

Usefulness of serum sFas and sFasL determinations as apoptogenic markers in liver disease

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ABSTRACT

This work aimed to investigate the biochemical changes of serum sFas/sFasL system and its usefulness as apoptogenic marker in patients with Bilharzial hepatic fibrosis and liver cirrhosis with and without hepatocellular carcinoma. Forty patients, 22 males and 18 females were selected for this study (age range: 30-67 years, average 48.4±8.9). They were clinically categorized as 15 patients with Bilharzial fibrosis, 15 patients with hepatitis C virus-related liver cirrhosis and 10 patients with hepatocellular carcinoma. Ten healthy subjects, age and sex-matched, were enrolled as controls. Statistical analysis showed that the mean value of serum sFas in Bilharzial fibrosis and liver cirrhosis, with and without hepatocellular carcinoma, was significantly higher than in control group ($P < 0.05$). Moreover, the mean value of serum sFasL was significantly elevated in all patients with liver cirrhosis, with and without hepatocellular carcinoma, and lower, but not significantly, in patients with Bilharzial fibrosis in comparison with the control group. We conclude that the biochemical changes in sFas/sFasL system might be considered a potentially useful tool as apoptogenic marker in some liver diseases.

INTRODUCTION

Yonehara et al. (1) reported an IgM monoclonal antibody that could kill several human cell lines and termed Fas (Fas7-associated cell surface antigen) the cell surface protein recognized by the antibody. The Fas protein is encoded by the tumor necrosis factor receptor super family 6 (TNFSF6) gene (2). When Fas ligand (FasL) or soluble Fas ligand (sFasL) binds the transmembrane receptor Fas/Apo-1, it induces apoptosis while soluble Fas (sFas), similar to Fas but lacking the transmembrane domain (3), blocks apoptosis by inhibiting binding between Fas and FasL or sFasL (4).

Apoptosis, or programmed cell death, and the elimination of apoptotic cells are crucial features in the maintenance of liver health. Apoptosis is tightly controlled and regulated via several mechanisms including Fas/FasL interactions, the effects of cytokines as transforming growth factor β (TGF- β) and the influence of pro- and anti-apoptotic mitochondria-associated proteins of the β -cell lymphoma-2 (Bcl-2) family. Liver disease is often associated with enhanced hepatocyte apoptosis as in viral hepatitis and schistosome infection (5,6). The disruption of apoptosis is responsible for other diseases as hepatocellular carcinoma (5).

Interaction between Fas and FasL plays an important role in cytotoxic T-lymphocyte and natural killer cell mediated apoptosis against cancer cells (7). Most of the tumors may escape the host immune attack by molecular mimicking the immune privileged sites by overexpression of the FasL, synthesized as a membrane bound protein that can be converted to sFasL (8). This study was

aimed to investigate the changes of serum sFas and sFasL concentrations and their usefulness as apoptogenic markers in patients with Bilharzial hepatic fibrosis (BHF), hepatitis C virus (HCV)-related liver cirrhosis (LC), with and without hepatocellular carcinoma (HCC).

MATERIAL AND METHODS

Forty patients from the Gastroenterology Department, Benha University Hospital, 22 males and 18 females, age range 30-67 years (average 48.4±8.9) and 10 healthy subjects, age and sex-matched, were enrolled in this study. The overall groups was represented by 10 healthy subjects (control group), 15 patients with BHF (hepatic fibrosis group), 15 patients suffering from HCV-related LC (LC group), 10 patients with HCC at different grading also related to HCV infection (hepatoma group).

All patients and controls were submitted to medical history recording, general and local examination, rectal biopsy for diagnosis of Bilharziasis, liver biopsy for histopathological examination and ultrasonography scanning of the abdomen. Only the patients with HCC were evaluated also by means of an abdomen computerized axis tomography scanning (CT-scan). Laboratory determinations included general testing such as urine and stool analysis (Kato Technique), blood cell count (Sysmex, Modl KX21), prothrombin time measurement (Clotting assay), serum glucose, total protein, albumin, creatinine and bilirubin concentrations, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and γ -glutamyl transferase (GGT) activities on RA50 analyzer (Bayer Diagnostics). More specialized

tests such as Bilharzial antigen (9) and antibodies detection (10) were also performed. Serological hepatitis markers such as hepatitis B surface antigen (HBsAg) and antibodies against HCV (HCV-Ab) were detected by immunochromatography technique (11,12). Qualitative HCV-RNA (only on patients positive for HCV-Ab) was performed by polymerase chain reaction (PCR) technique (13). Serum α -fetoprotein (AFP), sFas and sFasL determinations by ELISA methods (Biosource International Inc.) were also performed on all collected samples (14,15). About 9 mL of venous blood was drawn from each fasting subject and then divided into 4 tubes, the first (0.5 mL) containing K₂EDTA for complete blood cell count determination; the second (1.8 mL) containing 200 μ L of trisodium citrate for prothrombin time determination; the third (1.5 mL) (sterile) containing EDTA solution for qualitative determination of HCV-RNA by PCR; the remaining sample (5.2 mL), after 15 min at room temperature to complete clotting, was centrifuged and the serum used for determination of glucose, bilirubin, ALT and AST, alkaline phosphatase, GGT, total protein, albumin, creatinine, HBsAg, HCV-Ab and AFP. A serum aliquot was frozen at -80°C to assay serum sFas and sFasL.

RESULTS

Table 1 shows the range and mean values \pm SD of laboratory tests in all studied groups. Table 2 shows the mean values of serum sFas in patients with BHF, LC and HCC that were significantly elevated as compared to the controls. Moreover, the mean values of serum sFasL in patients with LC and HCC were significantly elevated as compared to the controls. The mean values of serum sFas and sFasL in LC patients with HCC were compared to the mean values of serum sFas and sFasL in LC patients without HCC revealing that serum sFasL had a significant elevation when HCC is present.

DISCUSSION

Epidemiological studies have associated infection with *Schistosoma Japonicum* and *Schistosoma Mansoni* with increased risk of cancers of the liver and colon, but the mechanism of carcinogenesis remains unestablished (6).

The major pathology resulting from *Schistosoma Mansoni* exposure is due to development of an immunomediated granulomatous response around *Schistosoma* eggs developing in the liver (16). Granulomas may injure host tissue by eliciting a T cell-mediated inflammatory response inducing tissue fibrosis and disease (17). Moreover, granuloma recruits, activates and further destroys antigen-reactive cells during the course of *Schistosoma Mansoni* infection (18). This selective destruction may result from antigen-driven Fas-FasL dependent apoptosis (19).

In an experimental model, Rumbley et al. (18) found that lymphocytes, unlike eosinophils, undergo apoptosis in the spleen and granulomas of *Schistosoma Mansoni*-infected mice and that, within the granuloma, lymphocy-

tes are more susceptible to Fas-FasL mediated apoptosis than in the spleen. Additionally, eosinophils isolated from spleens and granulomas of infected mice do not undergo apoptosis in vivo due to the absence of FasL expression rather than an anti-apoptotic signal provided by Bcl-2 or Bcl-xl proteins. Furthermore, Lundy (19) concluded that soluble egg antigens induced almost simultaneously CD4⁺ T cell activation and activation-induced cell death. During the early stages of granuloma development, increase of cytokines production by cell activation and cell proliferation is dominant, resulting in the observed increases in granuloma size and in inflammation intensity. However, the late stage of inflammation or infection is characterized by removal of neutrophils and other inflammatory cells by means of the induction of apoptosis. Fas/Apo-1 is a widely occurring apoptotic signal receptor molecule expressed by almost any type of cell, which is also released in a soluble circulating form (20). So, the increase of serum concentrations of sFas/Apo-1 from patients with BHF may contribute to the inflammatory response through accumulation of lymphocytes and other inflammatory cells at the sites of granuloma.

To date there are no reports about the biochemical changes in serum sFas/sFasL in patients with BHF. In this work, serum sFas was significantly elevated in BHF patients.

Persistence of HCV infection often progress to chronic hepatitis, LC and HCC. The mechanism involved in the development of infection persistence and the pathogenesis of the progression disease is still unclear (21). Apoptosis amplification has been shown to play major role in the development and in the pathogenesis of several diseases. The main mechanism of apoptosis is the molecular interaction between surface molecules known as "death receptors" and their ligands. The best studied death receptor and ligand system is the Fas/FasL system, in which FasL, a member of the TNF family of death-inducing ligands, activates death signals through the death receptor Fas, thereby resulting in the apoptotic death of the cell. Several cells in the liver had been shown to express Fas/FasL which plays a major role in the pathogenesis of many liver disease (22).

The results of this work showed that serum sFas and sFasL were significantly elevated in patients with LC due to HCV infection as compared to the control group.

More than 80% of HCV-infected individuals develop chronic disease, which can progress to LC. HCV can be replicated not only in hepatocytes but also in immune cells such as monocytes, B cells and T cells (23). As a part of defense mechanism of the host, cells infected by viruses are induced to start apoptotic cell death by signals delivered from cytotoxic T lymphocytes and natural killer cells, or by viral protein themselves, and apoptosis has been suggested to be a common pathway of virus clearance by the host (24). Lymphocytes found in the inflamed regions of the liver in HCV patients have been shown to express FasL (25). Presumably, it is FasL on these lymphocytes, which mediate the death of Fas positive hepatocytes contributing to liver injury resulting in

Table 1Range and mean \pm SD of laboratory tests in the studied groups

Biochemical parameters	Controls (n=10)	BHF (n=15)	LC (n=15)	HCC (n=10)
S-Total bilirubin (mg/dL)	0.5-1.0 0.77 \pm 0.16	0.8-4.0 1.67 \pm 0.98	3.0-16.0 8.80 \pm 4.92	3.0-23.0 11.20 \pm 6.05
S-Conjugated bilirubin (mg/dL)	0.10-0.20 0.18 \pm 0.04	0.1-2.0 0.71 \pm 0.59	2.0-13.9 6.61 \pm 4.30	1.50-13.0 4.25 \pm 3.57
S-ALT (U/L)	5-21 12 \pm 5	4-20 13 \pm 5	20-101 65 \pm 26	22-56 41 \pm 10
S-AST (U/L)	5-22 14 \pm 5	10-20 16 \pm 3	25-100 61 \pm 23	25-55 36 \pm 9
S-Alkaline phosphatase (U/L)	66-96 86 \pm 10	88-320 169 \pm 87	298-489 382 \pm 61	338-599 438 \pm 90
S-GGT (U/L)	26-45 37 \pm 7	26-48 38 \pm 7	112-345 238 \pm 74	22-89 52 \pm 23
S-Total protein (g/L)	71-81 77 \pm 3	57-74 67 \pm 5	59-70 66 \pm 4	40-66 55 \pm 7
S-Albumin (g/L)	40-51 46 \pm 4	29-43 38 \pm 4	30-39 35 \pm 3	19-31 25 \pm 4
P-Prothrombin time (sec)	11.1-13.3 12.0 \pm 0.8	13.3-18.6 15.1 \pm 1.4	14.3-20.2 17.0 \pm 1.7	17.5-24.6 20.7 \pm 2.3
S-AFP (μ g/L)	2.0-9.0 5.6 \pm 2.2	3.0-11.0 6.5 \pm 2.4	8.0-48.0 23.6 \pm 11.2	350.0-960.0 618.7 \pm 175.7

BHF: Bilharzial hepatic fibrosis, LC: Liver cirrhosis, HCC: Hepatocellular carcinoma

Table 2Mean \pm SD of serum sFas and sFasL in patients with BHF and LC, with and without HCC, compared with the control group

Biochemical parameters	Controls (n=10)	BHF (n=15)	LC (n=15)	HCC (n=10)
Serum sFas (μ g/L)	0.49 \pm 0.09	1.52 \pm 0.27 P <0.05	0.94 \pm 0.29 P <0.05	0.93 \pm 0.59 P <0.05 P1=NS
Serum sFasL (μ g/L)	0.45 \pm 0.11	0.39 \pm 0.11 P=NS	0.96 \pm 0.11 P <0.05	1.69 \pm 0.70 P <0.05 P1 <0.05

P: probability vs. control group, P1: probability vs. LC group

BHF: Bilharzial hepatic fibrosis, LC: Liver cirrhosis, HCC: Hepatocellular carcinoma

end stage liver disease (22). However, Fas antigen was not detected in patients without hepatitis infection. So, the presence of Fas antigen seemed to be essential for apoptosis of hepatocytes, which contributed to the elimination of infected cells. It has been suggested that the ratio of membrane-bound FasL to sFasL may be regulated to remove virally infected cells in chronic hepatitis HCV related (26).

In HCV infection, Fas expression is up-regulated in liver cells proportionally to the severity of the liver inflammation. When HCV-specific T cells migrate into hepatocytes and recognize the viral antigen via T cell receptor, they become activated and express FasL that transduces the apoptotic death signal to Fas-bearing hepatocytes resulting in their destruction (27). This may explain the significant increase in serum sFas and sFasL in patients with LC due to HCV infection compared with

the control group. These findings were confirmed by the results reported elsewhere (28,29). Moreover, our results revealed that serum sFas and sFasL were significantly elevated in patients with HCC compared with the control group. Comparing patients with HCV LC with and without HCC, serum sFas resulted unchanged while serum sFasL was significantly higher in HCC.

HCV is a widespread pathogen, with a worldwide seroprevalence of ~1%. It causes chronic liver inflammation, leading to HCC which occurs after many years of chronic hepatitis (30). The expression of Fas and FasL plays a role in apoptosis of cancer cells including HCC and it is associated with the worse prognosis of cancer patients (31). It is more likely that the massive apoptosis could be induced by inflammation enhanced by FasL (30). There are some hypotheses that explained the strong expression of Fas and FasL in patients with HCV-

related HCC. Firstly, because HCC cells can co-express Fas and FasL, they may undergo apoptosis induced not only by activated FasL-positive lymphocytes but also by their own FasL in an autocrine or paracrine manner. Secondly, FasL expressed on HCC cells might be important in their infiltration as well as dissemination into the liver. The third possibility is the so called "counterattack hypothesis". FasL expressed on tumour cells may be engaged with Fas receptors expressed on the surfaces of antitumour immune cells, causing them to undergo apoptosis (32).

The significant increase of sFas in our patients with HCC compared with the control group was in agreement with the results reported (4,33,34). The significant increase of serum sFasL in LC patients with HCC compared either with LC patients without HCC or the control group may be due to the ability of HCV core protein in the induction of functional FasL and apoptosis in a target T cell line expressing Fas as suggested also in other reports (34,35).

We conclude that the biochemical changes in serum sFas/sFasL system might be considered a potentially useful tool as apoptogenic marker in some liver diseases.

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