Serum profile of soluble Fas and soluble Fas Ligands in Iraqi patients with viral hepatitis type B and C

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Abstract:

Fas (CD95/APO-1) is a cell-surface membrane member of the tumor necrosis factor (TNF) receptor superfamily and mediate programmed cell death, or 'apoptosis', upon engagement by its ligand. Soluble forms of Fas and FasL may have a protecting function and may reduce tissue damage by competing for membrane bound FasL on infiltrating cytotoxic T cells and down modulating tissue Fas receptor. In this work serum levels of sFas and sFasL were assayed by immunoenzymatic methods in patients with HBV and HCV. sFas concentrations record a clear elevation in comparison to control healthy individuals 4.4 ±2.24 ng/ml in HBsAg positive sera and 6.2 ± 2.62 ng/ml in patients with HCV , while in control group 0.64 ± 0.42 ng/ml . Also levels of sFasL appeared to be elevated; 8.1 ±4.7ng/ml in HBsAg positive sera and 8.7 ±3.8 ng/ml in HCV patients which also record a clear elevation in comparison to control group 0.82 ±0.44 ng/ml. Our result suggest that the elevation of soluble forms of Fas and FasL participate in prolonging the disease and chronic form of infection by impairing the apoptosis .

Introduction:

Fas (CD95/APO-1) is a cell-surface membrane member of the tumor necrosis factor (TNF) receptor superfamily and mediate programmed cell death, or 'apoptosis', upon engagement by its ligand, FasL .Fas is widely expressed in numerous different cell types throughout the body, whereas FasL expression appears to be more restricted, different cell types within the immune system express FasL, including T and B cells. (Nagata, 1997 ; Connell ,2001).

Soluble Fas and soluble FasL is derived by specific proteolytic cleavage of the extracellular domain of membranous Fas and FasL by matrix metalloproteinases (MMPs), FasL elicit inflammation correlated with induce the apoptosis ,while sFasL opposed the role of FasL-mediated inflammation in vivo, sFas markedly reduce apoptosis inducing activity compared to membrane bound FasL(Hohlbaum *et.al.,* 2000).

Fas expression has been shown to be upregulated on hepatocytes from both hepatitis B and C (Okazaki *et.al.*,1996 ; Galle *et.al.*,1998; Pianko *et.al.*,2001).

Individuals with highest hepatocyte expression of Fas ligand could delete their HBV-specific CTL more efficiently and, therefore, could have a higher probability to become chronically infected. (Connell, 2001; Ferrari *et.al.*,2003).

Ruggieri *et.al.*,(1997) showed that infection of hepatoma cell line with hepatitis C core protein increased susceptibility to Fas-mediated death.

Lymphocytes found inflamed region of the liver infected with viral hepatitis express FasL which mediated the death of Fas+ hepatocytes that contributes the liver injury fig (1.3) (Pinkoski *et.al.*,2000) .

The concentration of sFas and sFasL in sera samples of HBV and HCV infected patients is elevated in comparison to healthy people (Lapanski *et.al.*,2004).

Materials and methods:

A cross sectional study was conducted on the groups of HBsAg and anti-HCV seropositve individuals in Al-Qadisiyia province in the period between July 2004 and December 2005.

A total sum of 217 individuals of both sexes gathered comprising as 131 HBsAg seropositve group, consisting of 120 males and 11 females. And 77 individuals were anti-HCV seropositve consisting of 55 males and 22 females. While only 9 individual showed both viral seromarkers.

Seventy five healthy individuals were randomly selected as normal control group .

Detection of Viral Markers :

ELISA for Detection of Hepatitis B surface antigen (HBsAg) :

This is a direct immunoenzymatic method of the sandwich type (Biokit , Spain) (Fields *et.al.*,1983; Giles *et.al.*,1999 ; Rose *et.al.*,2002).

ELISA for Detection of antibodies to HCV (Screening Test) :

This is an immunoenzymatic method (Biokit , Spain) (Uyttendaele *et al.*,1994).

Determination of human sFas and sFasL :

Serum levels of sFas and sFasL were measured quantitatively in patients of hepatitis C and B by the mean of enzyme linked immunosorbent assay method using ELISA kits (Bender MedSystems, Austria), measurement as recommended by the manufacture (Rose *et.al.*,2002; Hasegawa & Kojima, 1998).

Results:

Soluble Fas (sFas) :

In HBV positive sera samples the levels of sFas were significantly elevated ($p<0.01$). sFas levels (mean ± SD) was 4.4 ±2.24 ng/ml in comparison to control group 0.64 ±0.24 ng/ml as shown in fig (1).

HCV positive sera samples also showed a significant increment ($p<0.01$), sFas concentrations was 6.2 ± 2.62 ng/ml in comparison to control group 0.64 ± 0.42 ng/ml fig (1).

Soluble Fas Ligand (sFasL) :

In HBV positive sera levels of sFasL were significantly elevated ($p<0.01$), sFasL concentration (mean ± SD) 8.1 ±4.7ng/ml in comparison to control group 0.82± 0.44 ng/ml as shown in fig (2).

HCV positive sera also showed a statistically significant elevation ($p<0.01$), sFasL concentrations were 8.7 ±3.8 ng/ml which is higher than control group 0.82 ±0.44 ng/ml as shown in fig (2).

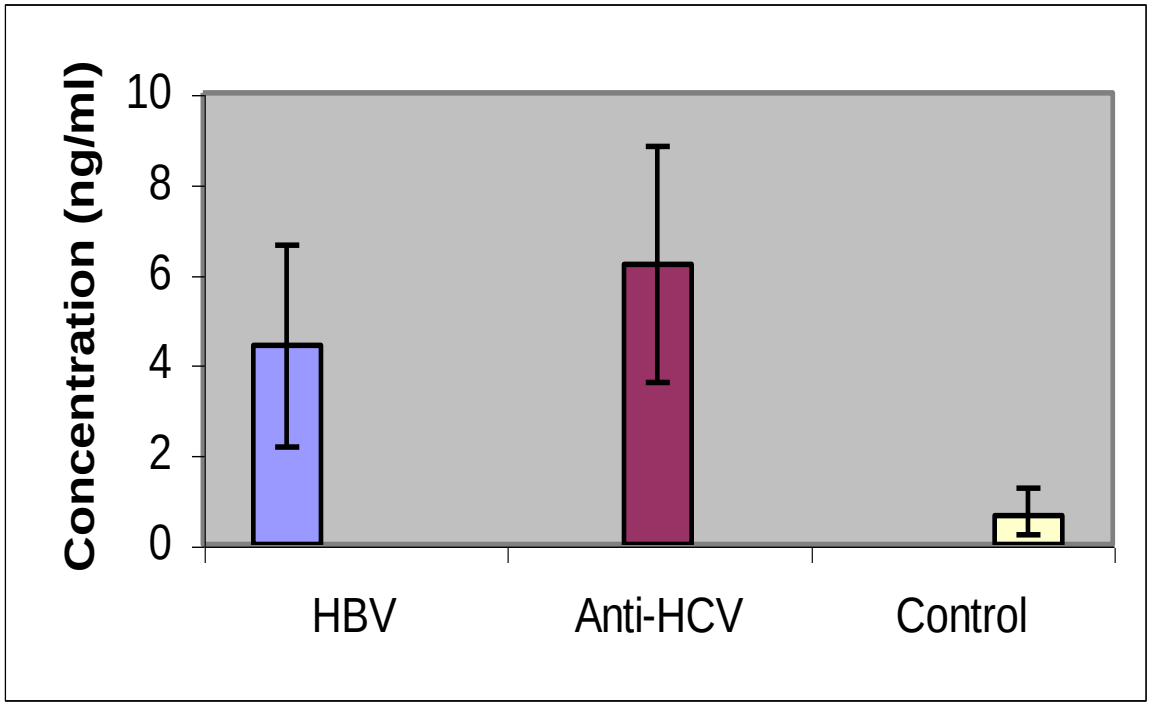

Fig.1: The serum levels of sFas in patients with HBV and HCV.

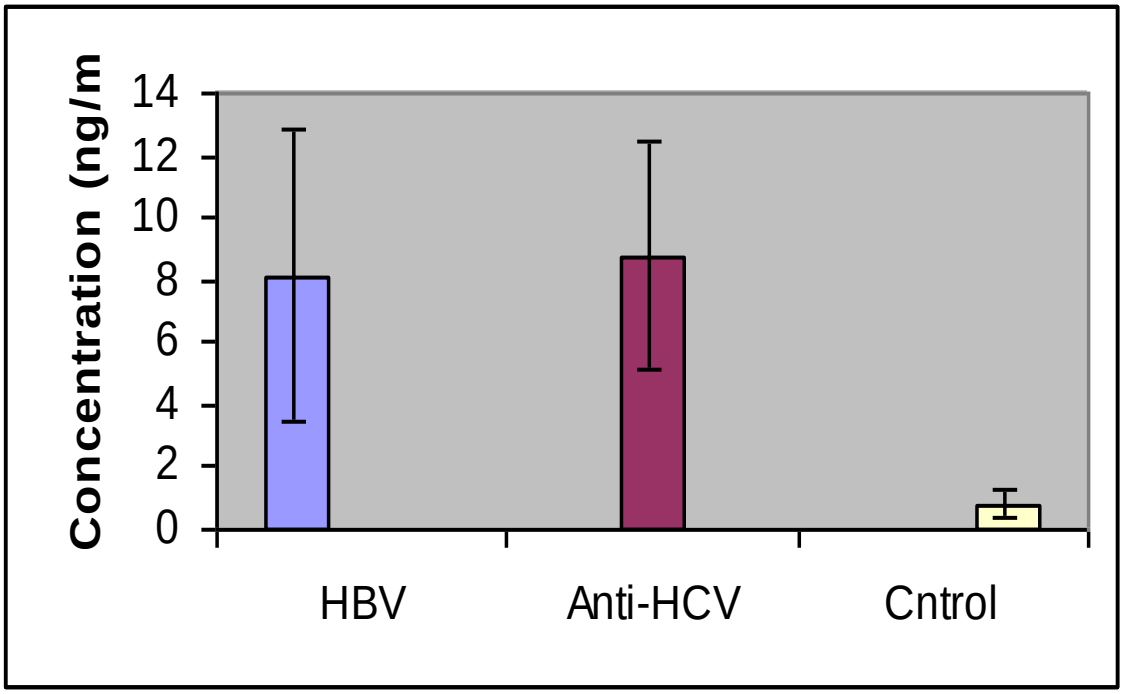

Fig.1: The serum levels of sFasL in patients with HBV and HCV.

Discussion:

This work showed a remarkable increment in the serum concentrations of sFas and sFasL. These results are similar to the results obtained by many previous studies.

Lapinski *et al.*, (2004) reported that the serum concentrations of sFas and sFasL in patients with hepatitis B and C were significantly elevated proposing the important role in the apoptosis .

Impaired of apoptosis lead to unfavorable course in hepatitis B and C, which may lead to prolonging the infection (Ozaslan *et al.*,2003).

The kupffer cells participate in the process, it stimulates the synthesis of the cytokines which regulate the biosynthesis of Fas in hepatocytes, recent studies indicates that it depends on liver or serum sFas concentrations and apoptosis activity as well as hepato-cytotoxic process in viral chronic hepatitis patients (Liaw *et al.*,2000 ; Xin *et al.*,2000).

HBV might stimulate the expression of FasL on lymphocytes, the complex Fas & FasL could activate apoptosis and cytotoxic lymphocytes

Activation of inflammation processes in the liver could influence FasL activity, associated with the Fas & FasL complex formation ,it could explain more uncommon occurrences of sFasL in patients with chronic hepatitis B than in healthy HBsAg carriers(Mita & Hayashi, 1997; Zhao *et al.*,2000) .

In patients with chronic hepatitis C, insufficient apoptosis activity could cause chronic inflammation, insufficient apoptosis activity could cause chronic inflammation (Zuckerman *et al.*, 2001) .

Ozaslan *et al.*,(2003) suggested a positive dependence on chronic hepatitis C intensity and serum sFas concentration.

Soluble Fas antigen can protect the cells against the Fas-mediated apoptosis, high level of sFas antigen characteristic for blood of patients with autoimmune diseases or cancer is believed to prevent the elimination of the autoimmune lymphocytes or tumor cells (Proussakova *et al.*,2003).

Pinkosk *et al.,* (2000) suggested that the sFas serum levels are significantly elevated in patients suffering from gut diseases such as viral hepatitis is positively correlated with the liver damage especially during acute self limiting and fulminant hepatitis.

Under these diseases conditions sFasL may have a protecting function and may reduce tissue damage by competing for membrane bound FasL on infiltrating cytotoxic T cells and down modulating tissue Fas receptor, whereas the conversion of membrane-bound cytotoxic FasL to its protective soluble form may represent an important regulatory mechanism by which sensitive tissues such as hepatocytes protects from excessive immune response (Pinkoski *et al.*,2000).

Neither CD95 expression nor the degree of liver injury correlate with intrahepatic viral load (Calabrese, *et al.* ,2000) , supporting the hypothesis of indirect immune-mediated mechanisms in hepatocyte apoptosis. In such a scenario, Th1 cytokines, such as tumor necrosis factor (TNF) or IFN-γ might upregulate CD95 in hepatocytes as well as CD95L in T-lymphocytes (Nuti, *et al.*,1998 ; Muschen *et al.*, 1999) .

Interestingly, CD95L may also exert proinflammatory activities by inducing the secretion of interleukin-1β and other cytokines that are responsible for leukocyte infiltration (Miwa, *et al.*,1998). Thus, the HCV-mediated immune response might be closely associated with CD95-triggered hepatocyte apoptosis (Bantel & Osthoff, 2003).

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