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Hepatitis C virus seropositivity and TNF superfamily receptors: sCD40, sFas – the new putative determinants of endothelial dysfunction in haemodialysis patients

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ABSTRACT

Introduction: Hepatitis C virus (HCV) infection occurs frequently among haemodialysis (HD) patients and increases the risk of atherosclerosis. CD40 and Fas belong to tumor necrosis factor receptor (TNFR) superfamily, which play a role in hepatocyte apoptosis during HCV infection. The aim of the present study was to determine whether anti-HCV-seropositivity constitutes an additional risk factor for endothelial dysfunction in HD patients, and whether sCD40 and sFas could be associated with endothelial dysfunction.
Materials and methods: A total of 69 stable HD patients and 28 healthy controls were included in this study. Patients were divided into anti-HCV-seropositive (HCV[+], n = 18) and anti-HCV- seronegative (HCV[-], n = 51). The plasma endothelial markers: von Willebrand factor (vWF), thrombomodulin (TM), soluble adhesion molecules – sICAM-1, sVCAM-1 and TNFRs were assayed.
Results: HD patients showed a significant increase in the levels of TM, sVCAM-1, sCD40 and sFas compared to controls, and all these parameters were higher in HCV[+] than in HCV[-] patients. sICAM-1 concentrations were higher in the HCV[+] group compared to controls and the HCV[-] group. vWF levels were higher in HD patients than in the controls, however there was no difference in this parameter between HCV[+] and HCV[-] group. The anti-HCV-seropositivity and sCD40 were determined as an independent variables of TM, whereas anti-HCV-seropositivity and sFas were found as independent determinants of sICAM-1 and sVCAM-1 levels.
Conclusions: This study showed that anti-HCV-seropositivity and TNF superfamily receptors: sCD40 and sFas are the novel determinants of the increased plasma endothelial dysfunction markers in haemodialysis patients.

1. Introduction

Hepatitis C virus infection (HCV) is one of the most frequent infections in haemodialysis (HD) patients. Accumulating evidence suggests that HCV increases the risk of atherosclerotic cardiovascular disease (CVD) [1] by causing insulin resistance and the release of proinflammatory cytokines [2,3]. Moreover, the chronic HCV infection also has propensities to develop the endothelial cell (EC) perturbation, reflecting by higher plasma levels of von Willebrand factor (vWF), thrombomodulin (TM) and soluble adhesion molecules both in general [4–7] and in uraemic [8–10] population.

Liver damage in chronic HCV-infected patients is mostly due to the host's mediated immune response with T lymphocytes, playing crucial role in the clearance of viral infection [11]. CD40 and Fas are membrane-bound glikoproteins belong to tumor necrosis factor receptor (TNFR) superfamily. In the liver, the Fas pathway is thought to be a key mechanism for hepatocyte apoptosis [12]. CD40 also plays a role, by inducing cell-surface Fas ligand expression [13]. The circulating, soluble forms both CD40 (sCD40) and Fas (sFas) are described as an apoptotic markers in anti-HCV- seropositive patients [14,15].

Endothelial dysfunction, an early abnormality for development of atherosclerosis, has been reported among HD patients, and elevated plasma levels of endothelial dysfunction markers, such as vWF, TM and soluble adhesion molecules – sICAM-1, sVCAM-1 have been described as the risk factors for the carotid atherosclerosis in this population [16,17]. The previous studies performed on HD patients showed also the increased plasma levels of sCD40 [18] and sFas [19]. Moreover, both sFas and sCD40 ligand were suggested as markers of coronary artery disease in these patients [20,21].

Taking all these data into consideration, the aim of the present study was to determine whether anti-HCV-seropositivity constitutes an additional risk factor for EC dysfunction and TNF superfamily receptors levels in haemodialysis patients. Moreover, we wanted to

Abbreviations: HCV, hepatitis C virus; HCV[+], anti-HCV-seropositive; HCV[-], anti-HCV- seronegative; EC, endothelial cell; vWF, von Willebrand factor; TM, thrombomodulin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; HD, haemodialysis.

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know if sCD40 and sFas could be associated with endothelial function in these patients.

2. Material

2.1. Subjects investigated

Sixty-nine adult patients on chronic maintenance HD were enrolled in this cross-sectional study. There were 43 male and 26 female patients. The patients were routinely haemodialyzed three times weekly for 4.0 hours with bicarbonate dialysate and low molecular weight heparin-enoxaparin as anticoagulation. The dialysate was endotoxin-free (Coatest Kabi Vitrum). The amount of delivered dialysis (Kt/V) was calculated using a single-compartment model. Body mass index (BMI) was calculated by dividing the dry weight in kilograms by the square of the height in meters. CVD was defined as documentation in the medical record of a history of myocardial infarction, ischaemic stroke, coronary revascularization procedures, angina pectoris, typical changes on coronary angiograms, typical ischaemic changes on electrocardiogram, peripheral artery surgery (not including the arterio-venous fistula), intermittent claudication or pain at rest. None of the patients received immunosuppressive or antiviral treatment, lipid-lowering agents, non-steroidal anti-inflammatory drugs, iron supplementation or antioxidants such as vitamin E, C or allopurinol during one month preceding the study. All medications received in the prior 3 months were recorded.

Positive anti-HCV antibody reaction was found in 18 (26%) patients. We therefore divided all patients into two groups: anti-HCV-seronegative (HCV[-], n = 51) and anti-HCV-seropositive (HCV[+], n = 18). Detailed characteristics of HCV[-] and HCV[+] groups were done in Table 1. Patients with other causes of chronic liver disease, alcoholism, haemochromatosis, autoimmune hepatitis or hepatitis B virus infection were excluded from the study.

Twenty-eight healthy volunteers matched for age (mean 58.21 ± 6.79 years) and gender (17 men/11 women) with HD patients served as controls for determination of endothelial function, sCD40 and sFas levels. Approval by our institutional ethical board (according to Declaration of Helsinki) was obtained and all patients and controls gave informed consent.

3. Methods

The HD patients were investigated on a mid-week day, before a dialysis session. Venous blood samples were collected both from HD patients and controls in the morning after an overnight fast. Citrated-plasma and serum samples were prepared conventionally, aliquoted and stored at -40 °C until the assay.

The plasma levels of: TM, vWF, sICAM-1, sVCAM-1 and sFas were determined by commercially available ELISA kits and standards (IMUBIND Thrombomodulin ELISA Kit, IMUBIND vWF ELISA from American Diagnostica Inc., Greenwich, CT; Quantikine Human sICAM-1/CD54, Quantikine Human sVCAM-1, Quantikine Human sFas from R&D Systems Europe Ltd, Abingdon, UK; respectively). Plasma sCD40 was determined using ELISA kit manufactured by Bender Medsystems GmbH, Vienna, Austria. The intra-assay coefficient of variation (CV) were 5.5%, 4.7%, 4.6%, 3.1% and 4% for sCD40, sFas, sICAM-1, sVCAM-1 and TM; respectively. The inter-assay CV were 7%, 7.9%, 5.5%, 7% and 5.2% for sCD40, sFas, sICAM-1, sVCAM-1 and TM; respectively.

Table 1
Clinical and biochemical characteristics of anti-HCV-negative (HCV[-]) and anti-HCV-positive (HCV[+]) haemodialyzed patients.

	HCV[-]	HCV[+]	p value
Age, years	64.12 ± 13.83	56.64 ± 15.24	0.1137
Males/females, n	32/19	11/7	0.9526
BMI, kg/m^2	25.97 ± 4.21	25.59 ± 6.29	0.8660
Haemodialysis duration, months	26.50 (3.0-118.0)	98.0 (4.0-241.0)	0.0010
Kt/V	1.23 ± 0.30	1.22 ± 0.20	0.8774
Systolic blood pressure, mmHg	133.9 ± 23.7	126.9 ± 21.2	0.2010
Diastolic blood pressure, mmHg	85.3 ± 11.2	78.8 ± 12.9	0.3160
Smokers, %	25.0	33.0	0.6341
Cardiovascular disease, %	86.0	78.0	0.8010
Erythropoietin treatment, %	78.0	83.0	0.8820
Erythropoietin dose, U/kg body weight/week	87.0 (18.7-160.7)	82.2 (17.5-206.8)	0.7856
ACE inhibitor, %	63.0	39.0	0.4723
Calcium channel antagonist, %	51.0	50.0	0.9673
β-blocker, %	53.0	56.0	0.9167
α-blocker, %	12.0	17.0	0.6448
Nitrates, %	20.0	11.0	0.5739
Total cholesterol, mg/dl	209.94 ± 51.02	235.46 ± 51.97	0.1315
HDL-cholesterol, mg/dl	43.39 ± 12.22	38.46 ± 8.71	0.1103
LDL-cholesterol, mg/dl	135.73 ± 46.69	163.77 ± 44.86	0.0617
Triglycerides, mg/dl	133.0 (54.0-475.0)	122.0 (44.0-421.0)	0.4468
Haemoglobin, g/dl	11.07 ± 1.23	10.96 ± 1.04	0.7452
White blood cells, x10^3/μl	6.16 ± 1.84	5.51 ± 1.74	0.2306
Platelets, x10^3/μl	208.61 ± 58.34	168.07 ± 68.12	0.0572
Bilirubin, mg/dl	0.46 ± 0.21	0.56 ± 0.31	0.2400
ALT, IU/L	15.0 (8.0-61.0)	17.5 (7.0-90.0)	0.2345
ALP, IU/L	92.0 (14.0-295.0)	128.0 (66.0-980.0)	0.0300
Total protein, g/dl	6.66 ± 0.51	6.91 ± 0.56	0.1564
Albumin, g/dl	3.94 ± 0.41	3.92 ± 0.40	0.9055
Fibrinogen, mg/dl	377.18 ± 81.49	289.07 ± 79.79	0.0024
aPTT, s	35.40 ± 6.07	38.44 ± 4.52	0.0536
PT, s	16.08 ± 3.12	17.39 ± 1.57	0.0419
INR	1.08 ± 0.26	1.19 ± 0.13	0.0436

Data are shown as mean ± SD or median (range) depending on their normal or skewed distribution. Arterial blood pressure values were calculated as a mean of 10 measurements. BMI = body mass index; ACE = angiotensin I-converting enzyme, ALT = alanine aminotransferase; ALP = alkaline phosphatise; aPTT = activated partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio; ACE = angiotensin I-converting enzyme.