with leptospirosis. In a restrospective study on the prognostic factors of leptospirosis conducted in a tertiary hospital in France, jaundice was one of the criteria that predicted development of severe leptospirosis[6]. The

physical examination may show a tender liver which is enlarged. Serum bilirubins may be high while aminotransferases and alkaline phosphatase are only mildly elevated. On liver histopathology, focal necrosis, foci of inflammation, plugging of bile canliculi, and hepatocytes apoptosis have been found. Leptospires have been shown to infiltrate Disse's space, and migrate between hepatocytes with detachment of the intercellular junctions and disruption of bile canaliculi, leading to leakage of bile[1].

Leptospirosis has a biphasic disease course. Presenting with nonspecific symptoms such as fever, headache, and myalgia, the first phase typically lasts up to seven days. The second phase can be classified into anicteric and icteric forms. While majority of the patients undergo the milder anicteric form, some may present with a severe icteric phase called Weil disease. Weil disease can lead to multiple organ damage including acute liver failure, acute kidney injury, rhabdomyolysis, and thrombocytopenia with possible hemorrhagic diathesis. Without treatment, the associated mortality rate ranges from 5% to 15%. Transaminase levels are moderately elevated in the 100s IU/L, with a mild increase of alkaline phosphatase.[7] In a study done by Chang in 2005, an aspartate aminotransferase–alanine aminotransferase ratio of >3 revealed a poorer prognosis.[8] Secondary to septic cholestasis, jaundice typically appears during day five to nine of the disease course. Serum bilirubin was shown to rise to as high as 30 to 40 mg/dl during the icteric phase.[7]

Implication and Importance: There have been no previous local studies describing at length the liver profile and ultrasonographic picture of patients with leptospirosis. Characterization of liver function tests and ultrasonographic findings that portend a poorer prognosis, may allow for earlier recognition, and may identify those

patients who will benefit from ICU admission. To disseminate this information, the authors plan to submit this study for publication in local and international journals.

RESEARCH QUESTION

Among Filipino adults diagnosed with leptospirosis, what are the liver biochemical profile, liver ultrasonographic findings, and clinical outcomes?

OBJECTIVES

General Objective:

To describe the liver profile and liver ultrasound findings in adult patients with suspected and confirmed leptospirosis.

Specific Objectives:

1. To describe the AST, ALT, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, prothrombin time, and international normalized ratio of Filipino adults with suspected and confirmed leptospirosis.
2. To describe the liver ultrasound findings of Filipino adults with suspected and confirmed leptospirosis.
3. To determine the AST, ALT, total bilirubin, direct bilirubin, and indirect bilirubin level at which jaundice occurs.
4. To determine if AST, ALT, total bilirubin, direct bilirubin, and indirect bilirubin levels in Filipino adults with leptospirosis are related to mortality.

METHODOLOGY

Study Design:

This was a retrospective correlational study. We collected all available data of all adult patients with leptospirosis from January 2008 to December 2019. Complete enumeration sampling was used and only available records of known patients with suspected or confirmed leptospirosis was analyzed. We chose a retrospective design because we wanted to obtain preliminary measures of association between patients with leptospirosis and their liver profiles and ultrasound findings.

Setting:

This study was conducted in the Philippine General Hospital (PGH), a tertiary, 1,500 bed capacity hospital in Manila, Philippines. As a major referral center, it caters to patients from all over the Philippines.

Study Population:

Patients with suspected and confirmed leptospirosis admitted at the Department of Medicine of the Philippine General Hospital from January 2008 to December 2019 were included in this study.

Inclusion criteria:

1. Adult patients more than 18 years of age with diagnosed or suspected with leptospirosis
2. Charts with available laboratories (at least AST, ALT, and prothrombin time)

Exclusion criteria:

Patients who's charts charts could not be accessed were excluded from this study.

Data Collection:

Medical records of suspected and confirmed cases of leptospirosis admitted since January 2008 to December 2019 were screened and reviewed. Detailed analysis of the clinical and laboratory profile with special emphasis on hepatic dysfunction were performed.

We used both the Leptospirosis census of the PGH - Infectious Disease Section (IDS) as well as the list of patients with leptospirosis (ICD Code A27.9) in the Medical Records Section.

Patient Information: We obtained the following demographics for each patient by chart review: (1) age, (2) sex, (3) exposure to contaminated water, (4) duration of symptoms prior to hospitalization, (5) duration of hospital stay and (6) the need for ICU admission, (7) infection with hepatitis B, (8) known chronic liver disease or risk factors such as alcoholism etc.

Clinical Manifestations*:* Signs and symptoms of the patient on admission were noted by chart review: (1) jaundice, (2) fever (T ≥ 38.0°C), (3) oliguria (urine output <400mL in a 24-h period or self-reported decrease or no urine output within the last 12 hours), (4) abdominal symptoms (abdominal pain, nausea, vomiting and diarrhea), (5) cardiac symptoms (chest pain, irregular rhythm), (6) conjunctival suffusion, (7) myalgia or calf tenderness, (8) mental status changes, and (9) pulmonary involvement as manifested by dyspnea, cough, tachypnea and/or hemoptysis.

Laboratory Findings and Imaging Studies*:* We recorded the following laboratory results within 24 hours of admission by chart review: (1) Serum Potassium (meq/L), (2) Serum Calcium (meq/L), (3) Serum Creatinine (mg/dL), (4) Aspartate aminotransferase (IU/L), (5) Alanine Aminotransferase (IU/L), (6) Computed AST/ALT

Ratio (7) Total Bilirubin (mg/dL), (8) Direct Bilirubin (mg/dl), (9) Indirect Bilirubin (mg/dl), (10) Viral Hepatitis Serologies, (11) White Blood Cell count, (12) Platelet count, (13) PT-INR, (14) PTT, and (15) Abdominal ultrasound.

***Patient outcomes**:* The following outcomes per patient were also noted: patients who expired and patients who survived until discharge. We correlated the liver function tests with the patient's survival.

DATA MANAGEMENT AND ANALYSIS

A total of 260 patients were included in the Leptospirosis registry of the IDS and the Medical Records Section from January 2008 to December 2019. Out of this, only 142 charts were available from the records section and retrieved. 119 of these patients where laboratory confirmed while 23 where suspected cases of leptospirosis. This process is outlined in Figure 1.

Each patient was assigned a code that remained confidential throughout the course of this study, and in the succeeding time frame. The code assignments were kept in a separate file which was password-protected, in a password-protected computer that only the investigators had access to. Each data sheet was kept in a secure, locked-cabinet. All data, both digital and in print, shall be destroyed by deletion or shredding three years after the completion of this study.

Table 1. Baseline characteristics and clinical profile

Characteristics (Median Range)	Patients N=142 (%)
Age: ≥ 40 years < 40 years	67 75
Males (%)	122 (85.9%)
Exposure to contaminated water (N [%])	113 (79.5%)
Duration of symptoms prior to admission (Mean days ± SD)	6.9 ± 4.4
Signs and Symptoms	
Fever	140 (98.6%)
Myalgia/Calf Tenderness	118 (83.6%)
Nausea/vomiting	93 (65.8%)
Conjunctival Suffusion	87 (61.6%)
Jaundice	81 (57.5%)
Oliguria	81 (57.5%)
Abdominal pain	75 (53.4%)
Diarrhea	70 (49.3%)
Tachypnea	48 (34.2%)
Cough	40 (28.8%)
Dyspnea	23 (16.4%)
Chest pain	15 (11.0%)
Mental Status Changes	9 (6.8%)
Hemoptysis	9 (6.8%)

An overwhelming majority of the patients were males with a history of exposure to contaminated water. They were symptomatic for an average of 7 days prior to being admitted. The most common clinical presentation was fever and myalgia. Jaundice occurred in 57.5% of patients.

Table 2: Laboratory parameters

Laboratory Parameters (Mean ± SD)	
Serum Potassium (3.5-5.1 mEq/L)	3.7 ± 0.9 mEq/L
Serum Creatinine (0.52-1.04 mg/dL)	7.4 ± 4.3 mg/dL
Serum Calcium (2.1-2.55 mEq/L)	2.2 ± 0.2 mEq/L
AST (14-36 IU/L)	100.33 ± 216.05 IU/L
ALT (<35 IU/L)	66.78 ± 47.15 IU/L
AST/ALT Ratio	1.42 ± 1.19
Direct Bilirubin (0-7 umol/L)	168.66 ± 166.74 umol/L
Indirect Bilirubin (0-19 umol/L)	36.54 ± 45.91 umol/L
ALP (38-126 IU/L)	176.06 ± 65.16 IU/L
PT-INR	1.25 ± 0.33

The mean AST was 2.78 times elevated from normal, the mean direct bilirubin was 24 times elevated from normal, and the mean indirect bilirubin was almost 2 times elevated from normal.

Of the 142 patients with clinical or laboratory confirmed leptospirosis, 11 expired and 131 survived. Only 3 whole abdominal ultrasounds were retrieved. All three ultrasounds had normal sized livers with homogenous parenchymal echopatterns, and non dilated biliary trees.

Table 3: Correlation of Jaundice with liver function tests

Group Statistics					
	Jaundice	N	Mean	Std. Deviation	Std. Error Mean
AST (U/L)	Yes	42	89.81	83.408	12.870
	No	64	110.50	290.154	36.269
ALT (U/L)	Yes	44	59.068	26.3018	3.9651
	No	66	70.282	50.9813	6.2754
DB (umol/L)	Yes	37	225.1757	196.44859	32.29595
	No	31	113.4190	113.70168	20.42142
IB (umol/L)	Yes	37	55.9114	57.33749	9.42623
	No	30	22.7857	25.97200	4.74182

Table 4: t-test to Correlate Jaundice

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
AST (U/L)	Equal variances assumed	-.449	104	.654	-20.690	46.036	-111.981	70.600
	Equal variances not assumed	-.538	77.965	.592	-20.690	38.485	-97.309	55.928
ALT (U/L)	Equal variances assumed	-1.343	108	.182	-11.2136	8.3478	-27.7604	5.3331

	Equal variances not assumed	-1.511	102.553	.134	-11.2136	7.4231	-25.9364	3.5091
DB (umol/L)	Equal variances assumed	2.797	66	.007	111.75664	39.95430	31.98531	191.52798
	Equal variances not assumed	2.925	59.188	.005	111.75664	38.21077	35.30216	188.21113
IB (umol/L)	Equal variances assumed	2.927	65	.005	33.12568	11.31683	10.52441	55.72696
	Equal variances not assumed	3.139	52.363	.003	33.12568	10.55171	11.95562	54.29575

The results were taken at 0.05 level of significance. Using independent-samples t-test (unequal variances assumed), the mean AST and mean ALT values of those with and without jaundice are not significantly different. On the other hand, the mean DB and IB levels of those with and without jaundice are found to be significantly different, i.e. those with jaundice exhibited, on the average, higher values of DB and IB. However, we cannot establish the cutoff values of DB and IB that will indicate/predict jaundice.

Table 5: Correlation of liver function tests with mortality

Group Statistics					
	Mortality	N	Mean	Std. Deviation	Std. Error Mean
AST (U/L)	Yes	11	92.45	43.778	13.199
	No	111	101.11	226.197	21.470
ALT (U/L)	Yes	11	51.455	16.9609	5.1139
	No	115	68.249	48.8608	4.5563
DB (umol/L)	Yes	9	226.6733	123.98916	41.32972
	No	72	161.4074	170.62558	20.10842
IB (umol/L)	Yes	9	49.0800	50.46430	16.82143
	No	71	34.9545	45.44436	5.39325

Table 6: t-test to Correlate liver function tests with mortality

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
AST (U/L)	Equal variances assumed	-.126	120	.900	-8.654	68.573	-144.423	127.116
	Equal variances not assumed	-.343	81.225	.732	-8.654	25.203	-58.797	41.490
ALT (U/L)	Equal variances assumed	-1.130	124	.261	-16.7942	14.8637	-46.2135	12.6252
	Equal variances not assumed	-2.452	30.492	.020	-16.7942	6.8492	-30.7727	-2.8156
DB (umol/L)	Equal variances assumed	1.109	79	.271	65.26597	58.86608	-51.90402	182.43597
	Equal variances not assumed	1.420	12.159	.181	65.26597	45.96188	-34.73131	165.26326
IB (umol/L)	Equal variances assumed	.868	78	.388	14.12549	16.27068	-18.26693	46.51792
	Equal variances not assumed	.800	9.718	.443	14.12549	17.66487	-25.38993	53.64092

To check for association between mortality and AST, ALT, DB and IB, independent samples t-test was utilized at 0.05 level of significance. The p-values indicated in the table above show that only the mean ALT value significantly differ by mortality status. However, it should be noted that death among the subjects in the study are relatively rare (small sample size).

Table 7: Association between Jaundice and Mortality

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	.918[a]	1	.338	.382
Continuity Correction[b]	.241	1	.623	
Likelihood Ratio	.892	1	.345	.647
Fisher's Exact Test				.382
Linear-by-Linear Association	.911[c]	1	.340	.382
N of Valid Cases	119			

Risk Estimate			
	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Mortality (No / Yes)	2.386	.383	14.854
N of Valid Cases	119		

Since the two variables are categorical variables, the Chi-square test for independence (with continuity correction) was used to check whether they are associated or not. The p-value indicates that there is no evidence of an association

between the two variables. The confidence interval estimate for the odds ratio indicate their lack of association (since the interval contains 1).

These results were recorded in an Excel file acessible only to the investigators. Statistical analysis was perfomed using the Statistical Package for the Social Sciences (SPSS) software.

DISCUSSION

Leptospirosos, considered as an emerging zoonotic disease[12], has long been implicated for multiple end organ damage and fatalities. This represents a public health concern specially in the urban areas of developing countries. However, to date, the seroepidemiology of *Leptospira*, the culprit bacterium, is largely unknown. Transmitted via contact with infected animals or contaminated environment, Leptospirosis manifests in different phases of its infective state and individual target organs. While most cases remain subclinical with mild illness not necessitating hospital admissions, complications arise and lead to clinically significant damage of predominantly affected organs like kidneys, lungs and liver.

Liver is commonly affected in leptospirosis. The spectrum of involvement includes acute hepatitis[13], gross enlargement of the liver, elevations of hepatic enzymes, damage to biliary tree and cholestasis. Locally, to the best of our knowledge, the magnitude of hepatic damage secondary to Leptospirosis has not been thoroughly investigated. This study thus aimed to determine the clinical and biochemical parameters of liver injury from leptospirosis. Patients would usually present with multiple organ injuries and rarely sole organ.

In this study, majority of infected patients presented with nonspecific symptoms like fever, myalgia and nausea/vomiting. Often mistaken for other acute febrile illness, symptoms of leptospirosis would manifest nonspecifically as observed in this study. These 3 leading signs and symptoms are often the basis for further evaluation especially among patients in endemic areas like the Philippines. As a result, laboratory support is essential. Muscle pain and tenderness is not unusual and typically involves the calves and lower back were high metabolically active tissues are. Conjunctival suffusion (dilatation of conjunctival vessels without purulent exudate), which occurs frequently in leptospirosis, is relatively uncommon in other infectious diseases. [14]

Jaundice was present in more than half of the subjects investigated similar to other studies observed abroad. In a retrospective study on the prognostic factors of leptospirosis conducted in a tertiary hospital in France, jaundice was one of the criteria that predicted development of severe leptospirosis[6]. The physical examination may show an enlarged and tender liver likely explains the occasional nonspecific abdominal pain of some. Serum aminotransferases and alkaline phosphatase are only mildly elevated. This somehow supports existing literature with liver histopathology showing necrosis, foci of inflammation, plugging of bile canaliculi, and hepatocytes apoptosis leading to release of aminotransferases. In other studies, Leptospires have been shown to infiltrate Disse's space, and migrate between hepatocytes with detachment of the intercellular junctions and disruption of bile canaliculi, leading to leakage of bile[1] hence the markedly elevated direct bilirubin levels. Coagulation parameters in terms of prothrombin time and INR were within normal limits and so are the other serum chemistry for renal function save for elevated creatinine.

Weil's disease, a severe form of leptospirosis, can lead to multiple organ damage including acute liver failure, acute kidney injury, rhabdomyolysis, and thrombocytopenia with possible hemorrhagic diathesis including pulmonary hemorrhage. Without treatment, the associated mortality rate ranges from 5% to 15%. Transaminase levels are moderately elevated in the 100s IU/L, with a mild increase of alkaline phosphatase.[7] In a study done by Chang in 2005, an aspartate aminotransferase–alanine aminotransferase ratio of >3 revealed a poorer prognosis.[8] Secondary to septic cholestasis, jaundice typically appears during day five to nine of the disease course. Serum bilirubin was shown to rise to as high as 30 to 40 mg/dL during the icteric phase.[7] In our study, the aminotransferase levels were about 2-3x elevated only and the bilirubinemia at 24x elevated (168.66umol or 9mg/dl) likely due to relatively moderate disease activity at the time of investigation. With the statistical analysis however, elevation of ALT, AST at 50-100IU/L and direct bilirubin at 226umol/L can be correlated to eventual mortality.

CONCLUSION

To date, no local studies yet have fully explored the liver profile of patients with leptospirosis. Our study is compatible with previous ones showing that leptospirosis presents with predominantly elevated direct bilirubin. The AST is elevated to a lesser degree with an AST:ALT ratio greater than 1 with mildly elevated alkaline phosphatase and coagulation parameter. Results also showed that an elevated ALT is associated with mortality suggestive of hepatocyte injury and apoptosis. Contrary to literature, our study found no association between jaundice and mortality.

ETHICAL CONSIDERATIONS

The authors claimed no conflict of interest in any form. The authors also certified that they have no affiliations with or involvement in any organization which would benefit from the research study.

Autonomy: Utmost care was dedicated into ensuring the confidentiality of identifiable patient information. There was a controlled access to patient information. Each patient was assigned with a code, and data were gathered using de-identified data collection forms. As the study is retrospective, there was no patient contact involved, and the analyzed data will be part of the routine evaluation of patients with leptospirosis. The study complied with the Philippine Data Privacy Act.

Non-maleficence: As a retrospective study, no direct harm were observed to the included population.

Informed Consent: As a retrospective study obtaining data through chart review, we requested that the informed consent process be waived.

Risks, Benefits, and Compensation*:* As the data were gathered via chart review with no actual interaction with the patients, there were no direct risks and benefits to the person of the patients. No compensation were provided to the patients whose data are used in this study.

REFERENCES

[1] Wagenaar J, Goris M. Leptospirosis. In: Harrison's Principles of Internal Medicine. 20th ed. 2019.

[2] Faine S, Adler B, Bolin C, Perolat P: Clinical leptospirosis in humans. In *Leptospira and leptospirosis* 2nd edition. MediSci, Mel- bourne; 1999.

[3] Victoriano AF, Smythe LD, Gloriani-Barzaga N, Cavinta LL, Kasai T, Limpakarnjanarat K, et al. Leptospirosis in the Asia Pacific region. BMC Infectious Diseases. 2009 Sep 4; 9: 147.

[4] Mendoza MT, Roxas EA, Ginete JK, Alejandria MM, Roman AD, Leyritana KT, et al. Clinical profile of patients diagnosed with leptospirosis after a typhoon: a multicenter study. Southeast Asian J Trop Med Public Health. 2013 Nov; 44(6): 1021–35.

[5] Republic of the Philippines Department of Health. Disease surveillance report: leptospirosis morbidity week 48 2018.

[6]Abgueguen P, Delbos V, Blanvillain J, Chennebault JM, Cottin J, Fanello S, et al. Clinical aspects and prognostic factors of leptospirosis in adults. Retrospective study in France. Journal of Infection. 2008; 57(3): 171–8.

[7] Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM. Peru-United States Leptospirosis Consortium. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis. 2003;3(12):757–771.

[8] Chang ML, Yang CW, Chen JC, Ho YP, Pan MJ, Lin CH, Lin DY. Disproportional exaggerated aspartate transaminase is a useful prognostic parameter in late leptospirosis. World J Gastroenterol. 2005;11(35):5553–5556.

[9]Opilla-Bautista I,Panaligan M. Predictors of mortality among patients with leptospirosis admitted at the JRRMMC. Phil J Microbiol Infect Dis 2002; 31: 145-9.

[10]Philippine College of Physicians. Philippine clinical practice guidelines on the diagnosis, management and prevention of leptospirosis. 2010.

[11]Levett P, Haake D. Leptospirosis species (Leptospirosis species). In: Mandell G, Bennett J, Dolin R, eds. Principles and practice of infectious diseases. 9th ed. Philadelphia: Churchill Livingston Elsevier, 2020: 2898-2905.

[12] Lau CL, Skelly C, Dohnt M, Smythe LD. The emergence of *Leptospira borgpetersenii* serovar Arborea in Queensland, Australia 2001 to 2013. BMC Infect. Dis., 2015;15: 230.

[13] Rizvi M, Azam M, Ajmal AR, Shukla I, Malik A. Prevalence of Leptospira in acute hepatitis syndrome and assessment of IL-8 and TNF-alpha level in leptospiral hepatitis. Ann. Trop. Med. Parasitol. 2011; 105(7):499–506.

[14] Haake D, Levett P. Leptospirosis in Humans. Curr Top Microbiol Immunol. 2015; 387: 65–97.

Appendix 1. Sample Data Collection Form

GENERAL INFORMATION					
Patient Code			CLD or Risk Factors		
Age			Sex		
Duration of Hospital Stay		days	Duration of Symptoms		days
Exposure to contaminated source	□ Yes	□ No			
SYMPTOMS AND SIGNS (check all that apply)					
□ Jaundice	□ Fever (T ≥ 38.0°C)		□ Oliguria (UO < 400ml/d, self-reported decrease in last 12 hrs		
□ Abdominal Symptoms □ Abdominal pain □ Nausea and vomiting □ diarrhea	□ Cardiac Symptoms □ Chest Pain □ Irregular rhythm		□ Pulmonary Symptoms □ Dyspnea □ Cough □ Tachypnea □ Hemoptysis		
□ Myalgia/Calf Tenderness	□ Mental status changes		□ Conjunctival suffusion		
LABORATORY RESULTS					
Potassium	meq/L	Corr. Calcium	meq/L	Creatinine	mg/dL
AST	IU/L	ALT	IU/L	AST/ALT Ratio	
Total Bilirubin	mg/dL	Direct Bilirubin	mg/dl	Indirect Bilirubin	mg/dl
PT-INR		PTT ref		PTT time	
HBSAg		WBC	x10^9/L	Platelet	x10^9/L
HBEAg		Anti HBC IgM		Anti HCV	
Anti HBCt		Anti HBS		Anti HBE	
Abdominal Ultrasound					
Confirmatory test result					

Patient Outcomes	
Classification of Leptospirosis (Mild, Moderate, Severe*) **Indicate if in Weil Syndrome*	
Final outcome (Mortality, Morbidity, Survived to discharge)	
a. If mortality, indicate primary cause of death	
b. Indicate morbidity, if applicable (e.g., intubated for respiratory distress, pulmonary hemorrhage, underewnt hemodialysis. etc)	
c. If survived, indicate number of days admitted	
Site of admission (ward vs. ICU)	

ICU Admission	Reason for ICU Admission: □ Oliguria/Anuria □ Pulmonary Symptoms □ Need for inotropic support

Date Accomplished		Accomplished By	

Abbreviations: UO: Urine Output; Corr.: Corrected; PCOD: Precipitating Cause of Death; CLD: Chronic Liver Disease;

Appendix 2. GANTT Chart

2019	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Protocol creation												
Technical review and ethics board approval												
2020	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
Data collection: **chart review**												
Data analysis												
Paper writing, revisions												
Submission for publication												

Appendix 3. Internal Operative Budget

A. Personnel Services	**Cost per unit**	**Total cost**
Data analyst (Statistician)	Php 4,000	Php 4,000.00
B. Supplies and Materials		
A4 80GSM Paper (500 pcs/ream)	PhP 1.00 / paper	Php 500.00
Photocopy	Php 2.00/ paper	Php 1,000.00
Printer ink	Php 1000/cartridge	Php 1000.00
Office supplies (paper envelopes, paperclips, staplewires, filing box)	Php 2,000.00	Php 2,000.00
C. Maintenance and other operating expenses		
Not applicable		
	TOTAL	**Php 8,500.00**

FIGURES

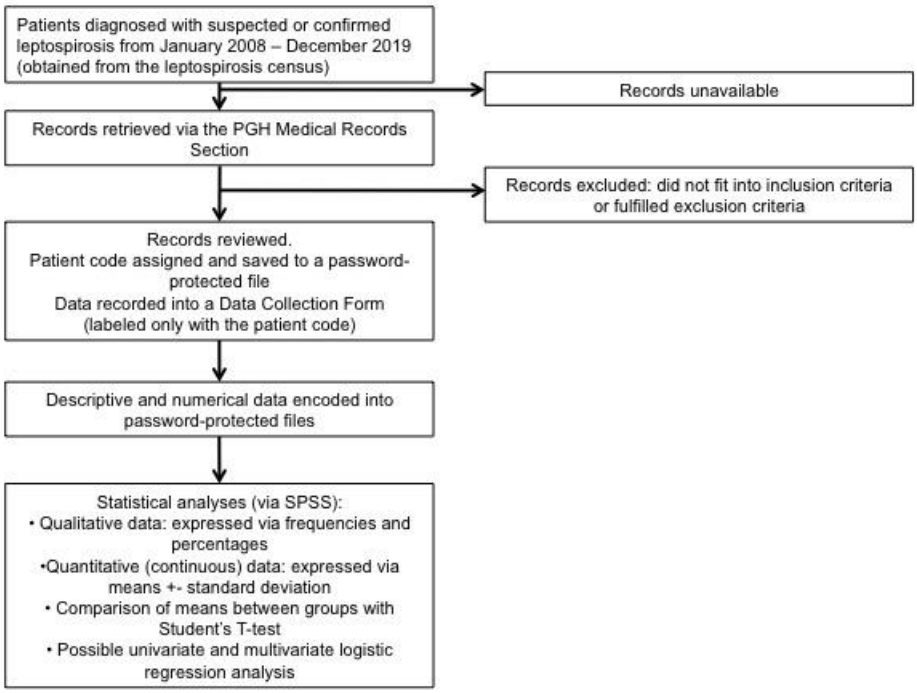

Figure 1. Diagrammatic work flow.