



Adhesive molecules and inflammatory markers among hepatitis C virus Saudi patients

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ABSTRACT

Background: Currently, about 2% of population are affected with hepatitis C worldwide, chronic hepatitis C (CHC) is the major cause of hepatic cirrhosis and referral for liver transplant. However, there is a high need for noninvasive methods for assessment of hepatocellular damage.

Objective: The purpose of this study was to determine the strength of the association between adhesive molecules and inflammatory markers among hepatitis C virus Saudi patients.

Methods: One hundred patients with chronic hepatitis C virus infection (64 males and 36 females, their age ranged from 28 to 53 years with circulating anti-HCV antibodies were equally categorized into two study groups: patients with CHC and patients with liver cirrhosis (LC). Also, one fifty healthy subjects were included as healthy controls. Serum alanine aminotransferase (ALT), soluble intercellular adhesion molecule1 (sICAM-1); Soluble vascular adhesion molecule 1 (sVCAM-1); Soluble E-selectin (s-E-selectin) and Tumor necrosis factor-alpha (TNF- α) were assayed for all participants. **Results:** We observed elevation with regard to the healthy controls group in the parameters of ALT, sICAM-1, sVCAM-1, s-E-selectin and TNF- α for patients with CHC and patients with liver cirrhosis (LC). Also, a significant positive correlation between serum TNF- α , sICAM-1, sVCAM-1 and ALT values was detected.

Conclusion: In conclusion, our results confirm that, in patients with chronic virus hepatitis and liver cirrhosis there is a significant positive correlation between serum TNF- α , sICAM-1, sVCAM-1 and ALT values. These findings suggest that serum TNF- α levels could be used as a sensitive predictor of liver inflammation, while serum ICAM-1 can be considered as a marker of hepatic necrosis and inflammatory activity in chronic hepatitis, while serum VCAM-1 is an indicator for the severity of liver cirrhosis.

Keywords: adhesive molecules, inflammatory markers, chronic hepatitis C, liver cirrhosis

INTRODUCTION

Chronic hepatitis C (CHC) is a global health problem, that affects over 3% of global population (1) and 3 to 4 million are newly infected each year (2,3) with an estimated 180 million people infected worldwide (4). Hepatitis C virus (HCV) infection is one of the main causes of chronic liver disease worldwide (5) and persistent infection occurs in 50 to 80% of those infected and may lead to the development of cirrhosis and subsequent hepatocellular carcinoma (2,6). In most cases of HVC infection, the host immune system fails to eradicate the virus and a sustained immune-inflammatory response takes place. Besides its ineffectiveness to eliminate HCV, the immune response also damages the liver tissue, leading to inflammation, fibrosis, and hepatocellular alterations (1).

Liver damage in chronic hepatitis C (CHC) is commonly attributed to immune-mediated mechanisms (7). Hepatic fibrosis is characterized by abnormal excessive accumulation of extracellular matrix accompanied by exaggerated cytokine release. However recruitment and trans-differentiation of peripheral blood cells, in particular, monocytes into injured liver may play a role in this respect (8). Chronic viral hepatitis is histologically characterized by intralobular infiltration of inflammatory cells which is considered as an ominous sign of deterioration and a criterion for disease activity (9).

Adhesion molecules are intimately involved in disease mechanisms of inflammation and cancers. Intercellular adhesion molecule-1 (ICAM-1) and leucocyte function-associated antigen-1, a ligand for ICAM-1, play a critical role in the interactions of cytotoxic T cells both with target cells and with other immune cells (10). Vascular cell adhesion molecule-

1 (VCAM-1) is important in the extravasation of circulating lymphocytes and in their infiltration into inflamed sites (11, 12). ICAM-1 is overexpressed in sinusoidal endothelial cells in liver tissues with hepatitis. Hepatocytes at the site of inflammation express ICAM-1 (13, 14). Recently developed enzymelinked immunosorbent assays (ELISA) for soluble forms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) in serum are useful for monitoring chronic hepatitis and hepatocellular carcinoma (15, 16).

ICAM-1 and VCAM-1 are strongly expressed on sinusoidal lining cells in chronic hepatic inflammation due to HCV infection and play a key role in leukocyte recruitment and extravasation (17). Moreover, it was found that HCV-infected hepatocytes but not normal hepatocytes express ICAM-1 (18). However, ICAM-1 has a restricted tissue distribution (19), but its expression can be up-regulated in response to proinflammatory cytokines as tumor necrosis factor-alpha (TNF- α) on various cells including hepatocytes (20).

Tumor necrosis factor-alpha (TNF- α) is a proinflammatory cytokine with a major role in both acute and chronic responses to viral, bacterial, fungal and parasitic infections (21). TNF- α is produced primarily by activated macrophages during the inflammatory reaction that follows recognition of viral antigens, but the precise stimulus for its enhanced production in HCV infection has not been clearly defined (22). TNF- α has been involved in the pathogenesis of several liver conditions including viral hepatitis (23). In patients with hepatitis C, TNF- α is an inducer of apoptosis in infected hepatocytes (24) and might also account for CTL damage to nearby non-infected hepatocytes (25).

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Table 1: Comparison of clinical data between healthy controls, patients with HCV infected individuals with or without cirrhosis

	Healthy controls	CHC without cirrhosis	CHC with cirrhosis
Age (year)	48.96 ± 5.18	50.73 ± 4.24	49.16 ± 5.71
BMI (kg/m ²)	28.15 ± 2.23	27.65 ± 2.46	27.18 ± 3.12
Waist circumference (cm)	82.51 ± 7.19	80.31 ± 6.52	82.13 ± 5.84
Fat mass (kg)	18.35 ± 2.44	17.98 ± 3.78	16.67 ± 2.81
FPG (mg/dl)	112.81 ± 18.54	116.50 ± 14.16	119.45 ± 13.95
Hb (gm/dl)	13.46 ± 1.82	11.25 ± 1.71	10.19 ± 1.93
AST(IU/l)	30.76 ± 5.22	45.25 ± 8.91*	81.73 ± 11.17 ^{*S}
Albumin (gm/dl)	4.15 ± 0.65	3.42 ± 0.58*	2.25 ± 0.41 ^{*S}
Total Bilirubin (mg/dl)	0.62 ± 0.18	1.23 ± 0.37*	2.76 ± 0.95 ^{*S}

BMI: Body Mass Index; AST: Aspartate aminotransferase; FPG= Fasting Blood Glucose; Hb: Hemoglobin; (*): indicates a significant difference relative to healthy controls; (S): indicates a significant difference relative to CHC, P < 0.05

The aim of this study was to determine the strength of the association between adhesive molecules and inflammatory markers among hepatitis C virus Saudi patients.

SUBJECTS AND METHODS

One hundred patients (64 males and 36 females, their age ranged from 28 to 53 (42.26 ± 5.17) years, were studied upon referral to Gastroenterology and Hepatology Department, King Abdulaziz University Teaching Hospital, Saudi Arabia. All these patients were anti HCV positive by enzyme-linked immunosorbent assay (ELISA). None of the patients included in this study had other potential causes of liver disease, such as alcoholism, autoimmune phenomena, or metabolic disorders. All participants had CHC infection with circulating anti-HCV antibodies and were equally categorized into two study groups: patients with CHC and patients with liver cirrhosis (LC). Patients diagnosis and classification were based on detailed medical history, thorough clinical examination, kidneys and liver function tests, procto-sigmoidoscopy, abdominal ultrasonography, parasitological examination and liver biopsies. None of the subjects gave history of medication with interferon and ribavirin or drugs known to have influence on the coagulation process within 8 weeks of study time or had a renal impairment based on normal creatinine clearance. However, one fifty healthy subjects were included as healthy controls. This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University. All participants were free to withdraw from the study at any time.

Laboratory Analysis

Ten milliliter blood samples were collected from each participant at study entry. The blood samples were obtained using disposable needles and heparinized vacuum syringes and stored at - 70°C until assayed. Serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by serum chemistry autoanalyzer (Model 736, Hitachi, Tokyo, Japan) using commercial reagents (Biomerieux, Marcy L'Etoile, France). However, Serum ICAM-1, VCAM-1 and E- Selectin located to the cell surface were measured after fixation with 4% formaldehyde and staining with anti-ICAM-1-FITC, anti-VCAM-1-FITC, anti-p-selectin-RPE and anti-Tissue-factor-FITC or the corresponding FITC- or RPE-labeled negative controls by a FACSCanto II flow cytometer (Becton Dickinson, USA). Also, tumor necrosis factor-alpha (TNF-α) level was measured from frozen plasma samples stored at -80°C. Enzyme-linked immunosorbent assays kits (ELISAs) were used to measure soluble levels of TNF-α (GE Healthcare Amersham, Biotrak Easy ELISA), which utilized the quantitative sandwich enzyme immunoassay technique.

Table 2: Mean value and significance of sICAM-1, sVCAM-1, sE-selectin and TNF-α of healthy controls, patients with HCV infected individuals with or without cirrhosis

	Healthy controls	CHC without cirrhosis	CHC with cirrhosis
ALT (IU/l)	31.26 ± 6.37	42.54 ± 7.21	49.91 ± 7.61
sICAM-1 (ng/ml)	213.65 ± 21.18	682.17 ± 65.32*	1012.43 ± 76.83 ^{*S}
sVCAM-1 (ng/ml)	338.74 ± 25.77	1355.16 ± 57.23*	1763.19 ± 82.46 ^{*S}
sE-selectin (ng/ml)	40.39 ± 7.26	94.81 ± 12.42*	116.27 ± 17.14 ^{*S}
TNF-α (pg/ml)	13.52 ± 3.63	32.26 ± 6.13*	75.16 ± 9.29 ^{*S}

ALT: Alanine aminotransferase; sICAM-1: Soluble intercellular adhesion molecule1; sVCAM-1: Soluble vascular adhesion molecule 1; s-E-selectin: Soluble E-selectin; TNF-α: Tumor necrosis factor-alpha; (*): indicates a significant difference relative to healthy controls; (S): indicates a significant difference relative to CHC, P < 0.05

Table 3: Shows the Pearson's correlation coefficients test value and the relationship between ALT and sICAM-1, sVCAM-1, sE-selectin and TNF-α in CHC patients with cirrhosis group than CHC patients without cirrhosis group

	Pearson's value (r)	
	CHC without cirrhosis ALT (IU/l)	CHC with cirrhosis ALT (IU/l)
sICAM-1 (ng/ml)	0.341*	0.391*
sVCAM-1 (ng/ml)	0.416*	0.388*
sE-selectin (ng/ml)	0.351*	0.436*
TNF-α (pg/ml)	0.427*	0.394*

ALT: Alanine aminotransferase; sICAM-1: Soluble intercellular adhesion molecule1; sVCAM-1: Soluble vascular adhesion molecule 1; s-E-selectin: Soluble E-selectin; TNF-α: Tumor necrosis factor-alpha; Significance was calculated by Spearman or Pearson correlation (2-tailed), *p < 0.05; r, correlation coefficient

Statistical Analysis

Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. Means of different groups were compared using one-way ANOVA. The relationship between continuous variables and ALT was assessed by Pearson or Spearman rank correlation. All data were expressed as the mean ± SD. P<0.05 indicated statistical significance.

RESULTS

The demographic and clinical characteristics of the all participants are shown in Table 1. The mean age of the healthy controls group was 48.96 ± 5.18 years, and the mean age of the CHC without cirrhosis group was 50.73 ± 4.24 years, where the mean age of the CHC with cirrhosis group was 49.16 ± 5.71 years. There was no significant age, body mass index, waist circumference, fat mass, fasting blood glucose and hemoglobin between the three groups. However, aspartate aminotransferase, albumin and total bilirubin were significantly different between the healthy controls, CHC without cirrhosis and CHC with cirrhosis groups.

The mean values of alanine aminotransferase, soluble intercellular adhesion molecule1, soluble vascular adhesion molecule 1, soluble E-selectin and tumor necrosis factor-alpha were significantly elevated in CHC patients with cirrhosis group and CHC patients without cirrhosis group when compared with Healthy controls group. Also, these parameters were significantly elevated in CHC patients with cirrhosis group than CHC patients without cirrhosis group (Table 2). The Pearson's correlation coefficients test for the relationship between Alanine aminotransferase and sICAM-1, sVCAM-1, sE-selectin and TNF-α in CHC patients with cirrhosis group than CHC patients without cirrhosis group showed a strong direct relationship (Table 3).

DISCUSSION

At present, the most reliable determination of severity and prognosis in chronic viral hepatitis is the histological staging of the disease which is an invasive procedure that is often not well accepted by patients. The search for alternative non-invasive methods is mandatory especially in follow-up after initial assessment by biopsy (26). Some authors reported that

circulating levels of adhesive molecules are related to degree of inflammatory activity and to histological score, suggesting a putative role in monitoring the follow-up (27, 28). Others have declared that their measurement adds little to the information provided by traditional biochemistry (29). The aim of this study was to determine the strength of the association between adhesive molecules and inflammatory markers among hepatitis C virus patients.

The results of our present paper showed that TNF- α levels in patients with chronic HCV and patients with liver cirrhosis are higher than in healthy controls. Thus, demonstrating that HCV individuals have a characteristic inflammation as compared to the normal population. Therefore, we proposed TNF- α as a marker of sustained response for the HCV population undergoing standard therapy. These results are in line with other studies as Neuman et al. who reported that serum TNF- α levels are significantly higher among patients with CHC compared to healthy volunteers (30). Also, Fallahi et al. stated that production of inappropriate levels of IL-6 and tumor necrosis factor-alpha (TNF- α) has been associated with the progression of chronic hepatitis C (31). However, Shapiro et al. proved that cytokines have been associated with liver injury in HCV and there is evidence that they can also be linked with response to therapy (32). Moreover, Neuman et al. demonstrated a correlation between TNF- α levels and the severity of HCV and the significance of TNF- α levels as mediators of inflammation, thus suggesting an immune-mediated response (33).

Moreover, our results also revealed marked elevation in circulating levels of soluble adhesive molecules sE-selectin, sICAM-1 and sVCAM-1 in both groups of patients with chronic hepatitis C liver disease compared to controls. Many previous studies reported increased sICAM-1 and sVCAM-1 values in patients with inflammatory diseases of the liver (34-38). While, elevated serum VCAM-1 in chronic hepatitis have been reported by some authors (39, 40). Also, many authors confirmed our data related to higher levels of sICAM-1 among patients with chronic HCV-related hepatitis than do control subjects (28, 41, 42). Moreover, similar results are reported in patients with CLD (34) and CHC (18, 25).

Our results also revealed marked elevation in circulating levels of ICAM-1 and VCAM-1 in cirrhotic patients compared to non-cirrhotic cases. Enhanced levels of these adhesion molecules may be the consequence of persistent activation of vascular endothelial cells which are able to produce connective tissue growth factor, a highly profibrogenic molecules involved in several fibrotic disorders, including those of the liver (43).

Such elevation in circulating ICAM-1 and VCAM-1 levels may be also attributed to increased levels of pro-inflammatory cytokines as TNF- α which was also reported in our study. TNF- α is a potent mediator of inflammation and sepsis (44) and has a pleiotropic effect on a wide variety of cells including endothelial cells (45). Furthermore, data revealed the circulating levels of TNF- α in both groups of patients were strongly correlated with each of sICAM-1 and sVCAM-1 values in these patients. These findings indicate that TNF- α might directly induce the expression of ICAM-1 and VCAM-1 in vascular endothelial cells (44).

Moreover, we found a significant positive correlation between adhesive molecules and ALT values. Then, our findings appear in agreement with literature data and suggest that VCAM-1, which is considered to reflect the degree of liver fibrosis (25, 28) and ICAM-1 levels, markers of liver inflammation, have significantly decreased in HCV patients with normal ALT levels following interferon administration (28,42,46,47). However, the relationship between ICAM-1, VCAM-1 and ALT values does not implicate a direct involvement of these adhesion molecules in physiopathology of hepatocellular damage, but it could only reflect the activation of undergoing immunological mechanisms.

Concerning the relationship between TNF- α levels and ALT, we found a positive relationship between them, these results are in line with Hassan et al. proved a positive relationship between TNF- α levels and aminotransferase levels (48), also Neuman et al. stated that TNF- α levels significantly decreased at the end of treatment and the decline paralleled those observed with aminotransferase levels (30). However, Durante-Mangoni et al. found an elevated serum levels of TNF- α have also been associated with hepatic steatosis (49). Moreover, Tilg et al. proved a positive correlation between TNF- α levels and inflammation and fibrosis in liver biopsies (50).

CONCLUSION

In conclusion, our results confirm that, in patients with chronic virus hepatitis and liver cirrhosis there is a significant positive correlation between serum TNF- α , adhesive molecules and ALT values. These findings suggest that serum TNF- α levels could be used as a sensitive predictor of liver inflammation, while serum ICAM-1 can be considered as a marker of hepatic necrosis and inflammatory activity in chronic hepatitis, while serum VCAM-1 is an indicator for the severity of liver cirrhosis.

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