Original Article
Peripheral sH2a in diagnosis and evaluation of fibrosis condition in liver cirrhosis

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Abstract: Asialo-glycoprotein soluble form of H2a (sH2a) is a liver specific protein, and it is presents in abnormal levels in the peripheral blood of liver disease patients. This study investigated if peripheral sH2a levels can be used for diagnosis and disease evaluation of liver cirrhosis. Enzyme-linked Immune Sorbent Assay (ELISA) measured sH2a content in the peripheral blood from liver cirrhosis patients and control people, along with serum albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Peripheral sH2a content was compared between liver cirrhosis patients at stage 1~2 and stage 3~5. Fibrosis parameters including hyaluronic acid (HA), laminin (LN), type IV collagen (IV-C) and type III collagen (PC III) were measured. The correlation between sH2a and HA, LN, IV-C and PC III was analyzed by Spearman method. Receiver operating characteristic (ROC) calculated area under curve (AUC) to evaluate sH2a in liver cirrhosis diagnosis. Compared to the control group, liver cirrhosis patients had significantly elevated serum ALT, AST, TBIL and DBIL, plus lower ALB and sH2a levels. Stage 3~5 liver cirrhosis patients had remarkably higher serum HA, PC III, IV-C and LN than stage 1~2 patients, whilst sH2a content was decreased to 67.2% (P<0.05). Significantly negative correlation existed between serum sH2a and HA, IV-C or LN (P<0.05) but not with PC III in liver cirrhosis patients. AUC of peripheral sH2a in liver cirrhosis diagnosis was 0.816 (95% CI: 0.782~0.850), suggesting moderate diagnostic value. Liver cirrhosis patients had abnormally decreased serum sH2a, which was correlated with clinical stages. sH2a is correlated with fibrosis, and has moderate diagnostic values. sH2a may have clinical implication in auxiliary diagnosis and disease evaluation in liver cirrhosis.

Keywords: Liver cirrhosis, liver fibrosis, diagnosis, ROC, sH2a

Introduction

Liver cirrhosis is a commonly occurring chronic digestive disease found in clinics. It is caused by repeated stimuli of virus (HBV or HCV) or ethanol to induce widely distributed hepatocyte denaturing or necrosis, which progresses into diffused liver fibrosis lesions, forming regenerative lesions and pseudo-lobule, eventually destructing normal hepatic lobular structure and further liver cirrhosis [1-3]. When liver fibrosis progresses into decomposition stage, multiorgan failure or dysfunction may occur [4-6]. Liver fibrosis is the pathological basis for multiple diseases including liver cirrhosis and liver cancer, and is a necessary step in these chronic liver diseases. Necessary measures are required to identify fibrotic changes, to remove factors causing liver fibrosis, and to correct further progression of fibrosis, and all of these

have critical implications for impeding liver disease progression and improving patient prognosis [7-10]. Therefore, dynamic monitoring and early diagnosis of liver fibrosis is of critical importance in clinics. Currently liver biopsy is still the gold standard for liver fibrosis diagnosis, but it is not widely applied due to major trauma for patients, and is not beneficial for early screening and diagnosis of fibrosis [11-13].

Human asialo-glycoprotein receptor (ASGPR) is a protein for clearing asialo-glycoprotein from plasma, and is specifically synthesized and expressed in hepatocytes. Asialo-glycoprotein soluble form of H2a (sH2a) is a soluble fragment of human ASGPR. Within the endoplasmic reticulum of hepatocytes, ASGPR is cleaved by 5 amino acids to be transformed into sH2a, which is subsequently released into the blood

circulation. Previous studies have shown abnormal changes of sH2a in the peripheral blood of liver disease patients [14, 15]. As sH2a has liver specificity, and can be detected within peripheral blood circulation, it may be used for auxiliary diagnosis of liver disease as a means of a non-invasive approach. This study compared peripheral sH2a levels in liver cirrhosis patients at different clinical stages, and analyzed the correlation with liver fibrosis related parameters of patients, in order to investigate the value of peripheral sH2a content in auxiliary diagnosis for liver cirrhosis.

Materials and methods

Clinical information

A total of 38 male and 18 female liver cirrhosis patients (42.5±11.8 years) were recruited from The Second People's Hospital affiliated to Luzhou Medical College in Neijiang city after liver biopsy and biochemistry examination. Among those patients there were 36 HBV induced liver cirrhosis patients, 12 alcoholic liver cirrhosis patients, plus 8 lipid liver cirrhosis patients. Clinical staging showed 24 patients at stage 1~2, and 32 patients at stage 3~5. Patient selection or inclusion criteria were defined according to the American Association for the Study of Liver Diseases Practice Guidelines. Exclusion criteria: (1) Liver cirrhosis caused by other viral hepatitis, autoimmune disorder, drug abuse or inheritance factors; (2) Those complicated with HIV or EB infection; (3) Complicated with severe heart, lung, kidney diseases that cannot comply to liver biopsy; (4) Pregnant or lactating women. Another cohort of 40 healthy control people were recruited in parallel, including 26 males and 14 females (average age = 42.5±11.8 years). No significant difference existed in age or gender between the two groups.

This study was pre-approved by the ethical committee of The Second People's Hospital Affiliated to Luzhou Medical College in Neijiang City. All subjects have signed the consent forms before recruitment in this study.

Blood biochemistry examination

Fasted venous blood samples were collected in the morning from all research subjects. Blood samples were incubated at room temperature for 2 h until blood clotting. Samples were centrifuged at 400 g for 10 min. The upper plasma phase was saved. AU800 fully automatic biochemical analyzer (Olympus) was used to determine serum albumin (ALB), total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Liver fibrosis parameters and sH2a assay

Radioimmune analyzing kit for determining hyaluronic acid (HA), type III pro-collagen (PC III), laminin (LN) and type IV collagen (IV-C) was purchased from Haiyan Biotech Institute (China). MAGLUMI 2000 Plus fully automatic chemiluminescence immunoassay apparatus was used to determine serum HA, PC III, LN and IV-C contents.

Human sH2a quantifying assay kit was provided by Weizhen Biomed (China). Following the manual instruction of the test kit, enzyme linked immunosorbent assay (ELISA) was used to determine serum sH2a concentrations.

Statistical analysis

All data were processed by SPSS 18.0 software. Measurement data were presented as mean ± standard deviation (SD). Student t-test was used to compare means between two groups. Spearman rank correlation analysis was used between parameters. Receiver operating characteristic (ROC) curve was used to analyze the value of sH2a in differential diagnosis of liver cirrhosis. A statistical significance was defined when $P<0.05$.

Results

Significantly decreased sH2a contents in peripheral blood of liver cirrhosis patients

Lab assay for liver functions showed that, compared to healthy control people, liver cirrhosis patients showed significantly elevated serum ALT, AST, TBIL and DBIL contents, whilst ALB level was remarkably decreased ($P<0.05$ in all cases, **Table 1**). ELISA results showed significantly decreased sH2a contents in peripheral blood samples from liver cirrhosis patients by 52.8% compared to healthy individuals ($P<0.05$, **Table 1**).

Correlation between liver fibrosis index, sH2a and disease stage in liver cirrhosis patients

Test results showed significantly higher serum liver fibrosis indexes including HA, PC III, IV-C

Table 1. Peripheral sH2a, liver function and biochemical indexes between two groups

	Control	Liver cirrhosis
sH2a (ng/mL)	126.3±38.7	59.6±21.4*
ALT (U/L)	31.4±4.5	142.6±26.8*
AST (U/L)	29.8±3.7	121.9±35.6*
TBIL (µmol/L)	13.2±2.8	52.6±8.1*
DBIL (µmol/L)	4.1±0.7	26.3±2.9*
ALB (g/L)	49.3±11.5	30.6±5.8*

*P<0.05 compared to control group.

Table 2. Fibrosis indexes and sH2a results in liver cirrhosis patients at different stages

	Stage 1~2	Stage 3~5
sH2a (ng/mL)	68.9±22.3	46.3±18.9*
HA (ng/mL)	271.3±44.3	386.5±56.9*
PC III (ng/mL)	18.5±3.1	26.7±4.5*
IV-C (ng/mL)	19.2±4.6	25.1±5.3*
LN (ng/mL)	144.6±39.4	203.9±52.2*

*P<0.05 comparing to stage 1~2 patients.

Table 3. Correlation between peripheral sH2a and liver fibrosis indexes in liver cirrhosis patients

Index	sH2a	
	r	P
HA	-0.584	0.039
PC III	-0.142	0.192
IV-C	-0.692	0.013
LN	-0.631	0.021

and LN in stage 3~5 liver cirrhosis patients compared to those in stage 1~2 patients (P<0.05, **Table 2**). ELISA results showed that, compared to stage 1~2 liver cirrhosis patients, stage 3~5 patients presented significantly lower peripheral sH2a contents, with about 67.2% (P<0.05, **Table 2**).

Peripheral sH2a in liver cirrhosis patients was correlated with liver fibrosis index

Spearman rank correlation analysis showed a significantly negative relationship between serum sH2a in liver cirrhosis patients and liver fibrosis indexes including HA, IV-C and LN (P<0.05), whilst no significant correlation was found between sH2a and PC III (P>0.05, **Table 3** and **Figure 1**).

*Relatively higher diagnostic value of peripheral sH2a on liver cirrhosis*

ROC curve was plotted for serum sH2a on liver cirrhosis patients and healthy control individuals to evaluate the diagnostic value of serum sH2a in differential diagnosis between healthy control and liver cirrhosis patients. Results showed that AUC of peripheral serum sH2a on liver cirrhosis was 0.816 (95% confidence interval: 0.782~0.850), with moderate diagnostic value (**Figure 2**).

Discussion

Liver cirrhosis mainly presents as liver dysfunction and portal artery hypertension, accompanied with multi-organ failure [16, 17]. When liver cirrhosis progresses into certain stages that go beyond the compensatory potency of hepatocytes, clinical presentation of a decompensation period also occurs including fatigue, mental depression, dry skin, face darkening, digestive tract hemorrhage, hypoproteinemia, ascites, blood clotting dysfunction, hepatic encephalopathy, and secondary infection; which severely affects patient health and life quality, and causes major economic burden for both family and society [18-20]. Liver fibrosis is a disease condition caused by imbalanced synthesis and degradation of the extracellular matrix (ECM) of liver tissues, and is featured as over-proliferation of hepatic mesenchymal tissues. Various factors including viral infection, alcohol abuse, autoimmune disorders and over-deposition of liver fatty acids may all contributed to liver fibrosis [21-23]. The onset and progression of liver fibrosis is a chronic process. If the damaged factor cannot be removed, persistent liver fibrosis may develop into liver cirrhosis, which is still reversible at an early stage. Therefore, early diagnosis of liver fibrosis and dynamic monitoring are of critical importance [24, 25].

Currently, various diagnostic approaches including tissue pathology, serum assays and imaging examinations are used for diagnosis of liver fibrosis. However, due to major trauma, deviation of sampling, lower reproductivity, complicated manipulation and expensive costs, their applications are largely limited. Hepatic ultrasound is one approach for qualitative diagnosis but with lower precision and sensitivity for early liver fibrosis during cirrhosis onset [26-
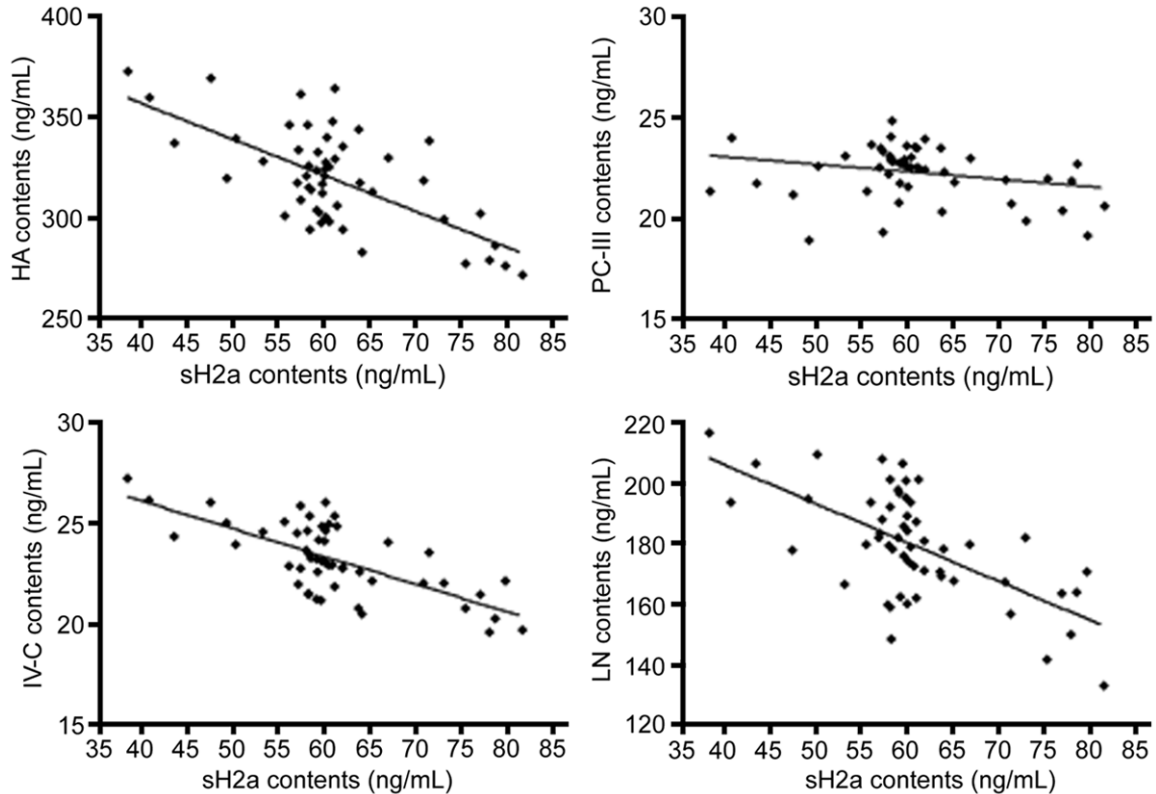
Figure 1. Correlation between serum sH2a and liver fibrosis indexes HA, IV-C, LN and PC III.
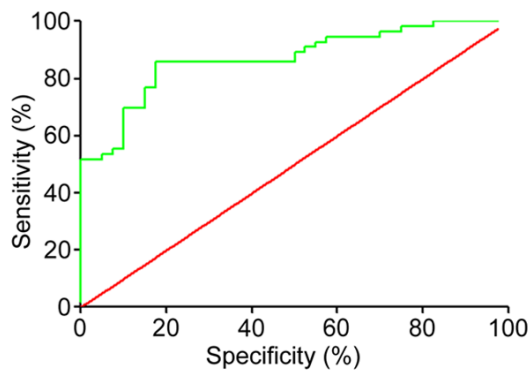
Figure 2. Diagnostic value of sH2a on liver cirrhosis by ROC analysis.

28]. Liver fibrosis parameters are a group of serum proteins to evaluate condition of liver fibrosis. All these serum proteins belong to ECM proteins or their degrading products, not liver-specific expression or secretory factors, whose expression levels can be deviated by extra-hepatic inflammation, causing lower specificity and sensitivity.

ASGFR is a functional protein responsible for clearing asialo-glycoprotein from plasma, with liver specificity. In developing liver tissues, ASGPR level is significantly lower than that in fully developed livers. ASGPR is composed of two related amino acid subunits, H1 (46 kD) and H2 (50 kD). H2a and H2b are two variable splicing isoforms of ASGPR H2 subunits, and are different only in the extra five amino acids within the extracellular domain adjacent to the transmembrane fragments. H2a can be rapidly cleaved into a 35 kD fragment at the site near those pentapeptides, including complete extracellular domain. Due to its secretory property, H2a can form a soluble form of receptor, or sH2a [14, 15]. Membrane binding H2a is unlike that of H2b, and it dose not participate in H1 receptor complex assembly. H2a is thus not a receptor subunit, but the precursor of a soluble secretory form, and does not participate in the assembly of the ASGPR H2 subunit.

This study compared peripheral sH2a content between liver cirrhosis patients and healthy people, and found significantly lower sH2a content in liver cirrhosis patients by 52.8%, and those patients with advanced clinical stage showed significantly lower peripheral sH2a con-

tent than those with early stages. These results suggested that liver cirrhosis patients presented lower sH2a content, which was further suppressed with disease progression. All these variations may be caused by damaged hepatocyte function, and suppressed synthesis and secretion of sH2a. Benyair et al found that compared to healthy control population, liver cirrhosis patients had lower serum sH2a contents [14], as similar with our results. As serum liver fibrosis parameters are widely applied noninvasive indexes evaluating liver fibrosis, this study further investigated the correlation between serum sH2a and liver fibrosis parameters including HA, IV-C, LN, and PC III. Spearman rank correlation test showed maximally negative correlation between serum sH2a and IV-C in liver cirrhosis between serum sH2a and IV-C in liver cirrhosis patients ($r = -0.584$), lower correlation with LN ($r = -0.631$), and minimal correlation with HA ($r = -0.584$), but not significant correlation with PC III. As a novel sH2 signaling protein family member, sH2a has not been fully investigated, with unclear function or clear boundary between normal and dysregulated concentrations. Routine Kappa consistent test cannot be used to reveal the consistency between diagnostic value of sH2a on liver cirrhosis and the gold standard of liver biopsy. Therefore, this study plotted ROC curves of serum sH2a between liver cirrhosis patients and healthy controlled individuals, in order to evaluate the diagnostic value of serum sH2a in differential diagnosis between liver cirrhosis and healthy people. Our results showed that AUC of peripheral serum sH2a for the diagnosis of liver cirrhosis was 0.816, indicating moderate diagnostic values.

This study recruited a limited sample number, and lacked large-sample study. Future study can be performed to enlarge the sample size, for further investigation of the relationship between sH2a and liver cirrhosis or fibrosis. It is still unclear if sH2a is under the influence of factors including virus, alcohol, or fatty acid liver. In the future, a large-sample patient cohort should be grouped based on disease condition for illustrating the correlation between sH2a quantity and those factors, thus rescuing the weakness of the current study. In summary, sH2a has satisfactory correlation with liver fibrosis condition, and moderately diagnostic value for liver cirrhosis. Due to con-

venient practice, sH2a may have clinical implications for auxiliary diagnosis and evaluation of liver fibrosis.

Conclusion

Liver cirrhosis patients had significantly decreased serum sH2a contents, which are correlated with clinical stages. sH2a has good correlation with liver fibrosis condition, and has moderate diagnostic value for liver cirrhosis. Due to the convenient operation, serum sH2a may have clinical implications in auxiliary diagnosis for liver cirrhosis and evaluation of liver fibrosis.

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Disclosure of conflict of interest

None.

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