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Analysis of Biochemical Markers in Sera of Patients with Febrile Disease During the Fall

Chong-Ho Kim[1,†], Seung-Taeck Park[2] and Young-Mi Seo[2]

[1]*Department of Clinical Laboratory Science, Wonkwang Health Science University, Iksan, Jeonbuk 570-750, Korea*
[2]*School of Medicine, Wonkwang University, Iksan, Jeonbuk 570-749, Korea*

We studied a comparison of the concentration of biochemical markers in sera of patients hospitalized with high fever (n=296) in Jeonbuk province during the last 2 years (2008 to 2009). The patients were divided into three patient groups of viral hemorrhagic fever (VHF) patient group tested positive for *Hantavirus* (n=53), leptospirosis (LEP) patient group tested positive for *Leptospira interrogans* (n=137) and scrub typhus (TSU) patient group tested positive for *Orientia tsutsugamushi* (n=106). We analyzed the concentration of ALP, AST, ALT, blood urea nitrogen, creatinine and glucose and compared the mean levels of them to normal range, the first sample and last sample. The frequencies of abnormal patient elevated above the upper limit of normal for ALP, AST and ALT were 18~43.4%, 78~97% and 62.3~92.7% in patient groups, and 24.5~47.4% (total protein) and 13.2~50.0% (albumin) of patients in patient groups had decreased below the lower limit of normal. The patients showed higher abnormal levels of glucose in patient groups were 58.5% (viral hemorrhagic fever patient group), 66.4% (leptospirosis patient group), 71.7% (scrub typhus patient group) and 66.9% (total patient group). There were significant difference between the first sample and the last sample in the mean levels of AST (decreased 22.2% in viral hemorrhagic fever patient group, 30.2% in leptospirosis patient group, 20.4% in scrub typhus patient group and 24.1% in total patient group), BUN (43.0% in viral hemorrhagic fever patient group, 41.6% in leptospirosis patient group, 47.4% in scrub typhus patient group and 43.0% in total patient group) and glucose (20.2% viral hemorrhagic fever patient group, 17.9% in leptospirosis patient group, 18.6% in scrub typhus patient group and 18.9% in total patient group) in the first sample and the last sample. According to these results, those diseases may cause liver damage and have high concentration of ALP, AST, ALT and glucose in blood even though the patients get out of the hospital.

Key Words: Biochemical markers, VHF, Scrub typhus, Leptospirosis

INTRODUCTION

The prevalent diseases among the acute febrile diseases during the fall in Korea are viral hemorrhagic fever (VHF), scrub typhus and leptospirosis (Song et al., 2002). The causative organism of VHF, leptospirosis and scrub typhus are *Hantavirus* (Schmaljohn and Hjelle, 1997), *Leptospira interrogans* (Levett, 2001) and *Orientia tsutsugamushi* (Mathai et al., 2001). VHF, leptospirosis and scrub typhus are well known that they are prevailed in Eastern and Southern Asia, Northern Australia, and on the islands of the Western Pacific region, including Taiwan (Munro-Faure et al., 1951; Kruger et al., 2001; Thammakumpee et al., 2005). VHF, leptospirosis and scrub typhus are common throughout in rural and in suburban areas in Korea, and is one of the most frequently reported infectious diseases during the months of October and November (Kim et al., 2004). The first patient with VHF, leptospirosis and scrub typhus in Korea were reported in 1951 (VHF and scrub typhus) and 1984 (leptospirosis), However, ever since these diseases were reported in Korea, the annual incidence of the diseases have increased steadily (Munro-Faure et al., 1951; Smadel,

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

1953; Song et al., 2002). The febrile diseases are characterized by fever, headache, pneumonitis, meningitis, renal failure and intravascular coagulation and leading to systemic multiorgan disfunction in untreated cases (Smadel, 1953; Ashford et al., 2000; Cracco et al., 2000). It is so difficult to make a correct diagnosis of VHF, leptospirosis and scrub typhus based on the clinical presentation and the history of a patient, because these febrile diseases show similar symptoms and signs. Physicians use the concentration of biochemical markers in blood for understanding the function of organs. The measurement of the biomarker concentration is carried for detecting, diagnosing, evaluating severity, monitoring therapy, and assessing the prognosis of the liver and kidney disease and dysfunction. The useful tests for diagnosis of liver function, kidney function and diabetes are the concentration of total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and glucose in serum (Gitlin et al., 1992; Dreisbach and Lertora, 2003; Pichette and Leblond, 2003; Engelgau, et al., 2000; Nathan et al., 2007). Even though the clinical symptoms, signs and diagnostic methods are studied well, the variations of the level of biochemical markers in serum of patients with the acute febrile diseases during the fall are not well demonstrated. Recent studies on these diseases have reported many serological and genetic methods, symptoms, complications and distribution of patients. It is necessary to determine the predictors that identify markers of severe febrile disease in order to reduce the mortality due to the high incidence of severe manifestations or complications and the delay in treatment. In this study, to identify biochemical markers in laboratory findings, we measured the concentration of the markers in serum of patients who were hospitalized from the VHF, leptospirosis and scrub typhus.

MATERIALS AND METHODS

Preparation of blood sample

This present study is a comparison of the concentrations of biochemical markers in sera of patients hospitalized with high fever (n=296) in Jeonbuk province during the last 2 years (2008 to 2009). The patients were divided into three patient groups of VHF patient group tested positive for *Hantavirus* (n=53), LEP patient group tested positive for *Leptospira interrogans* (n=137) and TSU patient group tested positive for *Orientia tsutsugamushi* (n=106) by serological immunoassay method as described previously (Griner et al., 1981; Linderholm and Elgh, 2001; Chappel et al., 2004). We collected blood two times from each fasting hospitalized patient. The first blood samples were obtained from patient right after entering a hospital and having diagnosed the disease. The last blood samples were collected from patients just before getting out of the hospital.

Analysis of biochemical marker

We isolated sera from bloods and analyzed the concentrations of biochemical markers in sera. The concentration of total protein, albumin, BUN, creatinine and glucose, and the activity of AST, ALT and ALP were determined by Hitachi automatic clinical analyzer (Hitachi High-Technologies Co., Tokyo, Japan).

Statistical analysis

The concentrations of biochemical markers in sera of the patient groups were compared to each other group and reference range. All data are expressed mean ± SD. Data analyzed by one-way ANOVA using the SPSS statistical software package, Version 8.0 (SPSS Inc., Chicago, IL, USA). A P value less than 0.05 was considered statistically significant.

RESULTS

Patients with abnormal biochemical marker

We studied the impact of the diseases of viral hemorrhagic fever (VHF), leptospirosis and scrub typhus on the concentration of biochemical markers in blood. We observed abnormal patients showed higher and/or lower level of biochemical markers in the first samples isolated from each patient right after entering a hospital than those of reference range (Fig. 1). Abnormal increase in levels of ALP, AST, ALT, blood urea nitrogen, creatinine and glucose was observed in 34.8%, 96.3%, 86.1%, 22.3%, 7.1% and

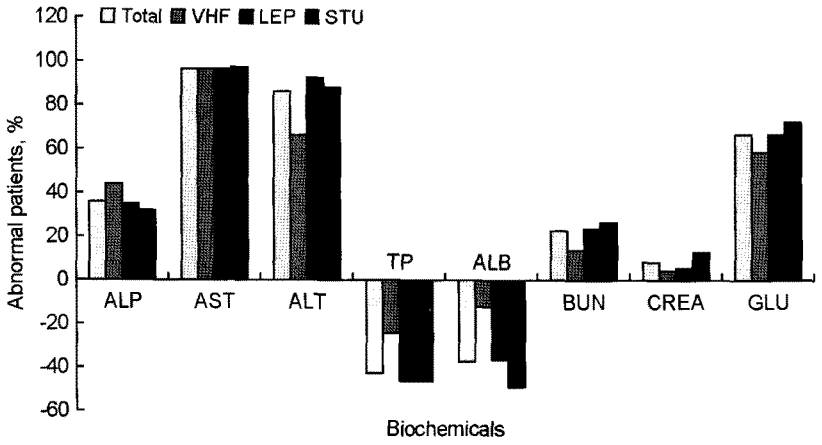

Fig. 1. The rate of abnormal patients in total patient group (Total), viral hemorrhagic fever patient group (VHF), leptospirosis patient group (LEP) and scrub typhus patient group (TSU). The data show higher (+ %) and/or lower (− %) concentration of biochemical markers in the first sample than those of reference range. The first samples were collected from the patient right at time of entering a hospital.

Table 1. The concentration of biochemical markers in sera of total patients, patients with vial hemorrhagic fever and reference

Biochemicals	Reference	Total			Vial hemorrhagic fever		
		First sample	Last sample	*P* value	First sample	Last sample	*P* value
ALP (U/L)	90±19	126.5±69.6	112.4±62.5	0.065	139.8±85.1	124.6±78.2	0.381
AST (U/L)	19±5.5	93.2±52.0	70.7±46.8	0.000	85.3±46.2	66.4±44.2	0.052
ALT (U/L)	25±7.5	73.9±46.6	69.4±55.9	0.377	68.7±41.3	64.5±44.3	0.617
TP (g/dL)	7.4±0.8	7.1±6.4	6.3±0.6	0.281	6.6±0.4	6.3±0.5	0.003
ALB (g/dL)	4.7±0.9	4.2±4.9	3.4±0.5	0.152	3.8±0.4	3.5±0.4	0.000
BUN (mg/dL)	13±3.5	16.5±6.8	9.4±3.7	0.000	14.9±4.4	8.7±3.1	0.000
CREA (mg/dL)	0.9±0.2	0.9±0.3	0.7±0.2	0.000	0.8±0.2	0.7±0.2	0.007
GLU (mg/dL)	90±8.0	119.3±31.3	96.8±24.7	0.000	121.4±36.6	96.9±23.4	0.003

*Data represents the means ± SD.

66.9% of the patients in total patient group, respectively and observed in 43.4%, 96.2%, 66.0%, 13.2%, 3.8% and 58.2% of patients in VHF patient group, respectively and observed in 34.3%, 95.6%, 92.7%, 23.4%, 4.4% and 66.4% In the LEP patient group. In the TSU patient group, 31.1% (ALP), 97.2% (AST), 87.7% (ALT), 25.5% (BUN), 12.3% (creatinine) and 71.7% (glucose) of patients were showed abnormal increase in levels, respectively. Abnormal decrease in levels of total protein and albumin were observed in 43.2% and 37.5% of patients in total patient group, 24.5% and 13.2% of patients in VHF patient group, 47.4% and 37.2% of patients in LEP patient group and 47.2% and 50.0% of patients in TSU patient group, respectively.

Statistical comparison of patient groups

We analyzed the concentration of biochemical markers in sera of patients in each group (Table 1 and Table 2). The average concentration of biochemical markers in the first samples in each patient group were compared with those in the last samples, and then we calculated the relative decrease rate (%) of the average concentration of ALP, AST, ALT, total protein, albumin, blood urea nitrogen, creatinine and glucose in the last sample groups to those in the first sample groups (Fig. 2). The average concentration of total protein, albumin, blood urea nitrogen, creatinine and glucose in the last samples of patients with viral hemorrhagic fever were significantly lower than those in the first samples of patients by 4.5% ($P<0.003$), 7.9% ($P<0.001$), 41.6% ($P<0.001$), 12.5% ($P<0.007$) and 20.2% ($P<0.003$), respectively. In the patient group with leptospirosis, the average concentrations of AST, BUN and glucose in the second samples of patients were significantly decreased by 30.2% ($P<0.008$), 39.1% ($P<0.001$) and 17.9% ($P<0.003$), respectively. The average concentrations of BUN, creatinine and glucose in sera of patients with scrub typhus were significantly decreased by 47.4% ($P<0.001$), 22.2% ($P<0.001$) and 18.6% ($P<0.001$), respectively. In total patient group, AST, BUN, creatinine and glucose were

Table 2. The concentration of biochemical markers in sera of patients with leptospirosis, scrub typhus and reference

Biochemicals	Reference	Leptospirosis			Scrub typhus		
		First sample	Last sample	*P* value	First sample	Last sample	*P* value
ALP (U/L)	90±19	112.4±47.9	105.8±60.0	0.476	128.5±72.1	108.6±50.5	0.129
AST (U/L)	19±5.5	94.9±53.1	66.2±41.8	0.008	97.8±56.1	77.8±53.0	0.052
ALT (U/L)	25±7.5	67.6±37.0	62.3±39.1	0.457	83.5±56.8	79.3±73.8	0.679
TP (g/dL)	7.4±0.8	6.4±0.6	6.3±0.7	0.581	8.0±10.4	6.3±0.6	0.361
ALB (g/dL)	4.7±0.9	3.7±0.6	3.5±0.5	0.106	4.9±8.1	3.2±0.5	0.250
BUN (mg/dL)	13±3.5	16.9±6.2	10.3±4.6	0.000	17.3±8.5	9.1±3.2	0.000
CREA (mg/dL)	0.9±0.2	0.9±0.2	0.8±0.2	0.056	0.9±0.3	0.7±0.2	0.001
GLU (mg/dL)	90±8.0	118.5±33.9	97.3±25.4	0.003	118.3±24.9	96.3±25.9	0.000

*Data represents the means ± SD.

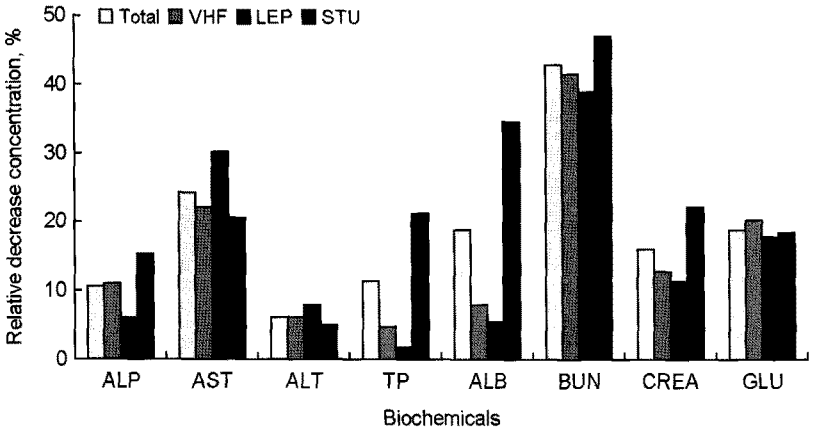

Fig. 2. The relative decrease concentration of biochemical markers in sera of patient groups. We compared the concentration of biochemical markers in the first sample to those in the last sample. The first samples were collected right after entering a hospital and the last samples were collected just before getting out of the hospital.

significantly decreased by 24.1% (P<0.001), 43.0% (P< 0.001), 15.9% (P<0.001) and 18.9% (P<0.001), respectively.

DISCUSSION

The liver function test is a routinely ordered clinical investigation used to assess hepatic function, disease severity (Sheehan and Haythorn, 1979; Lazo et al., 2008), and to evaluate the state of disease by measuring the levels of biomarkers of proteins in the blood such as ALP, AST, ALT, total protein and albumin. In the clinic, the results of these proteins are compared and interpreted according to reference charts containing internationally accepted normal-levels for these proteins. Elevation of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) indicates hepatic impairment. Elevated liver function test results may indicate a number of chronic and acute diseases and infections including liver cirrhosis, mononucleosis, hepatitis and heart disease (Williams and Hoofnagle, 1988; Memon et al., 2002). We estimated the frequencies of abnormal patient showed a abnormal level compare to those of reference range (Burtis and Ashwood, 2006) 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), BUN (6~20 mg/dL) and glucose (74~106 mg/dL). The frequencies of abnormal patient elevated above the upper limit of normal for ALP, AST and ALT were 18~43.4%, 78~97% and 62.3~92.7% in patient groups, and 24.5~47.4% (total protein) and 13.2 ~50.0% (albumin) of patients in patient groups decreased below the lower limit of normal. These data suggest that viral hemorrhagic fever (VHF), leptospirosis and scrub typhus disease may cause liver damage. Laboratory findings of blood urea and creatinine are useful to understand the kidney disease (Levey et al., 1999; Han et al., 2008). Physicians diagnose the kidney disease if the concentration of BUN and creatinine are elevated above the upper limit

of normal. Several investigators demonstrated that the levels of BUN and creatinine in a few patients showed a little increase, but did not show the significant difference between patient and normal (Lee et al., 1989; Kim et al., 2002). Our result of creatinine is similar to the studies above and a previous study on the one of rickettsiosis, leptospirosis (Kim et al., 2004), but the concentration of blood urea nitrogen showed the significant difference between patient and normal in total patient group ($P<$ 0.001), viral hemorrhagic fever patient group ($P<0.05$), leptospirosis patient group ($P<0.003$) and scrub typhus patient group ($P<0.007$). These data are identical with the report of abnormal increase in blood of leptospirosis patient (Edwards et al., 1990; Abdulkader, 1997). The fasting plasma glucose test is one of useful tests in diagnosis of diabetes. This test measures blood glucose levels after fasting. The normal fasting blood glucose level is 74~106 mg/dL. A fasting blood glucose level between 100 and 125 mg/dL signals pre-diabetes and A person with a fasting blood glucose level of 126 mg/dL or higher has diabetes (Graber et al., 2002; Sacks et al., 2002). In this study, the patients showed abnormal high glucose levels in patient groups were 58.5% (viral hemorrhagic fever patient group), 66.4% (leptospirosis patient group), 71.7% (scrub typhus patient group) and 66.9% (total patient group). These data suggest that the mechanism of inflammation of these diseases may be involved in the concentration of glucose in blood. In our studies, there were significant difference between the first sample and the last sample in the mean levels of AST (decreased 22.2% in viral hemorrhagic fever patient group, 30.2% in leptospirosis patient group, 20.4% in scrub typhus patient group and 24.1% in total patient group), BUN (43.0% in viral hemorrhagic fever patient group, 41.6% in leptospirosis patient group, 47.4% in scrub typhus patient group and 43.0% in total patient group) and glucose (20.2% viral hemorrhagic fever patient group, 17.9% in leptospirosis patient group, 18.6% in scrub typhus patient group and 18.9% in total patient group) in the first sample and the last sample. But our data show that the concentration of ALP, AST, ALT and glucose are not reduced to normal levels of those concentrations. These data suggest that many of patients have high concentration of ALP, AST, ALT and glucose in blood even though the patients with febrile disease of viral hemorrhagic fever, leptospirosis and scrub typhus don't have any symptoms of illness when they get out of the hospital. Also, we can suggest that it is possible to have high concentration of those chemicals in blood of patients before the development of those diseases in patient. In conclusion, our study was performed to investigate the variation of concentration of biochemical markers in blood of patients with vial hemorrhagic fever, leptospirosis and scrub typhus. We analyzed the concentration of ALP, AST, ALT, blood urea nitrogen, creatinine and glucose and compared the mean levels of them to normal range, the first sample and last sample. Our data suggest that viral hemorrhagic fever (VHF), leptospirosis and scrub typhus disease may cause liver damage and that many of patients have high concentration of ALP, AST, ALT and glucose in blood even though the patients don't have any symptoms of illness when they get out of the hospital. It is possible to have high concentration of those chemicals in blood of the patients before the development of those diseases.

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