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Research Article

BIOCHEMICAL CHARACTERIZATION, CLINICAL DIAGNOSIS AND HEPATIC COMPLICATION OF WEIL'S DISEASE AND OTHER CO-INFECTIONS

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ABSTRACT

Aim: The present study is focused with an objective to assess the Weil's disease and its association with hepatic damage and other co-infections. Methods: Leptospirosis was detected by microscopic agglutination test (MAT). These samples were assessed to find out the hepatic damage by liver markers such as amino transferase (ALT and AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilirubin and proteins specifically total protein and albumin quantification. Results: Widal test and semi quantitative slide test were carried out to confirm typhoid .Total and Direct bilirubin quantification were carried out to diagnose Jaundice. Conclusion: Based on the results, the patients were grouped into five categories as Normal, Patients with Leptospirosis, Patients with Leptospirosis and Typhoid, Patients with Leptospirosis and Jaundice, Patients with Leptospirosis, Typhoid and Jaundice. Out of 40 samples, 35 were diagnosed as Leptospirosis patients and 5 samples were considered as normal.

Key words: Leptospirosis; Microscopic Agglutination Test; Widal test; Liver markers; Typhoid.

INTRODUCTION

Weil's disease or Leptospirosis is a serious zoonotic disease caused by infection with *Leptospira* spp and it is presumed to be the most widespread zoonosis in the world. Leptospirosis has been identified as a re-emerging infectious disease and this has been demonstrated by the large outbreaks in Nicaragua, Brazil, India, Southeast Asia, Malaysia and in the United States[1-10]. Leptospirosis has a worldwide distribution and is more spread in tropical regions than in temperate countries [11,12]. This is attributed mainly to longer survival of *leptospires* in warm and humid environments. Leptospirosis is not limited to developing countries. Retrospective reviews of the disease epidemiology have been reported from Ireland, Denmark and Italy[13-15]. A pattern of disease seasonality has been described with a peak incidence occurring in summer or fall in temperate regions and during rainy seasons in warm climate regions[16]. Leptospirosis transmission in humans occurs by direct or indirect contact with urine, blood or tissue from an infected animal containing virulent *leptospires*. Infection may also arise from bathing or accidental immersion in the fresh water lakes, rivers or canals contaminated with the urine of the infected livestock that has been previously using the water[17]. After an incubation period that ranges from 1 to 3 weeks, a phase commences which is marked by fever, chills, headache and severe myalgias and arthralgias[18]. The first phase resolves, and the patient is briefly asymptomatic until the second phase begins. This is characterized by meningitis, liver damage (causing jaundice) and renal failure. The infection is often wrongly diagnosed due to the wide range of symptoms. This leads to a lower number of registered cases than exists. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice, red eyes, abdominal pain, diarrhea, and rash. Initial presentation may resemble pneumonia. The symptoms in humans appear after a 4-14 days incubation period[19, 20].

The disease was first described by Adolf Weil in 1886 when he reported on "acute infectious disease with enlargement of spleen, jaundice and nephritis". *Leptospira* was first observed in 1907 from a post mortem renal tissue slice[21]. In 1908, Inada and Ito first identified it as the causative organism[22] and in 1916 noted its presence in rats[23].

Annual rates of infection vary from 0.02 per 100,000 in temperate climates to 10 to 100 per 100,000 in tropical climates[24]. Nepal is experiencing a leptospirosis outbreak as of August 2010[25].having favourable conditions like warm temperatures and moist soils. Cases increase in summer months (monsoon), but no accurate case count has been made as most go unreported[26]. Almost all the higher animals and rodent hosts of Leptospira occur in Nepal.[26]

The Andaman and Nicobar Islands is among the worst affected in the tsunami attack and there is a serious risk of an outbreak of Weil's Syndrome (locally known as Andaman fever). The disease often occurs after natural catastrophes like tsunami and has been widely reported following floods and cyclones[27]. Leptospirosis in people is known by a variety of names reflecting the sources of infection. e.g. "Rice field fever", and "Cane-cutters fever" (transmission from contaminated water) and "Swine herder's disease" (transmission from contact with infected animals). Outside tropical areas Leptospirosis are caused due to distinct seasonality. Most of them occurring during August-September/February-March[28].

The diagnosis of Leptospirosis depends on detecting the *leptospires* in clinical specimens and demonstrating an increase in antibody titers to one or more leptospiral serovars. Most cases of Leptospirosis are diagnosed by serology. Dark field microscopy may provide a presumptive diagnosis of Leptospirosis in many cultures in India. *Leptospires* in smears of tissues or body fluids on slides can be stained using silver deposition methods[29].

The diagnosis of Leptospirosis is mainly based on serological testing, the microscopic agglutination test (MAT) considered as the gold standard methodology[30]. A variety of serological tests other than MAT have been developed for the diagnosis of leptospirosis. The MAT is a test which determines agglutinating antibodies in the serum of a patient by mixing it in various dilutions with live or killed formalized *leptospires*. Anti leptospiral antibodies present in the serum, cause *leptospires* to stick together to form clumps. This clumping process is called agglutination and is observed using dark-field microscopy. Agglutinating antibodies can be of both lgM and lgG classes used in ELISA to detect Leptospira - specific lgM and lgG in the sera of patients infected with Leptospiral serovars hardjo, Pomona or copenhageni[31,32]. All patients produced specific lgM and lgG antibodies, which are detectable by ELISA. IgM detection by ELISA is more sensitive than MAT, when the first specimen is taken early in the acute phase of the illness[33].

Rapid diagnosis of Leptospirosis in animals and men are evaluated by PCR. Urine and serum samples were checked by dark field microscopy (DFM) and PCR[34]. PCR assay of blood and urine samples is potentially useful quick diagnostic method for confirming active infection with *leptospires*. Nested-PCR method is also used for diagnosis. This approach was much more sensitive than traditional PCR[35]. Real time PCR assay successfully detected Leptospiral DNA from serum and urine samples of patients with leptospirosis. This assay has the potential to facilitate rapid, sensitive diagnosis of acute Leptospirosis[36].

MATERIALS AND METHODS

Collection of the sample

Samples were collected from patients with suspected clinical condition in Dr.Mehta Hospital, Chennai. The serum samples were prepared from the collected samples and used for the experiments.

Chemicals and reagents

All Chemicals and kits to quantify liver markers were obtained from Sigma Chemicals, Bangalore, India. All Chemicals and reagents are of analytical grade. *Leptospira* culture was obtained from the stock cultures of the Department of Microbiology CSMDRIA, Chennai, India.

Biochemical parameters

The leptospirosis was detected by microscopic agglutination test **(**MAT)[37]. Liver markers such as aspartate aminotransferase (AST) and alanine amino transaminase (ALT) were assayed by UV kinetic (IFCC) method[38,39]. Alkaline phosphatase(ALP) by pNPP method[40-42], Gamma glutamyl transferase(GGT) by SZASZ method[43], Total protein and albumin by Kjeldahl's method[44], Total bilirubin and direct bilirubin by van den Bergh method[45] to assess the hepatic damage. Typhoid was diagnosed by Widal test[46], quantified by rapid slide[47] and semi quantitative slide test[48].

Statistical analysis

All the grouped data were statistically evaluated with SPSS 16.00 software. Hypothesis testing methods included one-way analysis of the variance followed by least significant difference (LSD) test. $P<0.05$ was considered to indicate statistical significance. All results are expressed as mean ± standard deviation (SD).

Results & Discussion

Samples from 40 patients were collected and analyzed. Out of 40 samples, 35 were diagnosed as Leptospirosis patient and 5 samples were considered as normal. These were confirmed by microscopic agglutination test (MAT) and categorized into five different groups.

Group I : Normal (control)

Group II : Patient with Leptospirosis

Group III : Patient with Leptospirosis and Typhoid

Group IV : Patient with Leptospirosis and Jaundice

Group V : Patient with Leptospirosis, Typhoid and Jaundice

Table 1: Samples taken for analysis.

S.No.	Types of Sample	No. of Sample
1	Group I	5
2	Group II	12
3	Group III	10
4	Group IV	8
5	Group V	5

Plate 1: Detection of *Leptospires* by MAT

Antigen: Patient Serum Sample, Antibody: IgM, IgG

Patient Serum and Antibody were mixed. Clumps were formed by agglutination from positive patients and it was observed under darkfield microscope.

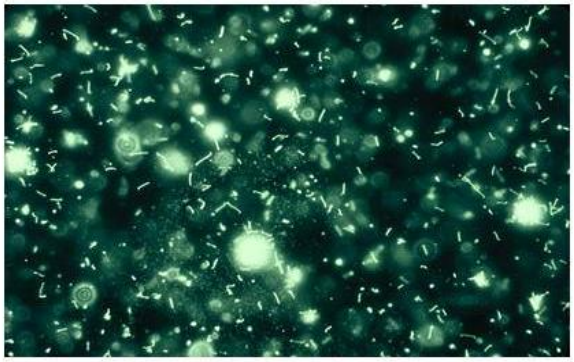

Fig 1: Detection of *Leptospires* by MAT.

Antigen: Patient serum sample.
Antibody: IgM and IgG.

Table 2: Statistical analysis of liver markers in Leptospirosis sample (Group II).

Liver Markers	Group II Sample (Mean ±S.D)	Control (Mean ± S.D)	Sample T-test	Control T-test
AST	61.45±11.76	40.74±1.71	6.035	2.074
ALT	59.45±13.15	40.35±6.37	4.526	2-074
ALP	201.61±52.46	171.46±48.45	1.462	2.074
GGT	46.15±12.84	28.15±15.66	3.076	2.074
Total protein	5.93±1.34	7.25±0.59	3.094	2.074
Albumin	3.88±0.86	3.98±0.91	0.274	2.074
TotalBilirubin	0.65±0.180	0.68±025	0.345	2.074
DirectBilirubin	0.35±0.10	0.34±0.13	0.217	2.074

S.D = Standard Deviation

Table 3: Semi/Quantitative slide test

Number of Sample	'O' Antigen					'H' Antigen				
	Test titres					Test titres				
	01:20	01:40	1:80	1:160	1:320	1:20	1:40	1:80	1:160	1:320
1)				✓					✓	
2)					✓				✓	
3)				✓					✓	
4)					✓				✓	
5)				✓					✓	
6)				✓				✓		
7)					✓					✓
8)					✓					✓

✓ -- Implies Agglutination.

Table 2 depicts the activity of pathophysiological liver enzymes such as AST, ALT, ALP, GGT and other liver markers –total protein, albumin, total bilirubin, direct bilirubin in the serum of control and Leptospirosis patient. AST level was increased significantly for all 12 samples, may be due to the leakage in cytosol of hepatocytes. ALT level was also increased significantly for all 12 samples, may be due to leakage in mitochondria of hepatocytes. ALP level was increased significantly due to obstruction of bile duct. GGT level was increased significantly due to leakage of hepatocytes. Total protein and Albumin levels were decreased significantly which indicates the early stage dysfunction of liver. Bilirubin levels were moderately increased due to leakage of hepatocytes which shows early stage liver dysfunction[49, 50].

From the statistical analysis it was proved that the difference is highly significant at 5% level for all the liver markers.

Leptospirosis with typhoid (Group III) patients were diagnosed for typhoid using Widal test.

Plate 2: Widal test

From this **Plate 2,** agglutination was observed in the circles where **"O"** antigen, **"H"** antigen, "**A(H)"** antigen and **"B(H)"** antigen added to the patient's serum sample. This shows that all the patients with typhoid have **"O", "H"** antigens

This table reveals that out of 8, four samples showed positivity for typhoid **"O"** antigen at **1:320** test titre. This indicates that 4 patients have severe typhoid with **"O"** antigen. Four samples were showed positivity for typhoid O antigens at **1:160** test titres. This indicates that four patients have moderate typhoid with **"O"** antigen. Out of 8, four samples showed positivity for typhoid H antigens at **1:320** test titre. This indicates that four patients have severe typhoid with **"H"** antigen. Four samples showed positivity for typhoid H antigens at **1:160** test titre. This indicates that four patients have moderate typhoid with "**H**" antigen.

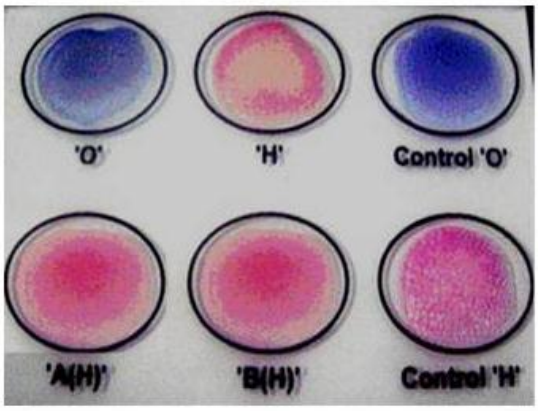

Fig 2: Widal test.

Table 4: Statistical analysis of liver markers in Leptospirosis with Typhoid sample (Group III).

Liver Markers	Group III Sample (Mean ± S.D)	Control (Mean ± S.D)	Sample T-Test	Control T-Test
AST	64.7±16.04	40.77±2.24	4.671	2.101
ALT	76.9±11.34	40.88±5.93	8.900	2.101
ALP	214.79±42.96	173.82±47.92	2.013	2.101
GGT	42.26±15.56	30.86±15.79	1.625	2.101
Total protein	6.46±1.45	7.34±0.73	1.701	2.101
Albumin	4.36±1.15	4.02±0.90	0.732	2.101
Total Bilirubin	0.82±0.19	0.53±0.24	2.880	2.101
Direct Bilirubin	0.41±0.13	0.33±0.13	1.350	2.101

S.D = Standard Deviation

Table 5: Statistical analysis of liver markers in Leptospirosis with Jaundice sample (Group IV)

Liver Markers	Group IV Sample (Mean ± S.D)	Control (Mean ± S.D)	Sample T-test	Control T-test
AST	66.72±17.0	38.45±4.35	4.555	2.145
ALT	77.75±22.51	38.75±5.59	4.740	2.145
ALP	221.03±45.93	169.87±45.13	2.510	2.145
GGT	63.8±12.0	32.82±14.81	4.595	2.145
Total protein	7±1.12	7.12±0.70	0.426	2.145
Albumin	3.95±0.97	4.1±0.83	0331	2.145
Total Bilirubin	2.23±0.435	0.68±0.237	8.870	2.145
Direct Bilirubin	0.88±0.203	0.33±0.140	6.321	2.145

S.D = Standard Deviation

Table 6: Statistical analysis of liver markers in Leptospirosis with Typhoid & Jaundice sample (Group V)

Liver Markers	Group V Sample (Mean ± S.D)	Control (Mean ± S.D)	Sample T-test	Control T-test
AST	101.58±12.15	40.84±1.95	11.02	2.306
ALT	96.42±15.57	40.84±1.95	7.39	2.306
ALP	272.74±9.98	173.66±50.60	4.28	2.306
GGT	62.3±10.83	30.86±16.75	3.52	2.306
Total protein	3.94±1.20	7.34±0.783	5.29	2.306
Albumin	2.98±1.28	4±0.940	1.42	2.306
Total Bilirubin	2.54±0.450	0.72±0.238	8.01	2.306
Direct Bilirubin	0.88±0.216	0.38±0.130	4.42	2.306

S.D = Standard Deviation

Table 3 shows that Serum aspartate transaminase enzyme level was increased significantly for all 10 samples may be due to leakage in cytosol of hepatocytes. Serum alanine transaminase enzyme level was increased significantly may be due to leakage in mitochondria of

hepatocytes. Alkaline phosphatase enzyme level was increased significantly due to obstruction of bile duct. Gamma glutamyl transferase enzyme was increased significantly due to leakage of hepatocytes. Total protein and albumin levels were decreased significantly which indicates the early stage dysfunction of liver. Bilirubin levels were moderately increased which shows early stage liver dysfunction[49, 50].

Typhoid fever can affect gastro intestinal tract which leads to gastro intestinal perforation. It is a serious complication of typhoid fever. Leptospira can also affect the gastro intestinal tract, because of these reasons patients with Leptospirosis were also borne to typhoid fever. Both *Leptospires* and S*almonella typhi* is water borne organism, and they spread out simultaneously. Hence Typhoid is considered as co-infection of leptospirosis.

Table 5 reveals that, **s**erum aspartate transaminase enzyme level was increased significantly for all 8 samples may be due to leakage in cytosol of hepatocytes. Serum alanine transaminase enzyme level was increased significantly may be due to leakage in mitochondria of hepatocytes. Alkaline phosphatase enzyme level was increased significantly due to obstruction of bile duct. Gamma glutamyl transferase enzyme was increased significantly due to leakage of hepatocytes. Total protein and albumin levels were decreased significantly, which indicates the early stage dysfunction of liver. Bilirubin levels are high in leptospirosis because it can cause dissociation of hepatocytes which leads to jaundice (severe form). The characteristic conjugated hyperbilirubinemia associated with Leptospirosis due to the degree of elevation of liver parenchymal enzymes. *Leptospires* can cause micro circulatory abnormalities due to failure of bilirubin excretion which leads to hyperbilirubinemia. Hence Jaundice is considered as co-infection of Leptospirosis[49-52].

Table 6 reveals that**,** Serum aspartate transaminase enzyme level was increased significantly for all 5 samples may be due to leakage in cytosol of hepatocytes. Serum alanine transaminase enzyme level was increased significantly may be due to leakage in mitochondria of hepatocytes. Alkaline phosphatase enzyme level was increased significantly due to obstruction of bile duct. Gamma glutaryl transferase enzyme was increased significantly due to leakage of hepatocytes. Total protein and albumin levels were decreased significantly, which indicates the severe stage dysfunction of liver. Typhoid fever can affect gastro intestinal tract which leads to gastro intestinal perforation. It is a serious complication of typhoid fever. Leptospira can also affect the gastro intestinal tract, because of these reasons patients with leptospirosis were also prone to typhoid fever. Both *Leptospires* and *salmonella typhi* were water borne organism, so these two spread out simultaneously. The characteristic conjugated hyperbilirubinemia associated with Leptospirosis is usually out of proportion to the degree of elevation of liver parenchymal enzymes. *Leptospires* can cause micro circulatory abnormalities due to failure of bilirubin excretion leads to increase in the bilirubin level. Hence typhoid and jaundice are considered as co- infection of leptospirosis[51, 52].

CONCLUSION

The result obtained from the present study describes the clinical and laboratory findings of patient with leptospirosis admitted to Dr.Mehta Hospital, Chennai, India. This clearly indicates that Leptospirosis patients have mild hepatic dysfunction. Leptospirosis with Typhoid patients has moderate hepatic dysfunction. Leptospirosis with Jaundice patients has severe hepatic damage. Hence patients with leptospirosis and co-infections (Leptospirosis with typhoid and Jaundice) have a severe hepatic damage compared to leptospirosis patients alone. The severity of the infection depends on the general health of the patient and causative agent **Serovar** (strain) of bacteria involved and the number of bacteria that enter the patient's body. Severe illness develops and starts progressing leading to organ failure and often death occurs if not treated with intervention and support. Hence the co-infection must be diagnosed early as possible before the severe hepatic dysfunction starts and to prevent the high risk.

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