

Analysis of Clinical Biochemical Components in Sera of Tsutsugamushi Disease Patients

Chong-Ho Kim^{1,†}, Seung-Taek Park² and Geum-Ga Oh³

¹Department of Clinical Pathology, Wonkwang Health Science College, Iksan 570-750, Korea.

²School of Medicine, Wonkwang University, Iksan 570-749, Korea.

³Department of Clinical Pathology, Namwon Medical Center, Namwon 590-702, Korea

The factors and mechanisms by infection of *Oriental Tsutsugamushi* caused disease are not well understood. The onset of tsutsugamushi disease is characterized by chilliness, fever, malaise, headache and generalized aching. Infection of tsutsugamushi is the cause of impairment of function of a major organ often complicate the picture and immediately change the prognosis for the worse. Tsutsugamushi disease is reported that this disease is characterized by the histopathogenesis of liver, kidney, heart, and lung, but the variation of biochemical components in serum of tsutsugamushi disease patient are not clear. We analyzed total protein (TP), albumin (AL), aspartic aminotranferase (AST), alanine aminotransferase (ALT), alkaline phosphotase (ALP), urea nitrogen (UN), creatinine (CRE), glucose (GLU), cholesterol (CHOL) and total bilirubin (TB) in sera of patients with tsutsugamushi disease. In comparison with reference, total protein and albumin were abnormally decreased in 19.6% and 39.2% of patients, respectively. AST, ALT, ALP, creatinine, UN, glucose, cholesterol and total bilirubin were abnormally increased in 94.1%, 72.5%, 25.5%, 15.7%, 9.8%, 62.7%, 25.5% and 6.0% of patients, respectively. The patients showed abnormal relative rate of protein electrophoretic fractions to total protein in serum compared to them of reference were 43.1% (albumin), 12.9% (α_1 -globulin), 58.8% (α_2 -globulin), 60.8% (β -globulin) and 70.6% (γ -globulin), respectively. These data suggest that infection of *Oriental Tsutsugamushi* causes impairment of function of a major organ and abnormal serum protein electrophoresis fractions to tsutsugamushi patients.

Key Words: Tsutsugamushi, Albumin, Aspartic aminotranferase (AST), Alanine aminotransferase (ALT), Urea nitrogen (UN), Creatinine, Glucose

INTRODUCTION

The etiologic agent of scrub typhus, tsutsugamushi disease was previously known as *Rickettsia tsutsugamushi* that is transmitted to humans by the mite vector, *Leptotrombidium deliense* (Gilbert et al., 1968; Traub et al., 1974; Iwasaki et al., 1991). The number of patients of tsutsugamushi disease in Korea reported for about 20 years since it was reported first in 1986 (Lee et al., 1986; Lee et al., 1994). The onset of tsutsugamushi disease is characterized

by chilliness, fever, malaise, headache and generalized aching (Caruana, 1974; Watt et al., 1994; Chi et al., 1997). A papule develops at the site of the mite bite which usually progresses to the formation of an ulcer, characteristically containing a black eschar. The eschar can occur anywhere on the body (Oaks et al., 1983; Kuroda et al., 1991; Seong et al., 2001). Investigators demonstrated that a rash, which is macular or maculopapular in type, involving the trunk and sometimes the face and the extremities, appears in most patients from the third to the sixth day of the disease. In untreated cases, the infection of *Rickettsia tsutsugamushi* leads to pneumonitis, meningitis, and disseminated multio-rgan vasculitis, which is caused by the destruction of endothelial cells and perivascular infiltration of leukocytes (Allen et al., 1945; Watt et al., 1994; Chi et al., 1997).

Complications were reported that the effects of the disease

*Received: October 22, 2007

Accepted after revision: November 26, 2007

†Corresponding author: Chong-Ho Kim, Department of Clinical Laboratory Science, Wonkwang Health Science College, Iksan 570-750, Korea.
Tel: 82-63-840-1213, Fax: 82-63-840-1219
e-mail: chkim@wkhc.ac.kr

were widespread throughout the body (Kim et al., 2000; Kim et al., 2006). Infection of tsutsugamushi was the cause of impairment of function of a major organ often complicated the picture and immediately changed the prognosis for the worse. Most of the patients suffering from scrub typhus developed myocarditis to some degree and showed cardiac enlargement. Some patients showed fluid retention characterized by oliguria and occasionally generalized edema and even ascites. Urinary findings of albuminuria and coarse granular casts were found. Occasional azotemia and even uremia occurred in some patients. Few patients examined at autopsy showed definite evidence of acute nephritis. Tenderness and enlargement, cloudy swelling and parenchymal degeneration of tissue of the liver were reported.

Laboratory findings in blood of this disease patient were reported that the activity of AST and ALT, creatinine and urea nitrogen were elevated, but albumin was decreased (Lee et al., 1994; Kim et al., 2000; Kim et al., 2001). Recently, scientists are interested in the mechanism of immune system, tests of rapid diagnosis and treatment. In this study, we compared the concentration of biochemical components and the protein electrophoresis fractions in sera of tsutsugamushi disease patients.

MATERIALS AND METHODS

Sera of tsutsugamushi patients (n=51) were obtained for analysis of the titer of antibody against tsutsugamushi protein by serological immunoassay, passive hemagglutination assay method using Genedia Tsutsu PHA 2 (Green Cross Co., Gyeonggi, Korea). The sheep red blood cells were coupled with the recombinant antigen as described by Hudson and Hay (1989) with modified method. The sensitized sheep red blood cells were suspended in a diluent buffer (PBS, pH 7.2, 0.5% rabbit sera, 0.002% Tween 20) and mixed with patient serum in a U-shaped 96-well micro-titer plate. Nonsensitized red cells, healthy human serum

and positive serum in each plate were used as negative control and positive control for the antigen, respectively. Specificity and sensitivity for the serodiagnosis of scrub typhus were calculated with 2×2 binary tables as described previously (Griner et al., 1981).

Total protein, albumin, AST, ALT, ALP, creatinine, UN, glucose, cholesterol and total bilirubin were determined by Hitachi automatic clinical analyzer 7600 (Hitachi High-Technologies Co., Tokyo, Japan). The concentration of chemical component that we analyzed was compared to reference range (Burtis et al., 1999). Sera isolated from patients with tsutsugamushi disease were carried out protein 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. Protein 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., 1999). All data were analyzed by SPSS software.

RESULTS AND DISCUSSION

We analyzed the each concentration of total protein, albumin, AST, ALT, ALP, creatinine, UN, glucose, cholesterol and total bilirubin in sera of patients with scrub typhus (Table 1 and 2). The data were compared to reference range (Burtis et al., 1999) of total protein (6.0~8.5 g/dL), albumin (3.5~5.2 g/dL), AST (10~30 U/L), ALT (10~40 U/L), ALP (53~128 U/L), creatinine (0.7~1.3 mg/dL), UN (6~20 mg/dL), glucose (74~106 mg/dL), cholesterol 130~230 mg/dL) and total bilirubin (0.3~1.2 mg/dL).

In comparison with the average of reference, the average of AST, ALT, ALP and glucose in sera of patients with scrub typhus were significantly increased by 324.5% ($P<0.001$),

Table 1. Chemical components in sera of tsutsugamushi patients (Mean ± SD)

Component	Total protein g/dL	Albumin g/dL	AST U/L	ALT U/L	ALP U/L
Reference	7.2±0.6	4.4±0.4	20±5.0	25±7.5	90±18.8
Patient (n=51)	6.5±0.7	3.6±0.5	84.9±58.0	70.5±51.8	123.9±71.9

Table 2. Chemical components in sera of Tsutsugamushi patients (Mean \pm SD)

Component	UN mg/dL	Creatinine mg/dL	Glucose mg/dL	Cholesterol mg/dL	Total bilirubin mg/dL
Reference	13.0 \pm 3.3	1.0 \pm 0.2	90 \pm 8.0	180 \pm 25.0	0.8 \pm 0.2
Patient (n=51)	15.7 \pm 7.3	0.9 \pm 0.3	114.1 \pm 20.1	135.4 \pm 31.5	0.8 \pm 0.4

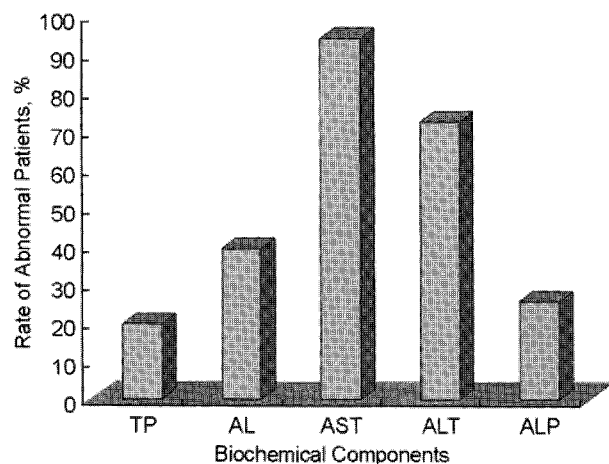


Fig. 1. The rate of patients showed abnormal level of biochemical components in sera of tsutsugamushi disease patients.

182.0% ($P<0.001$), 37.7% ($P<0.001$) and 26.8% ($P<0.001$), respectively. The average of total protein, albumin and cholesterol were significantly decreased by 9.7% ($P<0.001$), 18.2% ($P<0.001$) and 24.8% ($P<0.001$), respectively. UN, creatinine and total bilirubin were not significant in difference of average between patients and reference.

The frequencies of patient showed abnormal lower level of total protein or albumin than those of reference were in 19.6% and 39.2%, respectively. The patients showed abnormal higher concentration of AST, ALT, ALP, UN, creatinine, glucose, cholesterol and total bilirubin than those of reference were in 94.1%, 72.5%, 25.5%, 15.7%, 9.8%, 62.7%, 25.5% and 6.0% of patients, respectively (Fig. 1 and 2).

The hallmark of acute hepatocellular injury is elevation of AST and ALT level, typically more than eight times the upper reference limits, with no or minimal elevation of ALP. In children, only 1% have peak bilirubin greater than 10 mg/dL (Stewart, 1978), but in adults, jaundice develops in 20% to 70% of acute hepatocellular injury patients (Lednar, 1985; McMahon, 1985). In the common form induced hepatocellular injury by virus or some forms of drug, there is typically a gradual rise in cytoplasmic enzymes such as AST and ALT, followed after some days by an

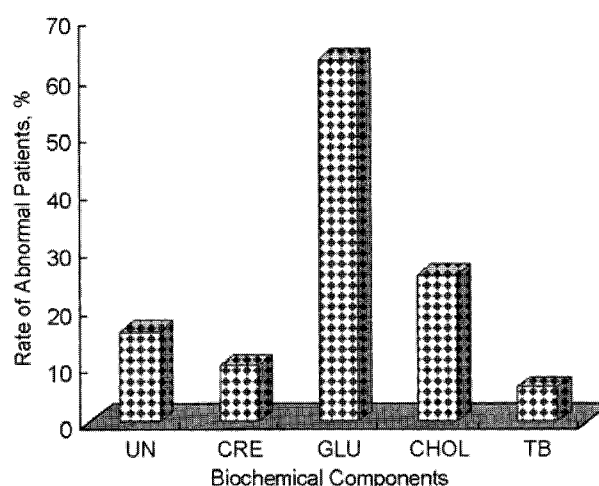


Fig. 2. The rate of patients showed abnormal level of biochemical components in sera of tsutsugamushi disease patients.

increase in bilirubin (Rozen, 1970). In this results, the significant elevation of AST, ALT and ALP suggests that the infection of *Rickettsia tsutsugamushi* is a common cause of acute hepatocellular injury.

Our results are similar to previous demonstrations that the elevation of AST, ALT and ALP were observed in Korean patients with tsutsugamushi disease (Lee et al., 1994; Kim et al., 2000; Kim et al., 2001; Kim et al., 2006). The level of total protein, albumin and cholesterol in plasma are depend on the function of liver because liver is the major site of synthesis for most plasma proteins and cholesterol except immunoglobulin. Therefore, the decreasing concentration of total protein, albumin and cholesterol in plasma is one of information to diagnose the liver disease (Fredrickson et al., 1967; Rothschild, 1972; Slater, 1975; Narayanan, 1979; Denke et al., 1993). We also observed the significant decrease of total protein, albumin and cholesterol. This tendency in our study was close to the previous demonstration (Kim et al., 2000; Kim et al., 2006). Several investigators demonstrated that the level of UN, creatinine and total bilirubin in a few patients showed a little increase, but did not show the significant increase (Lee et al., 1989; Kim et al., 2002).

Table 3. Relative serum protein electrophoresis fractions (%) to total protein in sera of patients with tsutsugamushi disease (Mean \pm SD)

Fractions	Albumin	α_1 -globulin	α_2 -globulin	β -globulin	γ -globulin
Reference	54.7 \pm 2.8	3.9 \pm 0.6	11.5 \pm 1.2	13.7 \pm 1.3	16.2 \pm 1.8
Patients	50.9 \pm 8.5	2.8 \pm 1.7	11.0 \pm 3.6	10.8 \pm 3.0	24.5 \pm 8.5

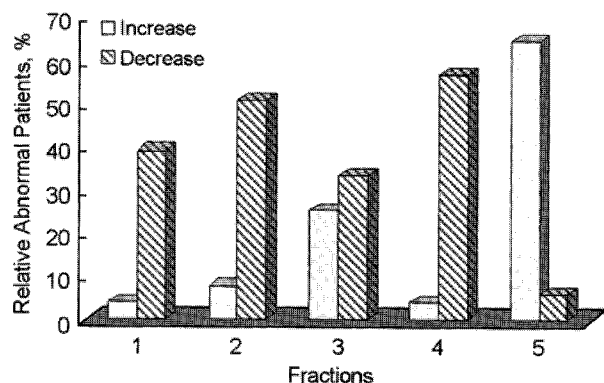


Fig. 3. The relative patients showed abnormal fraction of albumin (1), α_1 -globulin (2), α_2 -globulin (3), β -globulin (4) and γ -globulin (5) fractionated by cellulose acetate membrane electrophoresis method.

Our results are similar to the studies above and a previous study on the one of rickettsiosis, leptospirosis (Kim et al., 2004). 62.7% of patients with tsutsugamushi disease showed abnormal concentration of glucose suggests that the mechanism of inflammation of tsutsugamushi disease may be involved in the concentration of glucose in blood. We can't find any studies for interpretation of relationship between tsutsugamushi disease and the variation of electrophoretic serum protein fractions. So, we carried out electrophoresis of serum protein of patient with tsutsugamushi disease and analyzed the relative concentration of fractions to total protein (Table 3). The average of albumin ($P<0.03$), α_1 -globulin ($P<0.001$) and β -globulin ($P<0.001$) were increased and γ -globulin was decreased significantly than those of reference.

In comparison of patient's average to reference, albumin (6.9%), α_1 -globulin (28.2%), α_2 -globulin (4.3%) and β -globulin (21.2%) were decreased than the average of reference. The fractions of albumin, α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin were increased than the upper limit of reference value in 3.9%, 7.8%, 25.5%, 3.9% and 64.7% of the patients with tsutsugamushi disease, respectively. The decrease of albumin, α_1 -globulin, α_2 -globulin, β -globulin and γ -globulin than the lower limit of reference

showed in 39.2%, 51.0%, 33.3%, 56.9% and 5.9% of patients, respectively (Fig. 3). Electrophoresis of serum proteins is a commonly used clinical test. The patterns of fraction illustrate important pathological conditions. Some of investigators demonstrated that the patients with severe hepatitis showed low concentration of albumin, α_1 -globulin, α_2 -globulin, β -globulin but γ -globulin was high and in liver cirrhosis patients, albumin, α_1 -globulin and α_2 -globulin were decreased but β -globulin and γ -globulin were increased together (Agostoni, 1974; Kindmark, 1976; Burtis, 1999; Lewandrowski, 2002). In our results, high rate of patients showed a decrease of albumin, α_1 -globulin, α_2 -globulin, β -globulin and a increase of γ -globulin. This result suggests that the infection of *Rickettsia tsutsugamushi* is a cause of severe damage to liver.

Acknowledgements

This work was supported by grant from Wonkwang Health Science College (2007).

REFERENCES

- Agostoni A, Marasini B, Stabilini R, Del Ninno E, Pontello M. Multivariate Analysis of Serum Protein Assays in Chronic Hepatitis and Postnecrotic Cirrhosis. Clin Chem. 1974. 20: 428-231.
- Allen AC, Spitz S. A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial disease. Am J Pathol. 1945. 21: 603-681.
- Burtis CA, Ashwood ER. Tietz textbook of Clinical Chemistry. 1999. pp1831-1833. W.B. Saunders Company. Philadelphia, USA.
- Caruana S. Rickettsial diseases. Trop Dis Bull. 1974. 71: 781-786.
- Chi WC, Huang JJ, Sung JM, Lan RR, Ko WC, Chen FF. Scrub typhus associated with multiorgan failure: a case report. Scand J Infect Dis. 1997. 29: 634-635.
- Denke MA, Sempos CT, Grundy SM. Excess body weight: An underrecognized contributor to high blood cholesterol in

- white American men. *Arch Intern Med.* 1993. 153: 1093-1103.
- Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins-an integrated approach to mechanisms and disorder. *N Engl J Med.* 1967. 276: 34-42.
- Gilbert DN, Moore WL, Hedberg CL, Sanford JP. Potential medical problems in personnel returning from Vietnam. *Ann Intern Med.* 1968. 68: 662-678.
- Griner PF, Mayewski RJ, Mushlin AI, Greenland P. Selection and interpretation of diagnostic tests and procedures. *Ann Intern Med.* 1981. 94: 553-600.
- Hudson L, Hay FC. *Practical immunology*, 1989. pp 255-256. Blackwell Scientific Publications Ltd., Oxford, UK.
- Iwasaki H, Ueda T, Uchida M, Nakamura T, Takada N, Mahara F. Atypical lymphocytes with a multilobated nucleus from a patient with tsutsugamushi disease (scrub typhus) in Japan. *Am J Hematol.* 1991. 36: 150-151.
- Jeppsson JO, Laurell CB, Franzen B. Agarose Gel Electrophoresis. *Clin Chem.* 1979. 25: 629-631.
- Kim CH, Park ST, Oh GG. comparison of protein electrophoresis fraction in the leptospirosis patient serum. *J Exp Biomed Sci.* 2004. 10: 253-257.
- Kim DW, Lee KH, Lee SY, Min HG, Kim YJ, Kim YJ. A Case of Tsutsugamushi Disease with Atypical Symptom. *J Korean Acad Fam Med.* 2006. 27: 741-745.
- Kim HS, Jo KH, Kang SG, Cha MJ, Hwang HH. A clinical research of tsutsugamushi disease occurred in and around Jeoung-up in the autumn of 2000. *J Korean Acad Fam Med.* 2002. 23: 87-95.
- Kim IG, Lee SC, Kim JW, Seo KS, Park HB, Lee ST, Kim BC, Kim MK, Cho KH. Two cases of tsutsugamushi meningitis. *J Korean Neurol Assoc.* 2000. 18: 642-644.
- Kim KJ, Cho NS, Cho SH. Related Clinical Finding Result on Complication of Tsutsugamushi Patients. *J Korean Soc Emerg Med.* 2001. 12: 268-276.
- Kindmark CO. *Plasma Protein Pattern in Hepatitis A and B.* 1976. pp431-435. Pergamon Press. Oxford, UK.
- Kuroda T, Suzuki S, Konno M. A case of scrub typhus with disseminated intravascular coagulation, meningitis and pulmonary fibrosis. *Nippon Naika Gakkai Zasshi.* 1991. 80: 1816-1817.
- Lednar WM, Lemon SM, Kirkpatrick JW. Frequency of illness associated with epidemic hepatitis A virus infections in adults. *Am J Epidemiol.* 1985. 122: 226-233.
- Lee DG, Kim SH, Han BK, Lee KH, Hwang CH, Cho MK. The Clinical Study of 10 Cases of Tsutsugamushi Fever. *J Korean Pediatr Soc.* 1994. 37: 689-694.
- Lee JS, An YL, Kim YK, Lee MH. Thirteen cases of rickettsial infection including nine cases of Tsutsugamushi disease first confirmed in Korea. *J Korean Med Assoc.* 1986. 29: 430-438.
- Lee JS, Lee JH, Park JK, Kim SU. A clinical study of 40 patients with tsutsugamushi disease in Chungcheong province. *Korean J Derm.* 1989. 27: 272-282.
- Lewandrowski K. *Clinical Chemistry.* 2002. pp552-557. Lippincott Williams and Wilkins. Philadelphia, USA.
- McMahon BJ, Alward WL, Hall DB. Acute hepatitis B virus infection: Relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis.* 1985. 151: 599-603.
- Narayanan S. Lipoprotein-X. *CRC, Crit Rev Clin Lab Sci.* 1979. 11: 31-51.
- Oaks SC, Ridgway Jr RL, Shiral A, Twartz JC. Scrub typhus. *Bull Inst Med Res.* 1983. 21: 25-33.
- Rothschild MA, Oratz M, Schreiber SS. Albumin synthesis. *N Engl J Med.* 1972. 286: 748-753.
- Rozen P, Korn RJ, Zimmerman HJ. Computer analysis of live function tests and their interrelationship in 347 cases of vial hepatitis. *Isr J Med Sci.* 1970. 6: 67-79.
- Seong SY, Choi MS, Kim IS. *Orientia tsutsugamushi* infection: review and immune responses. *Microbes Infect.* 2001. 3: 11-21.
- Slater L, Carter PM, Hobbs JR. Measurement of albumin in the sera of patients. *Ann Clin Biochem.* 1975. 12: 33-41.
- Stewart JS, Farrow LJ, Clifford RE. A three-year survey of vial hepatitis in West London. *Q J Med.* 1978. 47: 365-384.
- Traub R, Wisseman CL. The ecology of chigger-borne rickettsiosis (scrub typhus). *J Med Entomol.* 1974. 11: 237-303.
- Watt G, Strickman D. Life-threatening scrub typhus in a traveler returning from Thailand. *Clin Infect Dis.* 1994. 18: 624-626.