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ORIGINAL ARTICLE

Implication of tumor necrosis factor alpha receptor 1 and hexosaminidase: relationship to pathogenesis of liver diseases

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Abstract Liver disease is the main cause of morbidity and mortality worldwide. The spectrum of the disease ranged from fatty liver to hepatic inflammation, necrosis, progressive fibrosis, and hepatocellular carcinoma. We evaluated the serum levels of soluble tumor necrosis factor alpha receptor 1, total B-hexosaminidase and its isoenzymes Hex A and B activities, and nitric oxide in patients with liver diseases and their association with aminotransferase level. Seventy patients and 12 healthy subjects were recruited. Patients were divided into three groups: chronic hepatitis group (20 patients), liver cirrhosis group (30 patients), and malignant liver group (20 patients). Serum levels of soluble tumor necrosis factor alpha receptor 1, total B-hexosaminidase and its isoenzymes Hex A and B activities, and nitric oxide were measured. Serum levels of soluble tumor necrosis factor alpha receptor 1, total B-hexosaminidase activity, and nitric oxide were significantly higher in the liver disease patients. Serum levels of isoenzymes Hex A and B were significantly higher in malignant liver patients. Total B-hexosaminidase and its isoenzyme Hex A activity levels were significantly higher in positive HBsAg and positive anti-HCV patients. Serum levels of soluble tumor necrosis factor alpha receptor 1 were positively correlated with aminotransferase level. Taken together, these findings suggested that these biochemical indices might reflect ongoing disease activity and played an important role in the pathophysiology of liver diseases.

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Introduction

Tumor necrosis factor alpha (TNF-α) is a multifunctional pro-inflammatory cytokine that plays a critical role in the inflammatory response with varied immune system functions (Ardebili et al. 2011; Ikeguchi et al. 2002; Li et al. 2012). TNF-α production is induced by multiple factors such as viruses, parasites, other cytokines, and endotoxins (Li et al. 2012). TNF-α is a homotrimer which may dissociate to monomers in the solution at physiological concentrations (Marusi et al. 2012). The action of TNF-α is mediated via two different cell surface receptors, TNF-α receptor 1 (R1) (p55) and TNF-αR2 (p75) (Belarbi et al. 2012; Li et al. 2012). TNF-αR1 has wide distribution, while TNF-αR2 is limited to cells of hematopoietic origins. TNF-αR1 produces the most part of TNF-α cellular responses, including activation of nuclear factor kappa beta and apoptosis (Kallinowski et al. 1998; Liu and Han 2001). These membrane receptors can be separated and become soluble receptors that combine with circulating TNF-α and restrain its activity. Because circulating soluble TNF-α receptor (sTNF-αR) levels have higher stability and longer half-life than TNF-α, it is postulated that sTNF-αR levels may serve as a sensitive monitoring of the activity of the TNF-α system (Moura et al. 2009; Shiraki et al. 2010).

Hexosaminidase (Hex) is a lysosomal heterodimer hydrolases enzyme, formed of two subunits, α and β subunits (Bateman et al. 2011; Matsuoka et al. 2011). In mammals, there are two major Hex isoenzymes, Hex A (αβ heterodimer) and Hex B (ββ homodimer). They catalyze the cleavage of *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine from nonreducing ends of oligosaccharide chains of glycoconjugates (Choromańska et al. 2011). Hex is abundant in liver hepatocytes and macrophages. Increased activity of Hex isoenzymes

Table 1 Demographic and clinical characteristics of the studied groups

Parameter	Healthy controls (*N*=12)	Chronic hepatitis (*N*=20)	Liver cirrhosis (*N*=30)	Malignant liver (*N*=20)
Age (years), mean ± SE	42.00±3.60	41.60±2.07	42.67±2.41	42.95±2.35
Sex				
Males/females	9/3	17/3	21/9	11/9
Special habits				
Goza smokers	8	9	18	14
Cigarette smokers	2	6	6	4
	2	5	5	2
Alcoholics	0	0	1	0
History of bilharziasis	No	11 (55 %)	18 (60 %)	6 (30 %)
Anti-HCV	Negative	19 (95 %)	12 (40 %)	10 (50 %)
HBs Ag	Negative	One (5 %)	2 (6.7 %)	One (5 %)
Bilirubin				
Total (μmol/L)	0.60±0.21	35.85±0.59*	44.39±6.47**	51.93±14.06**
Direct (μmol/L)	0.12±0.02	31.20±0.14**	35.52±3.00***	27.73±8.94**
Total proteins (gm/dl)	7.35±0.95	7.38±2.73	6.82±1.74	7.49±2.57
Albumin (gm/dL)	4.35±0.85	3.36±1.97	3.02±1.25	3.44±2.39
AST (IU/L)	32.35±0.65	230.85±28.05***	147.97±18.95**	177.30±11.09***
ALT (IU/L)	25.60±0.55	134.10±28.45**	99.89±6.16*	118.70±22.27*
ALP (U/L)	69.50±34.50	416.30±51.79***	290.60±34.30*	454.05±74.54***
Prothrombin concentration (%)	97.50±0.95	71.50±2.30***	58.00±2.82***	74.00±2.29***

All data are presented as mean ± standard error. Differences among the groups were compared by ANOVA test followed by Bonferroni multiple comparison tests

SE standard error, *Anti-HCV* hepatitis C virus antibody, *HBsAg* hepatitis B surface antigen, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase

*$p<0.05$; **$p<0.01$; ***$p<0.001$, versus the healthy controls group

was observed in liver disease and chronic alcoholism (Zylberberg et al. 1999). Hex was suggested being a potential marker in tumor diagnosis (Bierć et al. 2010; Moura et al. 2009; Szajda et al. 2011, 2010).

Nitric oxide (NO) is a short-lived, free radical, gaseous signaling molecule that exerts a wide variety of physiological functions including the regulation of blood vessel and airway tone, inflammation, neurotransmission, and apoptosis (Pérez et al. 2000). Nitric oxide is produced from L-arginine by one of three NO synthase (NOS) enzymes: two constitutive, neuronal and endothelial types, and one inducible (Ibrahim et al. 2010).

The aim of our study is to (1) investigate the role of soluble tumor necrosis factor alpha receptor 1, total B-hexosaminidase and its isoenzymes Hex A and B activities, and nitric oxide in the pathophysiology of liver diseases; (2)

Table 2 Serum levels of biochemical indices in the studied groups

Parameter	Healthy controls (*N*=12)	Chronic hepatitis (*N*=20)	Liver cirrhosis (*N*=30)	Malignant liver (*N*=20)
sTNF-αR1 (ng/mL)	1.75±0.11	4.49±0.68**	6.35±0.66***	6.65±0.65***
Total B-Hex (U/L)	474.50±17.22	665.50±11.21*	689.70±56.12**	859.00±46.26***
Isoenzyme Hex A (U/L)	167.70±15.32	290.50±22.20	239.30±47.70	390.10±54.90***
Isoenzyme Hex B (U/L)	315.60±20.90	392.80±25.35	408.40±28.62	537.80±64.02***
NO (μmol/L)	14.12±0.35	25.18±0.55***	20.86±1.18**	26.43 ±1.74***

All values are presented as mean ± SE. Differences among the groups were compared by ANOVA followed by Bonferroni multiple comparison test

sTNF-αR1 soluble tumor necrosis factor alpha receptor 1, *total B-Hex* total B-hexosaminidase, *NO* nitric oxide

*$p<0.05$; **$p<0.01$; ***$p<0.001$, versus healthy controls group

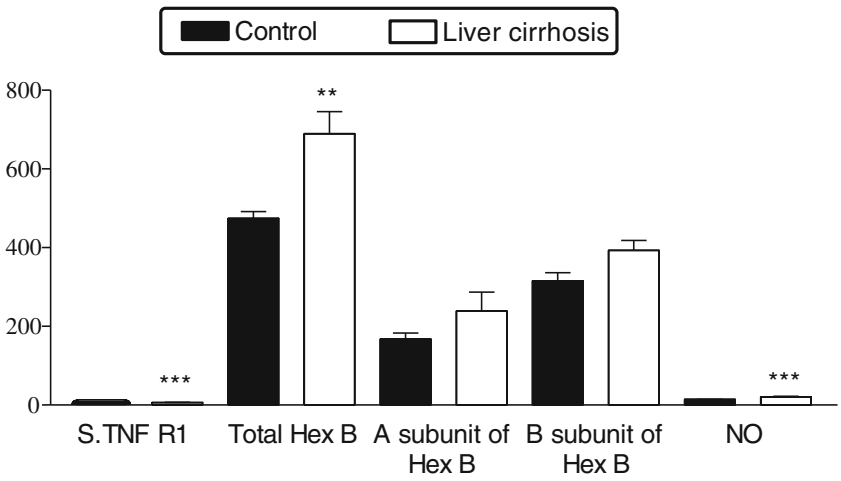

Fig. 1 Serum biochemical variables in liver cirrhosis group. *Bars* represent mean ± SE. ***$p<0.001$ versus the control group

study the association of these biochemical indices with hepatitis markers (HBsAg and Anti-HCV); and (3) evaluate the correlation of these biochemical indices with amino-transferase levels.

Material and methods

Patients

This study was carried out in the Assiut University Hospital, Egypt. The protocol was approved by the local ethics committee, and written informed consent was obtained from all patients.

The present study included 70 patients and 12 healthy subjects. They were divided into the following groups: group I (healthy controls); group II (chronic hepatitis) they have raised liver enzymes, tender hepatomegaly, and positive HBsAg or positive Anti-HCV for more than 6 months; group III (liver cirrhosis), diagnosed by clinical examination and abdominal ultrasound; and group IV (malignant liver) diagnosed by liver biopsy.

Blood sampling

Venous blood (10 cm^3) was obtained from the antecubital vein into sterile tubes without anticoagulant for serum collection. Care was taken to prevent any mechanical damage which might cause hemolysis of the blood. The tubes were allowed to stand in room temperature for few minutes, and then coagulated blood was centrifuged at 3,000 round per min (rpm) for 20 min. The clear, non-hemolyzed supernatant serum was quickly removed and kept at −70 °C until use.

Assay of liver functions and hepatitis markers

Standard parameters of liver function [total bilirubin, direct bilirubin, total proteins, serum albumin, serum aspartate transaminase (AST), serum alanine transaminase (ALT), alkaline phosphatase (ALP), and prothrombin concentration] and

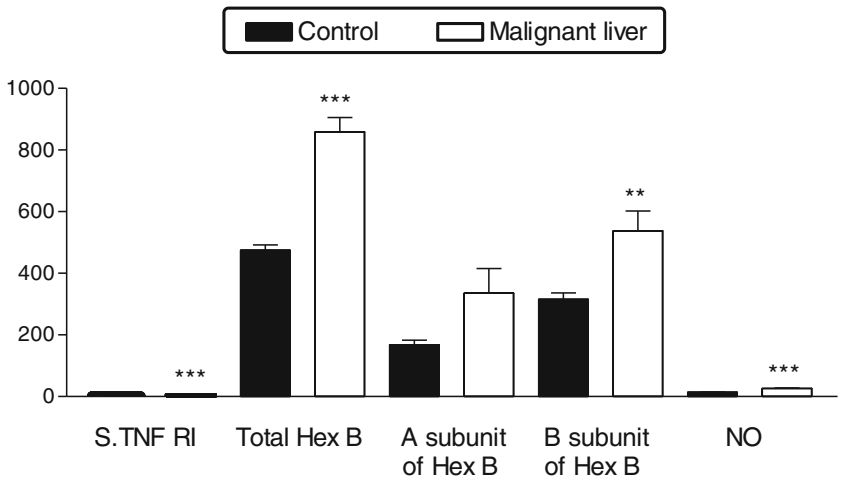

Fig. 2 Serum biochemical variables in malignant liver group. *Bars* represent mean ± SE. **$p<0.01$; ***$p<0.001$ versus the control group

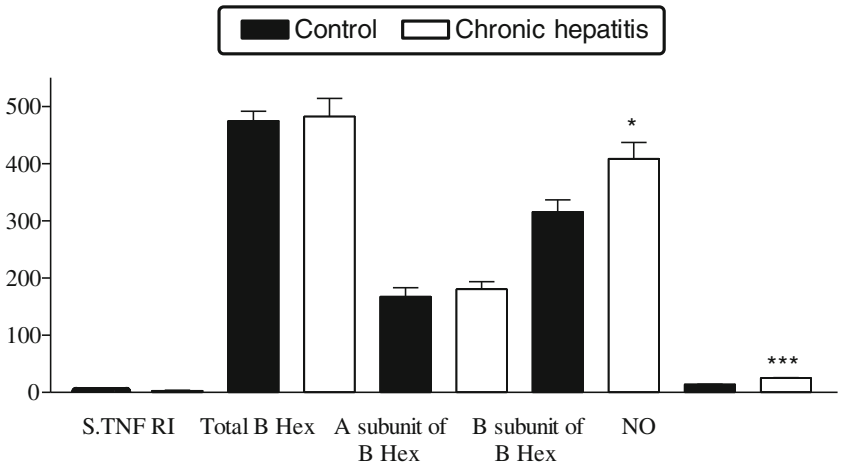

Fig. 3 Serum biochemical variables in chronic hepatitis group. *Bars* represent mean ± SE. *$p<0.05$; ***$p<0.001$ versus the control group

hepatitis markers (HBsAg and anti HCV) were measured by using an autoanalyser.

Determination of serum biochemical indices

Serum-soluble tumor necrosis factor alpha receptor 1 was determined by an enzyme-linked immunosorbent assays using kits supplied by Medgenix Diagnostics SA, Belgium, following the instructions supplied with the kit. Determination of activity levels of total B-hexosaminidase as well as its isoenzymes Hex A and B was done according to Chartterjee et al. (1979). Serum NO concentration was determined with an indirect method through the measure of serum concentrations of nitrates and nitrites. For NO

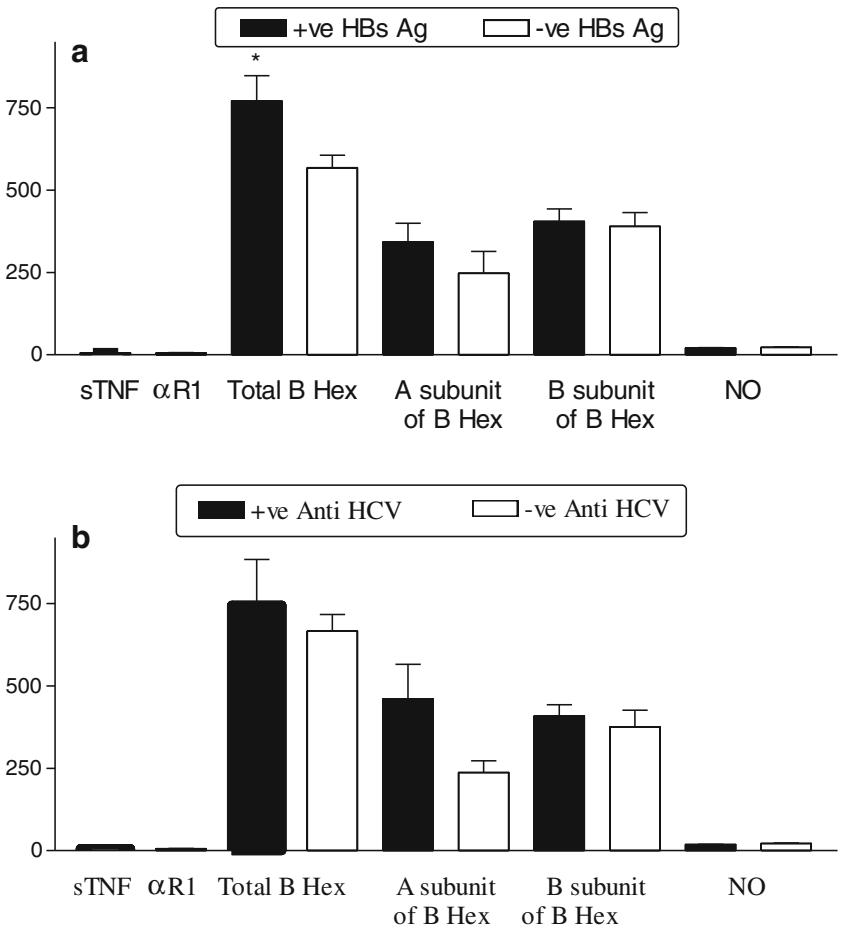

Fig. 4 Comparison of serum biochemical variables with HBsAg (**a**) and anti-HCV (**b**) in patients with liver cirrhosis. *Bars* represent mean ± SE. *$p<0.05$ versus the HBsAg-positive group

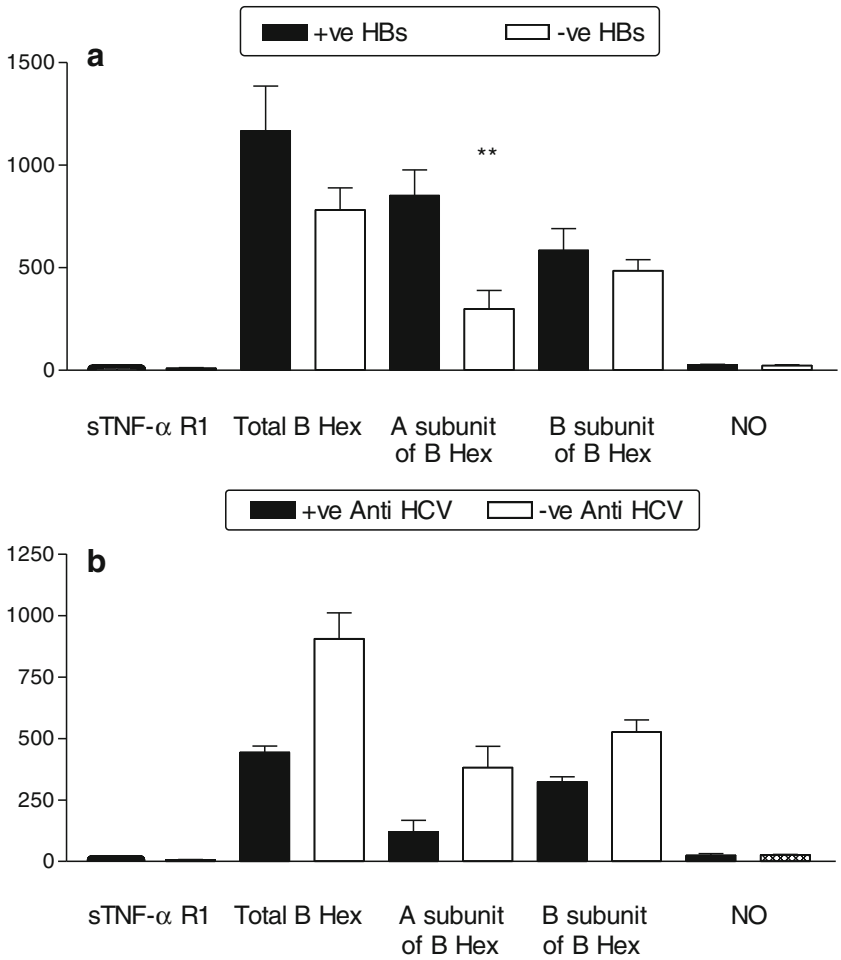

Fig. 5 Comparison of serum biochemical variables with HBsAg (**a**) and anti-HCV (**b**) in patients with malignant liver. *Bars* represent mean ± SE. $^{**}p<0.01$ versus the HBsAg-positive group

determination, the spectrophotometric reaction of Griess was used (Titheradge 1998).

Statistical analysis

Data were analyzed using GraphPad Prism version 5. All values were expressed as means ± standard error (SE) of the mean for *n* observations. Differences among the groups were compared by ANOVA test followed by Bonferroni multiple comparison tests. Correlations between indices were calculated using Spearman correlation. The level of significance was considered at $p<0.05$.

Results

Demographic and clinical characteristics of the studied groups

The demographic and clinical characteristics of the studied groups enrolled are shown in Table 1. Of the 12 healthy controls, nine were males, and three were females, with mean age of 42.00±3.60 years, without any history of liver disease. Of the 20 patients with chronic hepatitis, 17 were males, and 3 were females, with mean age of 41.60±2.07 years. Nineteen (95 %) were +ve Anti-HCV and one (5 %) were +ve HBs Ag. Of the 30 patients with liver cirrhosis, 21 were males and 9 were females, with mean age of 42.67±2.41 years. Twelve (40 %) were +ve Anti-HCV and two (6.7 %) were +ve HBs Ag. Of the 20 patients with malignant liver, 11 were males and 9 were females, with mean age of 42.95±2.35 years. Ten patients were with hepatocellular carcinoma, five with adenocarcinoma, two with cholangiocarcinoma, two with malignant lymphoma infiltrating the liver and one with angiosarcoma. Ten (50 %) were +ve Anti-HCV and one (5 %) were +ve HBs Ag.

Serum levels of biochemical indices in the studied groups

Serum levels of sTNF-αR1, total B-Hex, and NO were significantly higher in patients with chronic hepatitis, liver cirrhosis, and malignant liver compared to the healthy controls (Table 2 and Figs. 1, 2 and 3). Serum levels of isoenzymes Hex A and B were significantly higher in patients with malignant liver compared to the healthy controls.

Comparison of serum levels of biochemical indices with HBsAg and anti-HCV

Figure 4 shows a comparison of serum levels of biochemical indices with HBsAg in patients with liver disease. In malignant liver, serum levels of total B-Hex and its isoenzyme Hex A were significantly higher in HBsAg-positive patients in comparison with HBsAg-negative patients ($p<0.05$).

Figure 5 shows a comparison of serum levels of biochemical indices with anti-HCV. In patients with malignant liver, serum levels of total B-Hex and its isoenzyme Hex A were significantly higher in anti-HCV-positive patients in comparison with anti-HCV-negative patients ($p<0.05$).

Figure 6 shows a comparison of serum levels of biochemical indices with anti-HCV in patients with chronic hepatitis. Patients' serum levels of total B-Hex and its isoenzyme Hex A were significantly higher in anti-HCV-positive patients in comparison with anti-HCV-negative patients ($p<0.05$).

Correlation of serum levels of biochemical indices with aminotransferase level

To evaluate the clinical significance of alterations in serum levels of biochemical indices, their correlations with aminotransferase level were analyzed (Table 3). A positive correlation was found between serum sTNF-αR1 and elevated AST and ALT serum levels ($r=0.88$, $p<0.01$; $r=0.90$, $p<0.01$, respectively) in patients with malignant liver.

Table 3 Correlation between serum levels of biochemical indices and aminotransferase level in the studied groups

	Chronic hepatitis		Liver cirrhosis		Malignant liver	
	AST	ALT	AST	ALT	AST	ALT
sTNF-αR1 (ng/mL)	0.41	0.36	0.49	0.21	0.88**	0.90**
Total B-Hex (U/L)	0.39	0.34	0.38	0.42	0.49	0.26
Isoenzyme Hex A (U/L)	0.32	0.25	0.22	0.39	0.41	0.52
Isoenzyme Hex B (U/L)	0.23	0.24	0.20	0.32	0.43	0.33
NO (μmol/L)	0.36	0.31	0.44	0.42	0.47	0.46

Values represent correlation coefficient (r). The p values were calculated using Spearman's rank regression analysis

**$p<0.01$

Discussion

Liver damage induced an inflammatory response through activation of tissue macrophage Kupffer cells that released an array of cytokines, including TNF-α (Chen et al. 2011). TNF-α induced apoptosis in hepatocytes and might also activate cytotoxic T lymphocyte damage to near noninfected hepatocytes. Circulating levels of sTNF-αR reproduced TNF-α system activity and are considered as a superior marker of liver inflammatory activity (Moura et al. 2009).

The present study revealed that the serum levels of sTNF-αR1 were increased in patients with liver disease, without any difference between patients with positive and negative HBsAg and anti-HCV. Furthermore, the serum level positively correlated with aminotransferase levels (AST and ALT).

In accordance with our results, several studies demonstrated increased serum levels of sTNF-αR1 in the patients with chronic liver disease (Jorge et al. 2004), liver cirrhosis (von Baehr et al. 2000), and chronic hepatitis C virus infection (Zylberberg et al. 1999). Itoh et al. (1999) revealed a significant association of serum levels of sTNF receptors with alanine aminotransferase level. Trebicka et al. (2010) and Shiraki et al. (2010) demonstrated that soluble TNF-αR levels correlated directly with the severity of liver dysfunction. Contrary to our study, Zylberberg et al. (1999) found that serum levels of sTNF-α receptors are not correlated with anti-HCV.

Our results revealed that the serum levels of total B-Hex activity were increased in patients with liver disease.

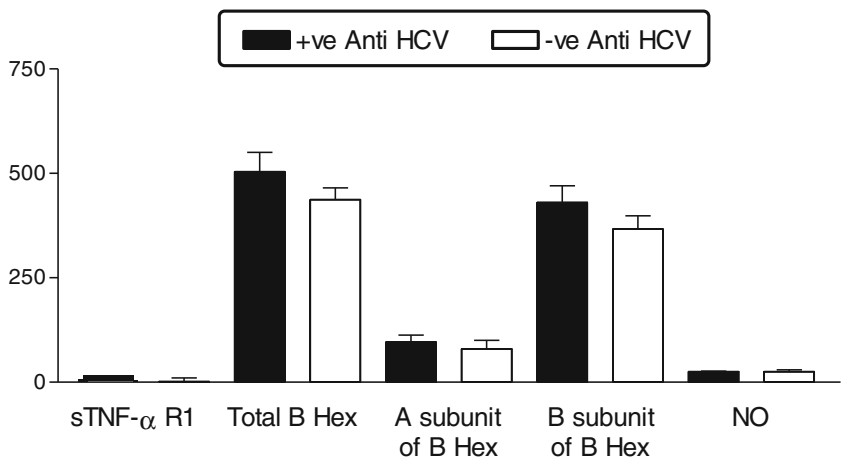

Fig. 6 Comparison of serum biochemical variables with anti-HCV in patients with chronic hepatitis. *Bars* represent mean ± SE. Patients' serum levels of total B-Hex and its isoenzyme Hex A were significantly higher in anti-HCV-positive patients in comparison with anti-HCV-negative patients ($p<0.05$)

Moreover, in patients with malignant liver, the serum levels of total B-Hex activity were higher in patients with positive HBsAg and anti-HCV. Isoenzymes Hex A and B were increased in patients with malignant liver, with higher levels of isoenzyme Hex A in HBsAg- and anti-HCV-positive patients. Furthermore, total B-Hex activity and its isoenzymes Hex A and B levels did not correlate with aminotransferase level.

Such result is in agreement with the findings of several studies. Hultberg et al. (1995) demonstrated increased serum levels of total Hex and its isoenzymes Hex A and B in human hepatoma cell line, and they speculated that increased concentration of ammonia in hepatic dysfunction interfered with the distribution pathway of the lysosomal enzymes. Pérez et al. (2000) revealed increased plasma activities of total Hex in liver cirrhosis. Napoleon et al. (2012) claimed that Hex contributed to local infiltration and metastasis of the cancer by breakdown of the basal membrane, extracellular matrix and cell surface glycoconjugates, and oligosaccharide and polypeptide chains.

Pathophysiological events leading to fibrosis and cirrhosis are characterized by overproduction of NO (Ergün et al. 2011). Our result revealed increased serum levels of NO in all patients with liver disease, without any difference between positive and negative HBsAg and anti-HCV patients and with no correlation with aminotransferase level.

This result is concomitant with other studies. López-Sánchez et al. (2010) found that inhibition of NO production alleviates hepatocellular injury. Tirapelli et al. (2011) concluded that NO is a marker of liver damage. Leung et al. (2011) employed that NO play a critical role in the succession of liver fibrosis and hepatocellular damage. Several studies found elevated nitric oxide serum level in patients with chronic liver disease and cirrhosis correlated with disease stage (Arkenau et al. 2001; Lee et al. 2010). Ikeguchi et al. (2002) revealed that the overproduction of NO correlated with carcinogenesis in cirrhotic liver, and they claimed this to inhibition of the immune defense mechanism and increased tumor blood vessels.

In conclusion, these results demonstrated increased serum levels of sTNF-αR1, total Hex B, and NO in patients with liver disease, and an association of sTNF-αR1 with serum aminotransferase supported their contention in the pathophysiology of liver disease. The observed increase of the activity of isoenzymes Hex A and B in patients with malignant liver highlighted their potential role in cancer progression.

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