

Full Length Research Paper

Predictive value of serum gelsolin in hepatitis B virus (HBV)-related chronic liver disease

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Gelsolin, an actin-binding protein, which serves as a substrate of caspase in tissue injury has been proposed as a prognostic marker in acute liver injury, but its relationship with human hepatitis B virus (HBV)-related hepatitis at various stages is still unclear. This study was conducted in order to investigate the predictive value of serum gelsolin in HBV-related chronic liver disease. The observation group included 101 patients with HBV-related chronic liver disease and 96 healthy adults were selected as the control group. The enzyme linked immunosorbent assay (ELISA) method was used to detect the gelsolin level in the serum. The concentration of serum gelsolin level of the observation group (54.099 ± 23.688 $\mu\text{g/ml}$) was much lower ($P = 0.000$) than that of the control group (186.372 ± 37.549 $\mu\text{g/ml}$). The concentration of serum gelsolin in chronic HBV hepatitis patients (64.158 ± 21.389 $\mu\text{g/ml}$), liver cirrhosis patients (50.067 ± 21.658 $\mu\text{g/ml}$) and acute-on-chronic liver failure patients (37.012 ± 21.231 $\mu\text{g/ml}$) was considerably different ($P = 0.013$) and decreased as the severity of the pathogenetic condition increased. Serum gelsolin levels of patients with HBV-related chronic liver disease had negative correlations with model for end-stage liver disease (MELD) scores ($r = -0.348$, $P = 0.001$) and Child-Pugh scores ($r = -0.487$, $P = 0.001$). The serum gelsolin level may be a potential marker of the severity of hepatic injury.

Key words: Gelsolin, hepatitis B virus (HBV)-related chronic liver disease, predictive value.

INTRODUCTION

Gelsolin is an actin-binding protein which was identified for the first time in rabbit lung macrophages in 1979. As a calcium ions (Ca^{2+}) dependent actin filament severing and capping protein, gelsolin is a member of the gelsolin superfamily (Young et al., 1994).

Studies have shown that gelsolin plays an important role in cell movement, apoptotic regulation, tumorigenesis regulation and organism anti-damage and is a prognostic marker for injury (Hui et al., 1999; Marrocco et al., 2010).

Actively searching for non-invasive serological markers that reflect the severity of liver injury in hepatic diseases has great significance when making an appropriate prognosis and selecting the correct treatment. Few studies have shown that the concentration of gelsolin is significantly reduced in patients suffering from acute liver injury and acute liver failure (Suhler et al., 1997; Ito et al., 1992). This suggests that gelsolin may be a potential marker of the degree of liver injury. At present, the gelsolin level in the serum of chronic liver disease patients arising because of various reasons has not been reported. Hepatitis B virus (HBV) infection is the most common cause of liver injury in Asia. Therefore, it is of clinical significance to explore the gelsolin level in the serum of patients with HBV-induced chronic hepatitis, hepatic cirrhosis and acute-on-chronic liver failure, and

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discuss the relation between the difference in gelsolin levels and the severity of liver diseases. This study made a preliminary approach in this regard.

MATERIALS AND METHODS

Study population

The observation group included 101 patients with the HBV-related chronic liver disease who were hospitalized at the Artificial Liver Center, Beijing You'an Hospital, Capital Medical University from June 2006 to April 2011. This study excluded patients with the history of recent myocardial infarction, myonecrosis, cancer, lung injury, septicemia, trauma, burns, hepatitis A, C, D, E virus infection, HIV infection, autoimmune, drug-induced or other causes of chronic liver disease, and other severe diseases and complications. This study was approved by the Ethics Committee of Beijing You'an Hospital of the Capital Medical University. All patients signed an informed consent form.

Patients of the observation group were divided into three groups according to the diagnostic criteria, Viral Hepatitis Diagnosis, Prevention and Cure Standard proposed at the 10th China National Viral Hepatitis and Hepatopathy Academic Conference held in Xi'an in 2000 (Chinese Society of Infectious Diseases and Parasitology and Chinese Society of Hepatology of Chinese Medical Association, 2000). The diagnosis of acute-on-chronic liver failure was according to the consensus of the Asian Pacific Association for the study of the liver (APASL) for acute-on-chronic liver failure in 2008 (Sarin et al., 2009).

The control group had 96 healthy volunteers from students of the Capital Medical University, and medical care personnel of the Artificial Liver Center, Beijing You'an Hospital of the Capital Medical University. The control group had 73 males and 23 females, aged between 19 and 50, with an average age of 27. The hepatitis markers A, B, C, D and E were negative in every case of the control group and the liver function was normal.

Preparation of the serum

All blood specimens were collected on an empty stomach and placed at room temperature for 20 min, then centrifuged for 20 min at 1,000 xg within an hour. Then, the supernatant was stored in a refrigerator at -80°C for testing.

Gelsolin detection

To detect the level of gelsolin in the serum of both the observation and control groups, the enzyme-linked immuno-sorbent assay (ELISA) method was adopted. The gelsolin ELISA Kit is a product of Beijing Cotimes Biotech Company Ltd.

Predictive value of serum gelsolin

The difference of gelsolin levels in the serum between patients with chronic HBV-related liver diseases and the healthy controls was compared. The MELD and Child-Pugh scores of the patients were calculated and their relationships with serum gelsolin were also determined. In addition, the concentrations of serum gelsolin between chronic HBV hepatitis patients, hepatic cirrhosis patients, hepatic cirrhosis patients together with hepatocellular carcinoma, and acute-on-chronic liver failure patients were compared.

Statistical analysis

Statistics software SPSS13.0 was used to carry out statistical

analysis of the data. The results were shown as $\bar{x} \pm s$. The Mann-Whitney test was used to compare the gelsolin level in the serum of the control group with the observation group. The Kruskal-Wallis test was used to compare the gelsolin level in the serum of different groups. The Spearman's Rank correlation was used to analyze the gelsolin level in the serum of patients with HBV-related chronic liver diseases in relation to the results of the Child-Pugh and MELD scores. The Mann-Whitney test was used to compare the level of the serum gelsolin of the surviving patients with the deceased and OLT patients within the acute-on-chronic liver failure patients. All $P < 0.05$ were considered as statistically significant.

RESULTS

Ninety six (96) serum specimens from the control group and 101 serum specimens of the observation group were collected to analyze the concentration of gelsolin in the serum. The patients' general information is shown in Table 1. The intergroup difference of age and gender was analyzed. There was no statistical intergroup difference. The concentration of serum gelsolin level of the observation group was much lower than that of the control group (Table 2). The concentration of serum gelsolin in chronic HBV hepatitis patients, liver cirrhosis patients and acute-on-chronic liver failure patients was considerably different and decreased as the severity of the pathogenetic condition increased (Table 3).

According to the clinical data and relevant laboratory index of the patients, the Child-Pugh and MELD scores were calculated. The Spearman's rank correlation showed that the gelsolin level in the serum of patients with HBV-related chronic liver disease had a negative relationship with Child-Pugh scores $r = -0.487$, $P = 0.001$ as well as with the MELD scores $r = -0.348$, $P = 0.001$ (Figures 1 and 2).

We also selected 21 acute-on-chronic liver failure patients, including 8 surviving patients, 9 who had received a liver transplant, and 4 deceased. The concentration of gelsolin in the serum of the survivors was 82.781 $\mu\text{g}/\text{m}$ while it was 30.725 $\mu\text{g}/\text{ml}$ in the deceased and orthotopic liver transplantation (OLT) group. The survivors had a significantly higher level of gelsolin than the deceased and OLT group with a significance of $P = 0.001$.

DISCUSSION

In this study, we tested the gelsolin levels in the serum of the control group and the observation group, and found that the serum gelsolin level of the observation group was lower than that of the control group. This result agrees with a previous report that demonstrated that the serum gelsolin levels of acute hepatic injury patients and acute liver failure patients declined (Ito et al., 1992). Gelsolin plays an important role in cell mobility, signal transduction of the cytoskeletal structure dynamics rearrangement process, apoptotic regulation, tumorigenesis modulation, organism anti-damage and is a prognostic marker for

Table 1. General information of patients with the HBV-related chronic liver disease.

Parameter	Diseases of the observation group			Total (n=101)
	Chronic HBV hepatitis (n=45)	Hepatic cirrhosis (n=35)	Acute-on-chronic liver failure (n=21)	
Gender				
Male (n)	36	30	16	82
Female (n)	9	5	5	19
Age	36.489±10.241	52.771±10.801	42.952±10.201	43.475±12.584
ALT (U/L)	259.949±350.981	103.249±159.181	373.871±500.657	229.334±351.171
AST (U/L)	148.972±182.714	99.112±123.504	357.886±580.065	260.633±455.502
TBil (µmol/L)	43.842±84.896	70.871±109.035	380.586±169.155	123.225±174.379
ALB(g/L)	40.540±5.116	30.534±7.547	30.590±4.498	35.004±7.712
CR (µmol/L)	72.111±14.537	76.923±36.395	92.229±44.293	77.961±31.409
INR	1.070±0.186	1.297±0.242	2.622±0.810	1.352±0.612

ALT: Alanine transaminase; AST: Aspartate aminotransferase; TBil: Total bilirubin; ALB: Albumin; CR: Creatinine; INR: International Normalized Ratio of plasmozyme.

Table 2. Comparison of serum gelsolin levels between the observation group and the control group (µg/ml).

Group	Observation group	Control group
Patient (n)	101	96
Mean ± standard deviation	54.099±23.688	186.372±37.549
Median	52.479	187.299
Minimum value	7.056	107.576
Maximum value	131.609	352.000

Comparison of serum gelsolin levels of the control group and patients with HBV-related chronic liver disease. The Mann-Whitney test was used to compare the two groups; U = 12.043, P = 0.000.

Table 3. Comparison of serum gelsolin levels of different HBV-related chronic liver diseases of the observation group (µg/ml).

Group	Chronic HBV hepatitis	Hepatic cirrhosis	Acute-on-chronic liver failure
Patient (n)	45	35	21
Mean ± standard deviation	64.158±21.389	50.067±21.658	37.012±21.231
Median	62.862	47.797	32.236
Minimum value	29.499	12.483	7.056
Maximum value	131.609	50.067	92.301

Comparison of serum gelsolin levels of different patients with HBV-related chronic liver disease. The Kruskal-Wallis test was used to compare the level of serum gelsolin of three groups. The results H = 8.745, P = 0.013 indicate a statistical significance.

injury (Mcgough et al., 2003; Hui et al., 1999; Marrocco et al., 2010). As the transcriptional start sites are different, two kinds of gelsolin, cytoplasm gelsolin and plasma gelsolin exist in vertebrates at the same time. Cytoplasm gelsolin is a kind of actin-binding protein, and can polymerize, depolymerize and shear actin filaments (Silacci et al., 2004; Liepina et al., 2003). Also, gelsolin is

one of the critical proteins in the process of apoptosis (Philchenkov 2003). Caspases-3 can break cytoplasm gelsolin at Asp352 and Gly353, and thus make gelsolin lose the cutting activity regulated by calcium and the ability to bind single actin, resulting in a change of cellular morphology and the enhancement of apoptosis (Koya et al., 2000). Cellular over-expression can inhibit the release

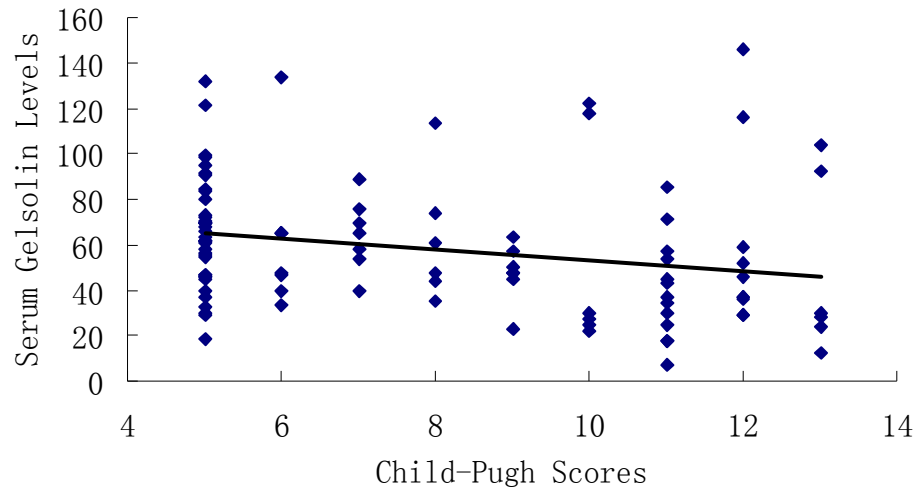


Figure 1. Analysis of the relationship between serum gelsolin levels of patients with HBV-related chronic liver disease and Child-Pugh scores. The Spearman's rank correlation indicates that the serum gelsolin levels of patients with the HBV-related chronic liver disease have a negative relationship with Child-Pugh scores ($r = -0.487$, $P = 0.001$).

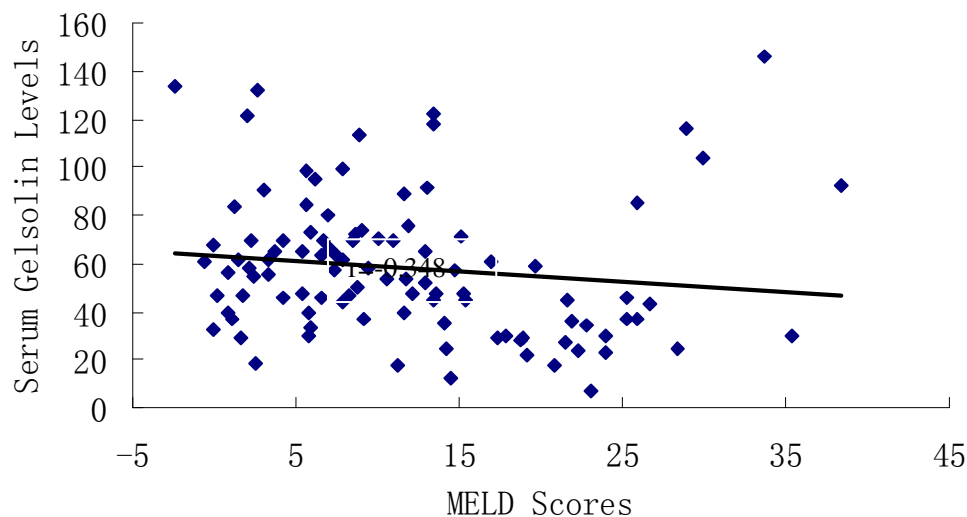


Figure 2. Analysis of relationship between serum gelsolin levels of patients with HBV-related chronic liver disease and MELD scores. The Spearman's rank correlation indicates that the serum gelsolin concentration of patients with HBV-related chronic liver disease have a negative relation with MELD scores ($r = -0.348$, $P = 0.001$).

of mitochondrial cytochrome C and restrain apoptosis by blocking the upstream pathway of CPP32 protein kinase (Ohtsu et al., 1997). Gelsolin is over-expressed in many cancers and plays an important role in cellular proliferation and migration. It is associated with tumor metastasis and may be a promising target for therapy and a potential biomarker for predicting the prognosis of cancer (Liao et al., 2011; Shao et al., 2011). Plasma gelsolin is mainly synthesized in skeletal and smooth muscles and the myocardium. Plasma gelsolin is also found in blood, lymph, bronchial epithelia, synovial fluids

and cerebro-spinal fluid. Plasma gelsolin is a secretion type protein existing in the plasma. Unlike cytoplasm gelsolin, plasma gelsolin has the Ca^{2+} insensitive actin microfilament breaking structural domain at the N-terminal and the Ca^{2+} sensitive actin microfilament binding structural domain at the C-terminal. This makes it possible for plasma gelsolin to clear the actin in the circulatory system, and maintain a stable internal environment (Bucki et al., 2008b). Actin is an abundant protein in mammalian cells (Janmey and Lind, 1987). In case of cell death or tissue injury, actin can be released

into the extracellular space and blood. Once an actin microfilament becomes 10 μm long in the blood, it can change blood characteristics or even block small vessels. In addition, actin can activate platelets and promote the formation of blood clots through direct interaction with *fibræ sanguis* (Calaminus et al., 2008), and has a major negative effect on endothelial cells and blood capillaries in the lungs (Cerecedo et al., 2006). Therefore, it is very important to clear actin in the blood. Research has demonstrated that plasma gelsolin levels of surgical patients in crisis as well as patients with severe external injury significantly decreased, and prognosis can be greatly improved after supplementing gelsolin (Lee et al., 2006; Rothenbach et al., 2004). Decreased levels of the gelsolin plasma isoform were also observed in patients with sepsis, myocardial infarction, liver failure, acute respiratory distress syndrome, inflammations and after burns (Ni and Zhao, 2007; Sadzyński et al., 2010). The degree of plasma gelsolin depletion could reflect the degree of tissue injury that may lead to significant exposure of actin to the extracellular space (Lee et al., 2008).

There are some studies on HBV-associated liver disease. The study of Marrocco et al. (2010) indicated that plasma gelsolin may be a candidate biomarker for hepatitis B-associated liver cirrhosis. But the aim of our study was to estimate the value of gelsolin in HBV-related chronic liver disease, including chronic HBV hepatitis, hepatic cirrhosis and acute-on-chronic liver failure. For patients with HBV-related chronic liver disease, as a result of hepatic tissue damage and other multiple tissue and organ damage caused by severe hepatopathy, a large number of actins may be released, which consumes gelsolin in the plasma. Therefore, the serum levels of gelsolin in these patients are significantly lower than in healthy people. The subjects of this study include chronic HBV hepatitis, hepatic cirrhosis and acute-on-chronic liver failure patients. We compared the serum gelsolin levels of these three groups of patients and found that the more severe the condition of the patient, the lower the serum gelsolin level. This indicates that the serum gelsolin level can reflect the severity of liver damage, and may be a potential marker to evaluate the severity of liver disease.

The pathogenesis of HBV-related chronic liver disease is extremely complicated, and has a broad impact on all organs of the body. Until now, the nature of hepatopathy has not been sufficiently studied. Developing a reliable marker to efficiently evaluate the level of severity of the patients' condition and prognosis will be helpful to establish a suitable therapeutic schedule for patients. At present, the prognosis models include the Child-Pugh Classification, Yale Model, Europe Model, Mayo Model, Model for End-stage Liver Disease (MELD) and Time-dependent Cox Regression Model. This study selected the Child-Pugh Classification and MELD models, which are well recognized and commonly used as prognostic

models in hepatopathy. Statistical analysis shows that serum gelsolin has some relation with these two prognostic models, which indicates that serum gelsolin can be used to evaluate the severity of liver disease, and be a potential prognostic tool. However, serum gelsolin is influenced by other damaged organs and may influence the evaluation of the severity of liver damage and the patient's prognosis. To avoid this interference, patients suffering from myocardial infarction, myonecrosis, cancer, lung injury, septicemia, trauma, burns, hepatitis A, C, D, E virus infection, HIV infection, autoimmune, drug-induced or other causes of chronic liver disease, and other severe diseases and complications should be excluded.

In this study, we also compared the gelsolin levels of acute-on-chronic liver failure patients with different prognoses, and found that the level of serum gelsolin of the survivors was higher than that of the deceased and OLT cases. This indicates that the level of serum gelsolin can be used to make an appropriate prognosis in acute-on-chronic liver failure patients, and can be chosen as one of the indicators for evaluating the need for liver transplants.

Until now, various functions of plasma gelsolin have not been clarified. Hepatic injuries have complicated pathogenetic conditions. In various infections and autoimmune diseases, gelsolin in the serum declines which may not simply result from its binding action to actin. The latest research shows that gelsolin can bind to lipopolysaccharides (LPS) generated by a variety of Gram-negative bacteria, and lipoteichoic acid (LTA) of Gram-positive bacteria, as well as bacterial lipids of Gram-negative or positive bacteria in a highly affinitive way and participates in the identification of LPS or LTA released by the outer bacterial membrane during the process of host immunity splitting of bacteria or drugs and immunity composition attack of bacteria (Bucki et al., 2008a).

This indicates that gelsolin may also play a role in the process of anti-inflammatory and immunological reaction. Further intensive study of the gelsolin level will help to understand the pathological and physiological mechanisms in hepatopathy, provide new clinical treatment, and offer new methods to evaluate the prognosis of patients with hepatic diseases.

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