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Effect of hepatitis C serology on C-reactive protein in a cohort of Brazilian hemodialysis patients

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Abstract

Hepatitis C (HCV) is not an uncommon feature in hemodialysis (HD) patients and may be a cause of systemic inflammation. Plasma cytokine interleukin-6 (IL-6) is mainly produced by circulating and peripheral cells and induces the hepatic synthesis of C-reactive protein (CRP), which is the main acute phase reactant. The aim of this study was to investigate the influence of HCV on two markers of systemic inflammation, serum CRP and IL-6, in HD patients. The study included 118 HD patients (47% males, age 47 ± 13 years, 9% diabetics) who had been treated by standard HD for at least 6 months. The patients were divided into two groups depending on the presence (HCV+) or absence (HCV-) of serum antibodies against HCV. Serum albumin (S-Alb), plasma high sensitivity CRP (hsCRP), IL-6, and alanine aminotransferase (ALT) were measured and the values were compared with those for 22 healthy controls. Median hsCRP and IL-6 values and hsCRP/IL-6 ratio were: 3.5 *vs* 2.1 mg/l, $P < 0.05$; 4.3 *vs* 0.9 pg/ml, $P < 0.0001$, and 0.8 *vs* 2.7, $P < 0.0001$, for patients and controls, respectively. Age, gender, S-Alb, IL-6 and hsCRP did not differ between the HCV+ and HCV- patients. However, HCV+ patients had higher ALT (29 ± 21 *vs* 21 ± 25 IU/l) and had been on HD for a longer time (6.1 ± 3.0 *vs* 4.0 ± 2.0 years, $P < 0.0001$). Moreover, HCV+ patients had a significantly lower median hsCRP/IL-6 ratio (0.7 *vs* 0.9, $P < 0.05$) compared to the HCV- group. The lower hsCRP/IL-6 ratio in HCV+ patients than in HCV- patients suggests that hsCRP may be a less useful marker of inflammation in HCV+ patients and that a different cut-off value for hsCRP for this population of patients on HD may be required to define inflammation.

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Key words
- Hepatitis C
- C-reactive protein
- Interleukin-6
- Hemodialysis

Introduction

Hepatitis C virus (HCV) is a common problem in patients with chronic kidney disease (CKD) (1,2). In Brazil the prevalence of HCV antibodies in hemodialysis (HD) patients is as high as 20-50%, depending on the region (3). The course of HCV may extend over decades in non-uremic patients, but in HD patients the long-term follow-up of HCV-infected patients is limited due to elevated morbidity and mortality (4).

C-reactive protein (CRP) is an independent predictor of mortality in HD patients (5,6). CRP expression *in vivo* is assumed to be restricted mainly to the liver (7) where CRP is produced under the control of various pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor α (TNF-α) (8). IL-6 is the prototypic pleiotropic cytokine commonly produced at local tissue sites, and circulating receptors modulate the biological effects of cytokines (9). The IL-6 receptor consists of two units: a ligand-binding part (gp-80) and a signal transducing part (gp-130) (10). High levels of IL-6 and its soluble receptor are present in cirrhotic as well as CKD patients (9,11-13). Nevertheless, there are few reports describing the CRP response to increased plasma IL-6 levels in HCV+ renal patients.

In response to various stimuli such as uremia, HCV infection, and HD itself, naive CD4-T cells differentiate into effector T helper (Th) cells and, according to the pattern of cytokine production, two T subsets of cells have been defined: Th1 cells, which produce interferon gamma and IL-2, and Th2 cells, which produce IL-4 and IL-10 and other cytokines such as IL-6 (14,15). A predominance of Th1 cytokine expression has been observed in patients with CKD, but it is not known if this imbalance could affect the production of CRP in the liver, especially in HD patients with liver injury such as hepatitis C.

The purpose of the present study was to investigate if HD patients with serological evidence of HCV (HCV+) have different levels of high sensitivity CRP (hsCRP), IL-6 and hsCRP/IL-6 ratio compared to HD patients without serological evidence of HCV (HCV-).

Subjects and Methods

Patients and study design

A total of 118 HD patients (56 males; median age, 47 years; range, 16-89 years) treated at three dialysis centers in the city of Curitiba (PR, Brazil) were enrolled in the study. The main inclusion criterion was HD treatment for at least 6 months. Patients with chronic inflammatory disease, i.e., rheumatic diseases, and active infection as well as hepatitis B, defined by the serum detection of HBs antigen, were excluded from the study. The causes of renal failure were as follows: chronic glomerulonephritis (N = 54), hypertensive nephrosclerosis (N = 32), diabetic nephropathy (N = 13), and other causes (N = 19). All patients were hemodialyzed three times weekly with modified cellulose membranes (cellulose acetate or derived cellulose). None of the patients had been treated with antiviral agents such as interferon or ribavirin. Twenty-two healthy controls were used for comparative analysis. The Ethics Committee of Hospital Evangélico de Curitiba approved the study protocol and all subjects gave written informed consent to participate in the study.

Biochemical analysis

HD patients were investigated during a mid-week session before dialysis. Venous blood samples were collected from the HD patients and control subjects in the morning after an overnight fast. Plasma was separated from blood cells and stored at -70ºC until the time for analysis. Serum albumin (S-Alb) was determined by the bromocresol

green method. Plasma hsCRP and alanine transferase were measured by the nephelometry method. Plasma IL-6 levels were measured by ELISA (Ortho, Raritan, NJ, USA). The diagnosis of hepatitis C was made based on the detection of anti-HCV antibodies by the anti-HCV test using second-generation ELISA (ELISA-2; Ortho) based on six consecutive measurements performed monthly before the beginning of the study.

Statistical analysis

Data are reported as means ± SD, or median and range as appropriate. A P value below 0.05 was considered to be significant. Comparisons between two groups were performed by the Student *t*-test for normally distributed variables, whereas the Mann-Whitney U-test was used for non-normally distributed variables. Categorical variables were analyzed using contingency tables. For non-normally distributed variables, correlations were calculated using the Spearman rank test. Log values of hsCRP and IL-6 were used for univariate regression analysis.

Results

Clinical and laboratory data for HD and controls

The clinical characteristics of HD patients and healthy controls are given in Table 1. In HD patients, a significant negative correlation was found between S-Alb and hsCRP (Rho = -0.21, $P < 0.05$). Positive correlations were found between IL-6 and age (Rho = 0.38, $P < 0.0001$) and IL-6 and hsCRP (Rho = 0.48, $P < 0.0001$), whereas an inverse correlation was found between IL-6 and S-Alb (Rho = -0.20, $P < 0.01$). On the other hand, no significant correlation was found between hsCRP and age (Rho = 0.13, $P = 0.15$). Whereas a significant negative correlation was found between hsCRP/IL-6 and alanine transferase (Rho = -0.23, $P = 0.01$), no significant correlations were found between the hsCRP/IL-6 ratio and age or time on dialysis, respectively.

Comparisons of patients according to HCV serology

The basal clinical and laboratory characteristics of the two groups are listed in Table 2. Sixty-two patients had anti-HCV antibodies. As expected, the mean time on HD treatment was significantly increased in the HCV+ group (6 ± 3 years) compared to the HCV- group (4 ± 2 years, $P < 0001$). The median level of serum hsCRP (mg/l) was lower in the HCV+ group than in the HCV-

Table 1. Clinical and biochemical characteristics of patients on hemodialysis and healthy controls.

	HD patients (N = 118)	Controls (N = 22)
Age (years)[a]	47 ± 13	32 ± 9*
Male gender (%)	47	50
Diabetes mellitus (%)	11	-
hsCRP (mg/l)[b]	3.5 (0.2-150)	2.1 (0.1-7.8)*
IL-6 (pg/ml)[b,c]	4.3 (0.9-19.6)	0.9 (0.3-2.7)*
CRP/IL-6 ratio[b]	0.8 (0.07-21)	2.7 (0.1-17)*
S-Alb (mg/dl)[a]	3.6 ± 0.1	4.6 ± 0.1*
Time on HD (years)[a]	5 ± 3	-

[a]Data reported as means ± SD; [b]data reported as median and range; [c]N = 115. HD = hemodialysis; hsCRP = high sensitivity C-reactive protein: IL-6 = interleukin-6; S-Alb = serum albumin.
*P < 0.05 compared to HD patients (Student *t*-test and Mann-Whitney U-test).

Table 2. Clinical and dialysis characteristics of the patients according to HCV serology.

	HCV+ (N = 62)	HCV- (N = 56)
Age (years)[a]	45 ± 11	49 ± 15
Prevalence of males (%)	48	47
Time on HD (years)[a]	6 ± 3	4 ± 2*
S-Alb (mg/dl)[a]	3.5 ± 0.4	3.5 ± 0.3
ALT (IU/l)[a]	30 ± 21	20 ± 24*
hsCRP (mg/l)[b]	6.6 (0.2-62)	11.8 (0.3-150)
IL-6 (pg/ml)[b]	4.7 (0.9-19)	4.2 (0.98-19.64)
hsCRP/IL-6 ratio	0.7 (0.07-4.2)	0.9 (0.1-21)*

[a]Data reported as means ± SD; [b]data reported as median and range. HCV+, HCV- = presence or absence of serum antibodies against hepatitis C virus; ALT = alanine aminotransferase. For other abbreviations, see legend to Table 1.
*P < 0.05 compared to HCV+ patients (Student *t*-test and Mann-Whitney U-test).

group, but this difference was not statistically significant (P = 0.08). There was no difference in IL-6 levels between the HCV+ and HCV- groups, but the mean hsCRP/IL-6 ratio was significantly lower in the HCV+ group (0.9 ± 0.8 *vs* 1.8 ± 3.2, P < 0.05; Figure 1). Significant correlations were found between log-hsCRP and log-IL-6 in HCV+ (r^2 = 0.26, P < 0.0001) and HCV- patients (r^2 = 0.27, P < 0.0001; Figure 2).

Discussion

Although we could not demonstrate any difference in hsCRP or IL-6 levels between HCV+ and HCV- patients, HCV+ patients tended to have lower CRP levels in relation to IL-6 levels than the HCV- patients and the hsCRP/IL-6 ratio was lower in HCV+ patients. The observed difference could imply that the liver response to IL-6 stimulation may be mitigated by the presence of HCV. Therefore, we may speculate that the hepatic injury caused by HCV could promote a disturbance in the production of CRP.

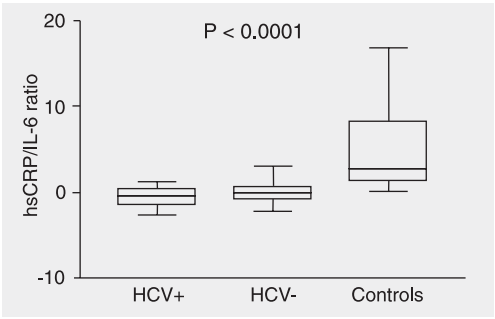

Figure 1. hsCRP/IL-6 ratio in HCV+ and HCV- patients and healthy controls. The horizontal lines in the box plots represent the medians. For abbreviations, see legend to Tables 1 or 2.

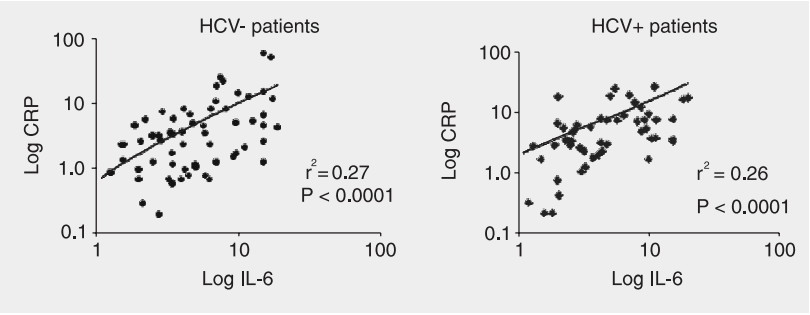

Figure 2. Correlation between serum high sensitivity C-reactive protein (CRP), and interleukin 6 (IL-6) in HCV+ and HCV- patients. The correlation coefficients were calculated by univariate regression analysis.

A decline in the release of acute phase reactants by the liver is observed postoperatively in non-uremic elderly patients undergoing major abdominal surgery (16). In these patients the decreased release of liver-synthesized acute phase reactants was associated with an impairment of hepatocyte function. In the present study, HCV+ patients tended to have lower hsCRP levels compared to HCV- patients. This finding agrees with several lines of evidence previously reported in the literature. Stevens et al. (17) showed that CRP levels were significantly higher in HCV- patients compared to HCV+ patients. Shima et al. (18) compared the expression of CRP by immunohistochemical analysis in HCV+ patients and in patients with hepatitis B and concluded that the intensity of CRP expression in patients with hepatitis B was closely associated with the progression of the disease but this finding was not reproduced in HCV+ patients. Finally, Lin et al. (19) evaluated non-uremic cirrhotic patients regarding the elevation of CRP in response to bacterial infection and showed that a higher CRP cut-off value had to be applied to detect these episodes. In the present study, the presence of a significant difference in the hsCRP/IL-6 ratio in HCV+ patients might indicate that hepatocellular injury could affect CRP production in HCV+ HD patients, although no difference in IL-6 levels could be detected between HCV+ and HCV- patients.

It remains controversial whether alterations in Th cell subpopulations contribute to the pathogenesis and clinical characteristics of chronic hepatitis C (20). Th1 and Th2 cells are involved in the response to various stimuli, such as infection, CKD and HD itself (21). More recently, it has been debated if a shift in the balance between the Th2 and Th1 immune responses may play a role in the progress of chronic hepatitis (15,22). Recently, in a study on non-renal HCV+ and HCV- patients with Sjögren syndrome, an

imbalance of the Th1/Th2 ratio was shown in the HCV+ group (23). The same study demonstrated a predominant Th2 pattern with higher circulating levels of IL-6, IL-10 and TNF-α in HCV+ patients compared to HCV- patients, possibly reflecting a more active systemic inflammatory response. On the other hand, modifications in the Th1/Th2 ratio provoked by ribavirin treatment leading to an imbalance towards the Th1 response may be associated with a more favorable outcome regarding HCV therapy (24-26).

The complexity of the cytokine profile in the HD population appears to be more intricate if we take into account that, besides the presence of HCV, both HD treatment *per se* and CKD might interfere with the balance between Th1 and Th2. In patients with CKD, there is an increased body of evidence indicating a predominance of the Th1 response (14,27). However, Spanakis et al. (22), in a study of the cytokine expression profile in HCV+ compared with HCV- HD patients, found that the virus provoked an induced Th2 immunosuppression, and both Th1 and Th2 were related to a progressive state of HCV chronicity. On the other hand, Rostaing et al. (28), using the cytokine flow cytometry assay, demonstrated no impairment in the production of cytokines in HCV+ dialysis patients. It seems difficult to establish if an imbalance in IL-6 production could have an influence on the production of CRP in the liver. In the present study, we did not detect a significant difference in IL-6 production between the HCV+ and HCV- groups. However, the significant differences in the hsCRP/IL-6 ratio according to HCV serology may imply that the local production of CRP might be impaired by the presence of HCV. Thus, based on these reports, it seems reasonable to propose that further studies are necessary to establish the complex mechanisms of the Th1 and Th2 response in the HCV uremic population.

Some limitations of the current study should be considered. First, the diagnosis of HCV infection was based on an indirect test and the presence of antibodies anti-HCV- with HCV RNA+ is not rare in HD patients (29). Second, the measurement of soluble receptor IL-6 might have been helpful as an indirect marker of cell hepatic responsiveness to IL-6 and therefore of hsCRP expression. Third, the patients were characterized with only one measurement of IL-6 and hsCRP. Finally, histological evaluation by liver biopsy might have better characterized the extension of liver injury and its association with HCV serology.

The finding that the hsCRP/IL-6 ratio was lower in HCV+ patients than in HCV- patients could indicate that hsCRP may be a less useful marker of inflammation in HCV+ patients. Due to a high prevalence of HCV throughout the world in HD clinics, another hsCRP cut-off level for clinically significant inflammation might be needed for dialysis patients with chronic hepatitis C. Further studies are necessary to determine whether the presence of HCV infection influences CRP levels in HD patients.

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