

Comparison of Protein Electrophoresis Fractions in the Leptospirosis Patient Serum

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The mechanisms by which leptospires caused disease are not well understood. A number of putative virulence factors have been suggested, but with few exceptions their role in pathogenesis remains unclear. In these days, many cases of leptospirosis are diagnosed by serological immunoassay. Leptospirosis is characterized by the histopathology of liver, kidney, heart, and lung, but the electrophoresis fractions of serum protein are not compared. We analyzed total protein, albumin, aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine and urea nitrogen (UN) in sera of patients diagnosed leptospirosis. Total protein and albumin were decreased in 18.5% and 31.2% of patients, respectively. AST, ALT, ALP, UN and creatinine were increased in 90.4%, 66.9%, 28.0%, 15.9% and 10.8% of patients, respectively. We performed cellulose acetate membrane electrophoresis (EP) on sera of patients increased both of AST and ALT, and of patients increased both of creatinine and UN. In patients increased both of AST and ALT, and in patients increased both of AST and ALT, the relative percentage of albumin to total protein in patient serum was decreased in 89.1% of patients. α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin were increased in 85.1%, 75.2%, 33.6% and 98.0% of patients, respectively. In patients increased both of creatinine and UN, the relative percentage of albumin to total protein in patient serum was decreased in 93.8% of patients. α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin were increased in 87.5%, 100%, 31.2% and 93.8% of patients, respectively. These data indicate that infection of *Leptospira* causes severe liver damage to most of leptospirosis patients, but doesn't cause renal damage to most of them. The relative rate of serum protein electrophoresis fractions to total protein are not identical with them of typical hepatitis patient.

Key Words: Leptospirosis, Albumin, AST, ALT, Creatinine, UN, EP

INTRODUCTION

Leptospirosis is characterized by the development of vasculitis, endothelial damage, and inflammatory infiltrates composed of monocyte cells, plasma cells, histiocytes, and neutrophils. On gross examination, petechial hemorrhages are common and may be extensive (Arean, 1962), and organs are often discolored due to the degree of icterus (Pierce et al., 1997). The histopathology is most marked in the liver, kidneys, heart, and lungs (Zaki et al., 1998), but other org-

ans may also be affected according to the severity of the individual infection. The overall structure of the liver is not significantly disrupted, but there may be intrahepatic cholestasis (Arean, 1962; Dooley et al., 1976). Hypertrophy and hyperplasia of Kupffer cells is evident (De Brito et al., 1967), and erythrophagocytosis has been reported (Arean, 1962; Dooley et al., 1976). In the kidneys, interstitial nephritis is the major finding, accompanied by an intense cellular infiltration composed of neutrophils and monocytes (Penna et al., 1963). Leptospires can be seen within the renal tubules (Arean, 1962; Penna et al., 1963; Zaki et al., 1998). By electron microscopy, the tubular cell brush borders are denuded, the tubular basement membrane is thickened, and tubular cells exhibit mitochondrial depletion (De Brito et al., 1965). In addition, minor changes are seen in the glomeruli, suggesting an anatomical basis for proteinuria in leptospirosis (De Brito

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Table 1. Chemical components in serum of leptospirosis patient (n=157)

Component	Total protein g/dL	Albumin g/dL	AST U/L	ALT U/L	ALP U/L	UN mg/dL	Creatinine mg/L
Reference range	7.2±1.3	4.6±0.6	20±10	25±15	90±38	13±7	1±0.3
Mean±SD	7.3±6.4	3.8±0.5	90.3±95.4	68.6±65.0	115.9±65.0	15.3±6.4	0.9±0.3

et al., 1965).

Pathological findings in the heart include interstitial myocarditis and coronary arteritis (Aean, 1957; Ramachandran et al., 1977; Estavoyer et al., 1980; Lee et al., 1986; De Biase et al., 1987; De Brito et al., 1987). In the lungs, pulmonary congestion and hemorrhage are common (Aean, 1962; Zaki et al., 1996). Recently, scientists are interested in the mechanism of immune system and tests of diagnosis. In this study, We compared the concentration of biochemical components and the protein electrophoresis fractions in sera of leptospirosis patients.

MATERIALS AND METHODS

Leptospirosis patients (n=157) were diagnosed by immunoassay kit (MRL Diagnostics, Cypress, CA, USA). Diluted serum was mixed with antigen-coated test cells in the well. The mixture was incubated at 25°C for 1h. Hemagglutination was read as described previously (Levett et al., 1998; Bajani et al., 2003).

Total protein, albumin, AST, ALT, ALP, creatinine and UN were determined by Hitachi automatic clinical analyzer (Hitachi High-Technologies Co., Tokyo, Japan).

The concentration of chemical components that we analyzed was compared to reference range (Burtis et al., 2001). Leptospirosis patients were classified for two study groups, a group of patients showed elevations in both AST and ALT, and the other group of patients showed elevations in both of creatinine and UN. Sera of leptospirosis patients in two groups were determined total protein and were carried out electrophoresis by cellulose acetate membrane electrophoresis technique with Helena Laboratory electrophoresis system (Jeppsson et al., 1979). Sera were loaded on the cellulose acetate membrane absorbed buffer. The membrane was run for 15 min. at 180 V and stained with Ponceau S. Fractions were analyzed with image analyzer (Vilber Lourmat, Cedex, France). The relative percent of the each fractions in the serum was calculated and compared to reference range (Jeppsson et al., 1979; Burtis et al., 2001). All data were analyzed by SPSS software.

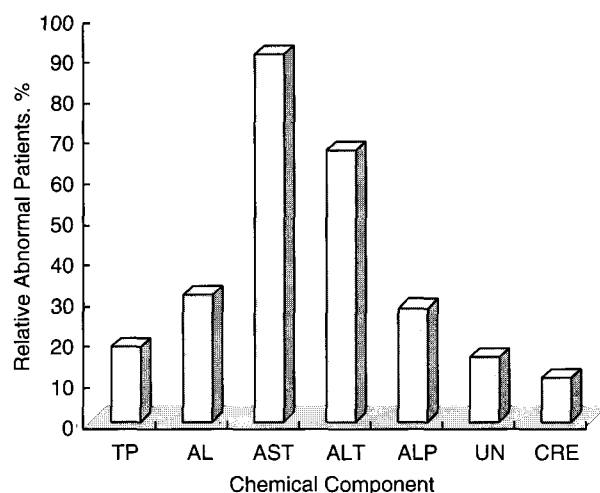


Fig. 1. Relative abnormal patients (%) showed lower level of total protein (TP) and albumin (AL) and showed higher level of AST, ALT, ALP, UN and Creatinine than reference range.

RESULTS AND DISCUSSION

We analyzed total protein, albumin, AST, ALT, ALP, UN and creatinine in sera of total 157 patients diagnosed leptospirosis (Table 1). The data were compared to reference range of total protein (96.0~8.5 g/dL), albumin (3.5~5.2 g/dL), AST (10~30 U/L), ALT (10~40 U/L), ALP (53~128 U/L), UN (6~20 mg/dL) and creatinine (0.7~1.3 mg/dL). The frequencies of patients showed lower level of total protein, and albumin than them of reference range were 18.5%, and 31.2%. The frequencies of patients showed higher level of AST, ALT, ALP, UN, and creatinine than them of reference range were 90.4%, 66.9%, 28.0%, 15.9%, and 10.8% (Fig. 1). Our Data shows that both of AST and ALT are increased in 64.3% of leptospirosis patients (Fig. 2). A mortality rate of 54% was reported in severe leptospirosis cases with myocarditis (Lee et al., 1986) and between 5 and 10% of all patients with leptospirosis have the icteric form of the disease (Health et al., 1965). The jaundice occurring in leptospirosis is not associated with hepatocellular necrosis, and liver function returns to normal after recovery (Ramos-Morales et al., 1959). Serum bilirubin levels may be high, and many weeks may be required for normalization (Edwa-

et al., 1990). There are moderate rises in transaminase levels, and minor elevation of the alkaline phosphatase level usually occurs. Our results shows that AST in serum of 90.4% of leptospirosis patients and ALT in 66.9% of patients are increased. These data indicate that most of patients with infection of *Leptospiras* have damage of liver as described by investigators above. ALP is increased only in

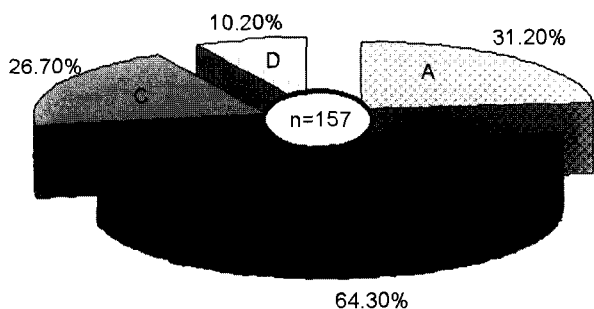


Fig. 2. Relative abnormal patients (%) in 157 leptospirosis patients. **A**, showed lower level of albumin (**A**); **B**, showed higher level of both AST and ALT; **C**, showed AST, ALT and ALP together, **D**, showed higher level of both UN and creatinine.

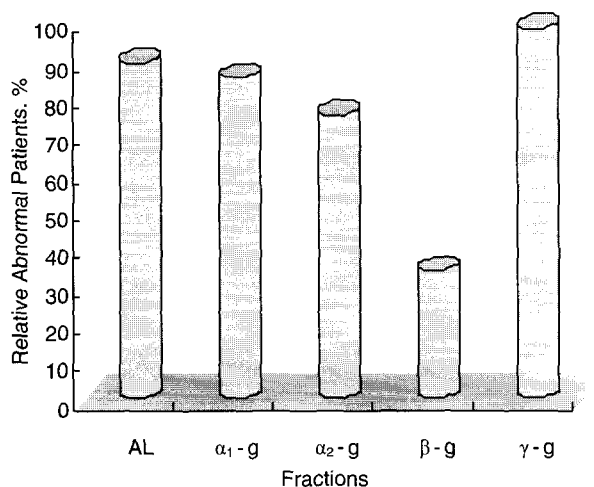


Fig. 3. Relative abnormal patients (%) to the leptospirosis patients increased both AST and ALT. The percentages were expressed the relative patients showed lower level of albumin (AL), and showed higher level of α_1 -globulin (α_1 -g), α_2 -globulin (α_2 -g), β -globulin (β -g) and γ -globulin (γ -g) than reference range.

28.0% of leptospirosis patient. This result isn't identical with the report (Arean, 1962; Dooley et al., 1976).

We analyzed the relative concentrations of electrophoretic fractions in serum proteins of each patients with increase of both AST and ALT (Table 2, A group). The leptospirosis patients had the most marked decrease in albumin and the increase in α_1 -globulin, α_2 -globulin and γ -globulin, having an average albumin, α_1 -globulin, α_2 -globulin and γ -globulin value of $39.1 \pm 8.7\%$, and $6.4 \pm 1.9\%$, $14.4 \pm 2.0\%$, and $28.3 \pm 6.4\%$, respectively. The relative patients showed the decrease of albumin, and the increase of α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin in leptospirosis patients with increase of both AST and ALT are 89.1%, and 85.1%, 75.2%, 33.6%, and 98.0%, respectively (Fig. 3). A increase and decrease of protein electrophoretic fractions in serum were demonstrated that the hepatitis patients and liver cirrhosis patients have a increase of AST, ALT and showed a increase of β -globulin and γ -globulin and a decrease of albumin (Agostoni, 1974; Kindmark 1976; Burtis, 1999; Lewandrowski, 2002). But our results shows that α_1 -globulin

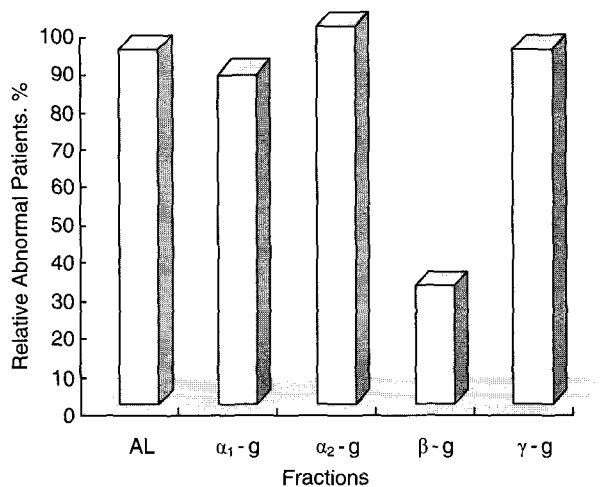


Fig. 4. Relative abnormal patients (%) to the leptospirosis patients increased both UN and creatinine. The percentages were expressed the relative patients showed lower level of albumin (AL), and showed higher level of α_1 -globulin (α_1 -g), α_2 -globulin (α_2 -g), β -globulin (β -g) and γ -globulin (γ -g) than reference range.

Table 2. Relative serum protein fractions (%) of total protein in leptospirosis patient

Fractions	Albumin	α_1 -globulin	α_2 -globulin	β -globulin	γ -globulin
Reference range	54.7 ± 5.6	3.9 ± 1.2	11.0 ± 2.9	13.7 ± 2.6	16.2 ± 3.5
A group Mean \pm SD	39.1 ± 8.7	6.4 ± 1.9	14.4 ± 2.0	15.4 ± 3.1	28.3 ± 6.4
B group Mean \pm SD	41.5 ± 5.7	6.3 ± 1.3	17.2 ± 2.8	13.9 ± 2.1	29.5 ± 5.3

A, leptospirosis patient showed elevation of both AST and ALT; **B**, leptospirosis patient showed elevation of UN and creatinine

and α_2 -globulin are also increased in patients showed high level of AST, ALT. These data indicate that even though infection of *Leptospiras* causes the liver damage, The serum protein electrophoretic patterns are not same as the patterns in hepatitis or liver cirrhosis patients. We also analyzed the relative concentrations of protein electrophoretic patterns in serum of leptospirosis patients with increase of both UN and creatinine. The leptospirosis patients in this group had the decrease in albumin and the increase in α_1 -globulin, α_2 -globulin and γ -globulin, having an average albumin, α_1 -globulin, α_2 -globulin and γ -globulin value of $41.5 \pm 5.7\%$, and $6.3 \pm 1.3\%$, $17.2 \pm 2.8\%$, and $29.5 \pm 5.3\%$, respectively (Table 2, B group). The relative patients showed the decrease of albumin, and the increase of α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin in leptospirosis patients with increase of both UN and creatinine are 93.8%, and 87.5%, 100%, 31.0%, and 93.8%, respectively (Fig. 4). Scientists reported that leptospirosis is a common cause of acute renal failure, which occurs in 16 to 40% of cases (Ramachandran et al., 1976; Winearls et al., 1984; Edwards et al., 1990; Abdulkader, 1997). Serum protein electrophoretic patterns were demonstrated that the α_2 -globulin region is noticeably increased, and albumin is markedly decreased (Burtis, 1999; Lewandrowski, 2002).

Only 10.2% of leptospirosis patients are increased both of UN and creatinine in our data indicate that leptospirosis is not common cause of acute renal failure. Our data indicate that the patients with damage of kidney by *Leptospiras* have the marked decrease of albumin and the increase of α_1 -globulin, α_2 -globulin and γ -globulin. We can conclude that the concentration of biochemical components and electrophoretic serum protein fractions in serum of leptospirosis patient are not identical with those of patients with hepatitis or renal failure.

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