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The Levels of Soluble Intercellular Adhesion Molecule, Vascular Adhesion Molecule and Se-Selectin Levels in Patients with Non-Alcoholic Fatty Liver Disease

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Abstract

Background: High levels of adhesion molecules are known to result in inflammation of the vascular cells through endothelial dysfunction and thus atherogenesis. To this end, this trial was performed to investigate whether a cause of the accelerated atherogenesis in cases with non-alcholic fatty liver disease (NAFLD) was the increase in the levels of the adhesion molecules.

Material and Methods: 53 cases with NAFLD diagnosed using ultrasonography and biopsy and 46 control cases were enrolled in the trial. Soluble intercellular adhesion molecule (sICAM-1), Vascular adhesion molecule (sVCAM) and sE-selectinconcentrations were determined by using an enzyme linked immunosorbent assay (ELISA) kit from Biosource (Bender MedSystems GmbH, Vienna Austria) according to the manufacturer's instructions.

Results: The levels of these adhesion molecules in patients with NAFLD were higher than those in the control subjects but only a significant difference in sE-selectin levels between the NAFLD and control groups was observed ($p<0.05$). However, there were no statistically significant differences in sICAM-1 and sVCAM-1 levels between the two groups ($p>0.05$).

Conclusions: The high levels of adhesion molecules and particularly of the sE-selectin statistically suggest that endothelial dysfunction and inflammation occur, which may represent a major factor in accelerating the atherogenesis process. We believe that detection of a high sE-selectin level would be leading the way when developing new therapeutical modalities for cases with NAFLD.

Keywords: sICAM-1; sVCAM; sE-selectin; NAFLD

Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a wide range of liver diseases that range from from fatty liver alone to nonalcoholicsteatohepatitis (NASH). NAFLD is considered the hepatic manifestation of the metabolic syndrome. It also is associated with an increased risk for cardiovascular disease [1,2]. A recent meta analysis suggests that patients with NAFLD have significantly greater carotid intima-media thickness and carotid plaques than patients without NAFLD, independently of the classical risk factors of the Metabolic Syndrome [3]. Similarly, patients with NAFLD have an increased prevalence of vulnerable coronary plaques [4]. Additionally, a strong relation between NAFLD and endothelial dysfunction was previously described [5]. Finally, cross-sectional studies suggested that the presence of cardiovascular complications in patients with NAFLD increases with the histological severity of the disease [6]. Despite the above mentioned evidence, it is still controversial whether NAFLD is merely associated with the cardiometabolic risk factors, or is an independent causal factor that promotes by itself a systemic proatherogenic and inflammatory state. This debate is further motivated since the pathogenic pathways and molecular processes that link NAFLD and cardiovascular disease remain unidentified. Nevertheless, while still inconclusive, several studies showed the association of NASH with elevated circulating levels of inflammatory biomarkers (C-reactive protein, tumor necrosis factor alpha, interleukin 6 (IL6), chemokine ligand, plasminogen activator inhibitor-1 (PAI-1) and fibrinogen [2,7-10]) and endothelial dysfunction (soluble intercellular adhesion molecule-1, sICAM-1) [11]. Among many molecular mediators implicated in the atherosclerotic process, some of them merit particular attention in the evaluation and stratification of cardiovascular risk. For example, circulating levels of sICAM-1 are associated with endothelial dysfunction [12].

There is increasing evidence that the production and secretion of proinflammatory factors in endothelial cells play a key role in atherogenic process [13]. E-selectin is a major Endothelial Cell adhesion molecule which regulates the endothelial adhering and extravasation of leukocytes in order to reach to the sites of inflammation. When Endothelial Cells are activated in response to

cytokines, the expression of cell adhesion molecules on their surface is significantly increased [14]. The appearance of soluble cell adhesion molecules (i.e. intercellular adhesion molecule-1 [ICAM-1], vascular adhesion molecule-1 [VCAM-1], and E-selectin) in the circulation is thought to be the consequence of their release from the surface of activated Endothelial Cells due to increased expression [15].

Thus, this trial was designed to investigate whether the elevated levels of adhesion molecules, leading to endothelial dysfunction and inflammation of the vascular cells also exist in NAFLD cases.

Material and Methods

This study included 53 patients diagnosed with NAFLD and 45 healthy controls. Subjects enrolled in the study were admitted to Buca State Hospital Internal Medicine and Izmır BozyakaTrainig Research Hospital Outpatient Clinics between May 2010 and July 2011 with elevated or normal serum aminotransferase values and found to have hepatosteatosis by ultrasonography. Routine biochemical analyses including serum ALT, AST, bilirubin, albumin, fasting blood glucose (FG), serum insulin level, BUN and creatinine levels, lipid profile, viral markers, TORCH group hepatotropic viruses, HCV RNA, autoimmune markers (ANA, SMA, AMA, LKM1), whole blood count, serum iron level, iron binding capacity, transferrin saturation index, ferritin level, serum Cu and ceruloplasmin level, α-1 antitrypsin, thyroid function tests (sT3, sT4, TSH, anti-M), serum insulin level, PT, PTT, and IgG, IgA, and IgM measurements were obtained. Age, sex, smoking status and drug use, history of surgical operations, height, weight, and body mass index of the patients were recorded. Other conditions such as diabetes mellitus, hyperlipidemia, and hypertension were investigated. Patients with a history of drug use, gastrointestinal surgical operation, alcoholic liver disease, viral hepatitis (B,C,D, and TORCH), cholestatic liver disease, hemochromatosis, liver disease secondary to drug use, Wilson's disease, α-1 antitrypsin deficiency, and history of alcohol intake >20 g/day were excluded from the study.

Hepatic ultrasonography was performed by a radiologist with an Hitachi 6500 EUB Doppler ultrasonography device using a 3.0- to 6.0-mHz convex probe. Findings were as follows: grade I, there was a diffuse hyperechoic (shiny liver) hepatic appearance; grade II, liver echogenicity was increased compared to the kidneys; and grade III, mural echoes of hepatic vascular structures were indistinct and deep attenuation was observed.

Liver biopsy was performed with ultrasound guidance and modified 1.4mm diameter Menghini needles (Hepafix, Braun, Germany) under local anesthesia on an outpatient basis. Liver biopsy specimens were routinely fixed in 40 g/l formaldehyde (pH 7.4) embedded in paraffin and stained with hematoxylin and eosin, Masson trichrome and silver impregnation for reticular fibers. The same liver pathologist, who was blinded to patient details, read all the biopsies. All the biopsies were at least 2 cm in length and contained a minimum of 8 portal tracts. The degree of steatosis was assessed on the percentage of hepatocytes containing macrovesicular fat droplets. NAFLD was defined as steatosis plus mixed inflammatory-cell infiltration, hepatocyte ballooning and necrosis, glycogen nuclei, Mallory's hyaline, and any stage of fibrosis, including absent fibrosis.

Venous fasting blood samples were collected from an antecubital vein. All blood samples were collected under minimal tourniquet pressure. Blood samples were allowed to clot for 15 to 30 minutes, and were centrifuged at 1500g for 10 minutes. The serum was then separated and stored at -20°C until the analysis. Samples were thawed only once.

Serum sICAM-1, sVCAM-1 and sE-selectin concentrations were determined by using an enzyme linked immunosorbent assay (ELISA) kit from Biosource (Bender MedSystems GmbH, Vienna Austria) according to the manufacturer's instructions. The sensitivity of the sICAM-1, sVCAM-1 and sE-selectin assays wsd 2.2 ng/mL, 0.6 ng/mL and 0.3 ng/mL respectively. The intra-assay coefficients of variation (CV) for sICAM-1, sVCAM-1 and sE-selectin were 4.1, 3.1 and 5.4% respectively and the inter-assay coefficients of variation (CV) for them were 7.7, 5.2 and 6.0% respectively, according to the manufacturer.

Statistical analyses

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 11.0 for Windows, Chicago, Ill, USA). Data were expressed as mean±standard deviation (SD). A "p" value<0.05 was accepted as significant. Kolmogorov-Smirnov test was performed to assess the normality of the variables. According to Kolmogorov-Smirnov test, the distribution of sVCAM-1 sE-selectin and fasting blood glucose were normal (p>0.05) whereas the distribution of sICAM-1 and other parameters were not normal. Normally distributed variables were compared using the Student's t-test whereas sICAM-1 and the others were compared with Mann-Whitney U test. Differences in the categorical variables were measured by Chi-Square test.

	NAFLD	Control	P value
n	53	45	
Age (y)	58.8 ± 5.9	56.2 ± 6.1	0.212
Sex (female/male)	32/21	26/19	0.399
BMI (kg/m2)	32.0 ± 4.1	32.8 ± 3.0	0.402
ALT (IU/L)	44.1 ± 28.7	23.0 ± 3.1	<0.001
AST (IU/L)	37.6 ± 19.6	22.0 ± 3.3	<0.001
FG (mg/dL)	90.3 ± 9.0	87.1 ± 7.0	0.098
TG (mg/dL)	188.7 ± 88.6	187.1 ± 25.8	0.111
T.Chol (mg/dL)	199.5 ± 30.4	190.2 ± 12.7	0.127
HDL-C (mg/dL)	42.6 ± 10.0	40.6 ± 4.6	0.198
LDL-C (mg/dL)	119.5 ± 23.5	112.3 ± 11.5	0.101

NAFLD: Non Alcoholic Fatty Liver Disease; BMI: Body Mass Index; ALT: Alanineaminotransferase; AST: Aspartate aminotransferase; FPG: Fasting Plasma Glucose; TG: Triglyceride; T.Chol: Total Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol

Table 1: Clinical and biochemical characteristic of patients with NAFLD and control subjects. Results were presented as mean ± SD

Results

The clinical and biochemical characteristic of the 53 subjects with non-alcoholic fatty liver disease and 45 healthy controls, with a mean age of 58.8 ± 5.9 and 56.2 ± 6.1 respectively, are summarized in Table 1. Age, sex, BMI were similiar among the groups. The levels of ALT, AST were significantly higher in patients with NAFLD (<0.001). But

other biochemical analyses including fasting plasma glucose and serum lipid profile in NAFLD group were not significantly different when compared with control group (p>0.05).

Serum sICAM-1, sVCAM-1 and sE-selectin concentrations are presented as mean ± SD in Table 2. The levels of these adhesion molecules in patients with NAFLD were higher than control subjects but only a significant difference in sE-selectin levels between NAFLD and control groups was obtained (p<0.05). However, there were not statistically significant differences in sICAM-1 and sVCAM-1 levels between two groups (p>0.05) (Table 2 and Figure 1).

	NAFLD	Control	P value
sICAM (ng/mL)	551.21 ± 143.79	490.99 ± 89.73	0.085
sVCAM (ng/mL)	619.19 ± 142.39	617.69 ± 153.57	0.969
sE-selectin (ng/mL)	50.74 ± 18.43	33.64 ± 15.30	0.000

Table 2: The levels of adhesion molecules of patients with NAFLD and control subjects (Results were presented as mean ± SD, sICAM: Soluble intercellular adhesion molecule; sVCAM: Soluble vascular adhesion molecule.)

Discussion

Leukocyte adhesion to vascular endothelial cells is an important inflammatory process. Adhesion is mediated via a number of receptors on the leukocyte surface-associated antigen-1 (LFA-1), a member of the CD18 family of adhesion molecules, which binds to ICAM-1 on the target cells.

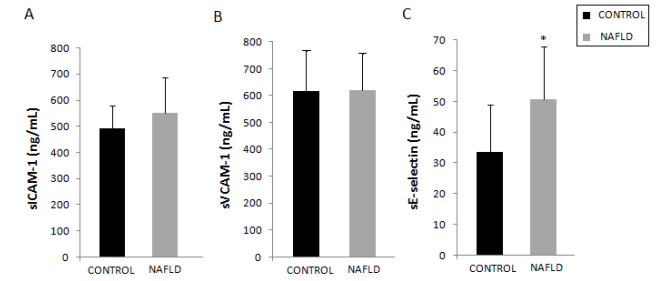

Figure 1: Serum sICAM-1, sVCAM-1 and sE-selectin levels in control subjects and patients with NAFLD (A. Serum sICAM-1 levels and B. serum sVCAM-1 levels; (B) were elevated in patients with NAFLD but the differences did not reach significance; C. The levels of sE-selectin were significantly high in patients with NAFLD (*p<0.001)).

Intercellular adhesion molecule-1 is a cellular surface glycoprotein expressed on endothelial cells, epithelial cells, hepatocytes, fibroblasts, and hematopoietic cells. Expression can be increased in vitro by proinflammatory cytokines such as interferon γ, IL-1, and TNF-α.

The previous data from epidemiological studies suggesting that NAFLD patients have an elevated risk for cardiovascular disease [16,17]. Unfortunately, data from prospective studies is not currently available, and the evidence about the incidence of cardiovascular complications in patients with NAFLD is indirectly estimated from large studies designed to evaluate the relation between type 2 diabetes and cardiovascular risk [18]. Additional evidence is supported by a long-term clinical cohort study of patients with NAFLD that suggested a high mortality related to cardiovascular disease [16].

E-selectin is a specific endothelial adhesion receptor which is induced by pro-inflammatory stimuli. It mediates the adhesion of leukocytes to endothelial cells allowing their extravasation into inflamed tissues. Inflamed vascular endothelium has been recognized as an attractive site for targeted delivery of therapeutic and imaging agents because of significant difference in the expression of surface receptor proteins between normal and inflamed endothelium. Expression of e-selectin on endothelial cells is transcriptionally induced in the presence of inflammatory stimuli, and subsequently, E-selectin expression is commonly seen in pathological inflammation, including cancer [7]. Additionally, following membrane sorting upon cytokine stimuli, E-selectin undergoes a recycle phase, rapid internalization to endosomes and subsequent partial lysosomal degradation. These characteristics, inflammation- dependent expression and internalization, make E-selectin an attractive target for intracellular delivery of therapeutics to inflamed vasculature.

In this study, we investigated the serum levels of the adhesion molecules in NAFLD cases. While there is literature data reporting elevated levels of sICAM, we detected sICAM and sVCAM levels within the normal limits. On the other hand, a high level of s-E-selectin is not only significant with respect to inflammation but its increased expression observed in case of pathological inflammation in cancer patients is also important. We believe that detection of an elevated sE-selectin level would be leading the way when developing new therapeutical modalities for NAFLD patients.

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