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ORIGINAL ARTICLE

Is there any relationship between serum levels of interleukin-10 and atherosclerosis in hemodialysis patients?

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Abstract

Objective. Cardiovascular complications due to atherosclerosis (AS) are the major cause of mortality in hemodialysis (HD) patients. Inflammation may play an important role in the development of AS. Several studies have demonstrated an association between AS and acute-phase proteins and cytokines in the general population and in HD patients. Interleukin-10 (IL-10) is an anti-inflammatory cytokine. The aim of this study was to compare serum levels of inflammatory and anti-inflammatory indicators in HD patients according to the presence or absence of AS. *Material and methods*. A total of 33 HD patients were studied. AS was defined as the presence of plaques as detected by Doppler ultrasonography. The patients were subgrouped according to the presence or absence of plaques. Serum levels of IL-1, -2, -6 and -10, C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) were measured. Risk factors for AS, such as age, gender, hypertension, hyperlipidemia and duration of HD, were also evaluated. *Results*. Patients with AS had significantly higher high sensitivity (hs)-CRP and lower IL-10 levels. Blood pressure was also elevated in patients with AS. There was an inverse correlation between CRP and IL-10 levels in patients with AS. *Conclusion*. Patients with AS undergoing HD had low serum levels of the anti-inflammatory cytokine IL-10 and high serum levels of hs-CRP. These results may suggest that limitation of the anti-inflammatory response in atherosclerotic uremic patients is a triggering or contributory factor for AS.

Key Words: *Atherosclerosis, hemodialysis, high sensitivity C-reactive protein, inflammation, interleukin-10*

Cardiovascular disease (CVD) associated with atherosclerosis (AS) is the major cause of mortality in uremic patients [1]. The increased risk of AS in this group is partly explained by contributory mechanisms such as malnutrition, oxidative stress, genetic factors and inflammation [2]. Chronic systemic inflammation has been known to play an important role in the pathogenesis of CVD in uremic and hemodialysis (HD) patients [3]. Increased production of inflammatory cytokines, such as interleukin-1 (IL-1), -2 and -6 or tumor necrosis factor-α (TNF-α), and elevation of acute-phase proteins such as C-reactive protein (CRP) are the markers of inflammation. Uremia itself and the frequent contact between blood and membranes or endotoxins from the dialysis water supply during HD treatment induce inflammation, and in this way enhance the production of cytokines. In recent studies, an association between high CRP levels and cardiovascular mortality has also been shown in HD patients in the general population [4].

AS, which is characterized by the presence of plaques in the arteries, has been defined as an inflammatory disease because the lesions of AS represent a series of specific cellular and molecular responses. The monocyte/macrophage cell type inflammation plays a role in AS. These cells produce proinflammatory cytokines which then infiltrate atherosclerotic plaques [5]. It is clear that detectable signs of inflammation accompany the progression and instability of vascular plaques [6]. The inflammatory response is downregulated by the anti-inflammatory cytokine IL-10 [7]. The purpose of the present study was to correlate the presence of (ultrasonographically proven) AS plaques and serum levels of inflammatory cytokines, including IL-1,-2

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and -6 and TNF-α, and the anti-inflammatory cytokine IL-10 in HD patients.

Material and methods

A total of 33 chronic HD patients (16 males, 17 females; mean age 43.9±12.6 years) were included in this cross-sectional study. The patients suffered from chronic renal failure due to glomerulonephritis ($n=9$), interstitial nephritis ($n=10$), hypertension ($n=5$), diabetes mellitus ($n=3$), polycystic kidney disease ($n=1$) or small kidneys of unknown origin ($n=5$). The mean time spent on dialysis was 49.8± 36.1 months. A total of 23 patients were dialyzed three times weekly and the rest twice weekly. Semi-synthetic membranes (polysynthane) and bicarbonate-containing dialysis solutions were used for dialysis treatment. Patients with evidence of a malignant disease, systemic inflammatory diseases or a duration of HD of <6 months or who were receiving immunosuppressive therapy were excluded. Blood pressure (BP) measurements were obtained prior to each HD session using a conventional sphygmomanometer. The predialysis systolic BP (SBP) and diastolic BP (DBP) were the means of four separate measurements. The pulse pressure (PP) and mean arterial pressure (MAP) were calculated. Medications being taken by the patients included antihypertensive drugs, vitamin B, C and D supplementation, calcium acetate and/or aluminum hydroxide, iron-sucrose and erythropoietin.

Blood samples were collected prior to the initiation of each dialysis session and prior to heparin administration. Levels of IL-1, -2, -6 and -10 and TNF-α were measured by means of an enzyme-linked immunosorbent assay system. The serum concentration of high sensitivity (hs)-CRP was analyzed by means of a nephelometric immunoassay. Serum albumin, blood urea nitrogen, total cholesterol and triglyceride were measured. HD adequacy (kt/V) was assessed according to the Dialysis Outcome and Quality Initiative (DOQI) guidelines.

AS was defined as the presence of plaques as detected by carotid Doppler ultrasonography (Acuson Aspen; Acuson Corp., Mountain View, CA). All patients were subgrouped according to the presence or absence of plaques. First, a transverse B-mode scan from low on the neck to behind the angle of the mandible was performed to locate bifurcation and plaques. Then, longitudinal color scans were taken to identify areas of abnormal flow and disease and spectral samples were taken in both normal and abnormal vessels, representing standard readings and areas of disease, respectively.

Statistical analysis was performed using SPSS software (version 9.05; SPSS Inc., Chicago, IL). As many of the variables were not normally distributed, non-parametric statistical methods were used. $p<0.05$ was considered significant.

Results

The HD patients were subgrouped according to the presence ($n=15$) or absence ($n=18$) of AS. The patient characteristics are shown in Table I. There was no significant difference between the subgroups according to age, sex or duration of HD. BP values (SBP, DBP, MAP and PP) were significantly higher in AS-positive patients (Table I). Measurements of kt/V were slightly lower in AS-positive compared to

Table I. Comparison of clinical, biochemical and cytokine profiles in patients with and without AS plaques. The values are presented in the following way: mean±SD; median (range).

Characteristic	AS-positive	AS-negative	*p*
Age (years)	47.6±10.2; 45.0 (30–65)	40.8±13.8; 39.0 (20–80)	NS
Sex (M/F)	6/9	10/8	NS
HD duration (months)	41.3±28.2; 36.0 (10–84)	57.8±41.5; 63.5 (7–144)	NS
SBP (mmHg)	151.0±13.7; 150.0 (130–180)	127.2±18.8; 125.0 (100–155)	0.000
DBP (mmHg)	91.0±7.8; 90.0 (70–100)	78.8±13.2; 80.0 (60–100)	0.003
PP (mmHg)	59.3±13.4; 60.0 (30–80)	47.7±11.5; 47.5 (30–80)	0.012
MAP (mmHg)	110.7±9.2; 110.0 (95–130)	94.8±14.6; 90.0 (80–120)	0.001
kt/V	0.8±0.18; 0.85 (0.5–1.18)	1.03±0.2; 1.04 (0.5–1.4)	NS
Albumin (g/dl)	3.8±0.3; 3.9 (3.2–4.6)	4.1±0.2; 4.1 (3.6–4.5)	NS
Hs-CRP (mg/l)	9.0±7.6; 5.5 (3.0–27.0)	4.2±1.5; 3.6 (3.0–7.9)	0.02
TNFα (pg/ml)	11.1±14.1; 2.9 (1.2–42.0)	6.5±7.6; 5.3 (1.0–31.7)	NS
IL-1 (pg/ml)	11.0±1.5; 11.0 (8.1–14.0)	10.7±2.5; 10.5 (5.0–15.6)	NS
IL-2 (pg/ml)	18.0±16.4; 17.0 (3.1–67.0)	15.3±15.3; 15.9 (3.0–64.7)	NS
IL-6 (pg/ml)	10.2±10.5; 9.9 (1.0–39.7)	9.7±9.4; 6.5 (1.0–38.8)	NS
IL-10 (pg/ml)	102.4±55.5; 82.9 (15.9–186.6)	489.7±229.7; 487.7 (68.0–853.7)	0.000
Total cholesterol (mg/dl)	160.9±45.6; 150.0 (109.0–265.0)	154.4±47.9; 145.0 (89.0–310.0)	NS
Triglyceride (mg/dl)	197.8±109.1; 131.0 (82.0–390.0)	196.7±90.7; 198.5 (63.0–384.0)	NS

AS-negative patients, but the difference was not significant.

The serum concentration of hs-CRP was found to be significantly increased in AS-positive patients (9.0 ± 7.6 vs 4.2 ± 1.5 mg/dl; $p = 0.02$) (Figure 1). The serum level of TNF-α was higher in AS-positive patients, but the difference was not significant (Figure 2). Serum levels of cytokines, including IL-1, -2 and -6, were slightly higher in AS-positive patients (Figure 2). Serum levels of IL-10 were significantly lower in AS-positive and higher in AS-negative patients (105.7 ± 59 vs 489.7 ± 29.7 pg/ml; $p = 0.000$) (Figure 3). Also, there was an inverse correlation between CRP and IL-10 levels in AS-positive patients ($r = -0.45$; $p = 0.009$) (Figure 4). While no correlation between albumin and IL-10 levels was found, there was a positive correlation between kt/V and IL-10 ($r = 0.42$, $p = 0.015$). There were no correlations between kt/V, albumin and hs-CRP. Total cholesterol and triglyceride levels were similar in AS-positive and -negative patients.

Among AS-positive patients ($n = 15$), 4 (26.7%) had a long (>48 months) and 11 (73.3%) had a short (<48 months) duration of HD. In the AS-negative group ($n = 18$), 11 patients (61.1%) had a duration of HD of >48 months and 7 (38.9%) had a duration of HD of <48 months. Serum levels of IL-10 and hs-CRP were not correlated with the duration of HD. The number of patients with a long duration of HD was found to be significantly higher in the AS-negative ($n = 11$) compared to the AS-positive group ($n = 4$) ($p = 0.048$).

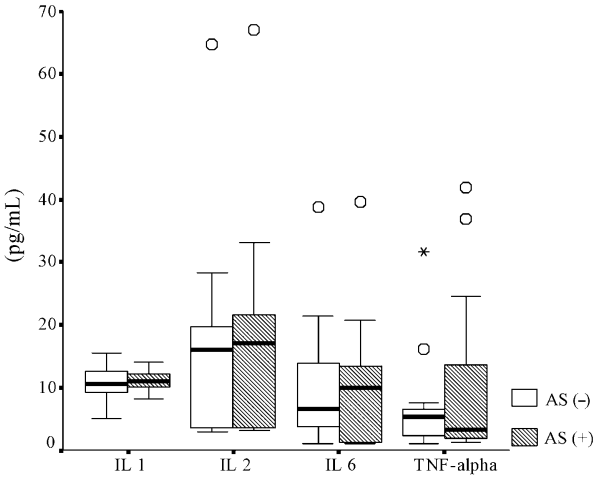


Figure 2. Serum levels of IL-1, -2 and -6 and TNF-α in AS-positive and -negative patients.

Discussion

CVD is the commonest complication of HD and a cause of high mortality in HD patients, with approximately half of all deaths in this population being due to CVD [8]. In addition to traditional vascular risk factors, such as age, gender, hypertension, hyperlipidemia and physical inactivity, uremia and dialysis-related factors play a role in AS and CVD in HD patients [9]. Chronic inflammation is known to increase the incidence of AS [5]. Indicators of systemic inflammation are elevation of acute-phase proteins such as CRP and high levels of cytokines such as TNF-α and interleukins. An association between high levels of CRP or IL-6 and cardiovascular mortality has been shown in HD patients [4,10]. IL-10 is a counter-regulatory cytokine which limits the inflammatory response. A high serum level of IL-10 was found to be a protective factor in patients with acute coronary syndrome [11].

In this study, contrary to expectations, the duration of HD was slightly shorter in the AS-positive

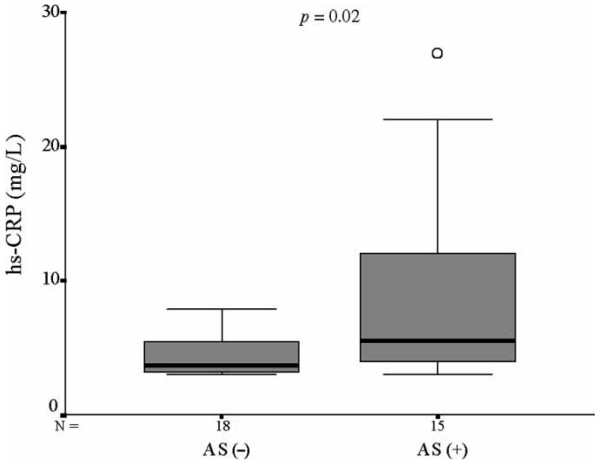


Figure 1. Box plots showing plasma hs-CRP concentration according to the presence or absence of plaques.

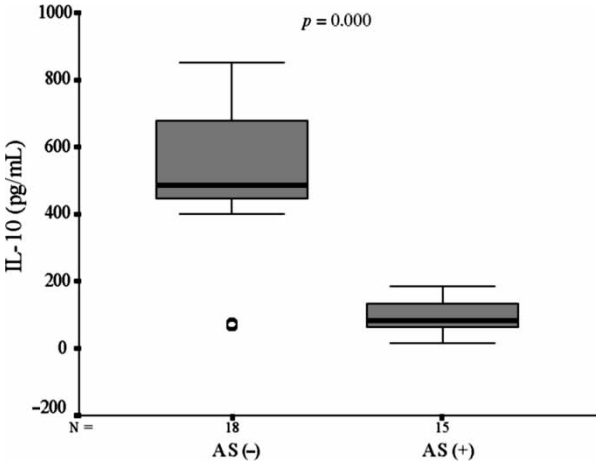


Figure 3. Serum levels of IL-10 in AS-positive and -negative patients.

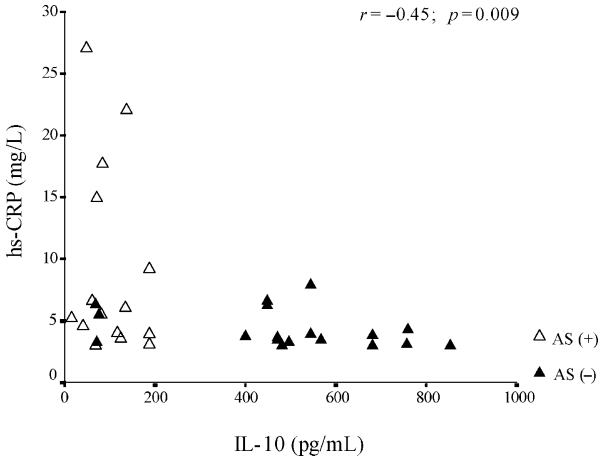


Figure 4. The correlation between serum levels of IL-10 and hs-CRP in AS-positive and -negative patients.

group. Low albumin levels and low kt/V ratios, which were considered to be indicative of inadequate dialysis, were found in patients with AS. Another unexpected observation was that there were fewer male than female patients in the AS-positive group. Interestingly, levels of total cholesterol and triglyceride were similar in AS-positive and -negative patients. Moreover, levels of the inflammatory cytokines IL-1, -2 and -6 and TNF-α were similar in AS-positive and -negative patients. In contrast, the plasma concentration of hs-CRP was increased in AS-positive patients, as in non-uremic patients with coronary disease [12]. More interestingly, the level of the anti-inflammatory cytokine IL-10 was lower in AS-positive compared to AS-negative patients. Because the risk factors for CVD were similar between the two groups, the association of low levels of IL-10 with AS-positive patients may explain the true pathogenetic link between the anti-inflammatory cytokine IL-10 and AS.

In this preliminary study, the duration of HD was not longer in AS-positive compared to AS-negative patients. When a duration of HD of $>$ or <4 years was taken into consideration, the frequency of AS did not differ.

In the literature, the -1082 A allele, which is associated with low production of IL-10, has been reported to be a predictor of higher cardiovascular mortality in HD patients [13]. We did not perform a genotype analysis of IL-10. However, there was no difference regarding risk factors for CVD in our AS-positive and -negative HD patients. Low production of IL-10 in AS-positive patients may be an important risk factor for CVD in HD patients, as was seen in our patients. Low albumin and kt/v levels in our AS-positive patients might have triggered the inflammation cascade and helped to the development of AS.

In conclusion, despite the relatively small number of patients in our study we can say that HD patients with atherosclerotic plaques had significantly higher hs-CRP levels, as is the case for non-uremic patients. Universal contributory factors for AS, such as older age, male gender, hyperlipidemia, duration of HD and even inflammatory cytokines (IL-1, -2 and -6 and TNF-α), did not differ between AS-positive and -negative patients in this small group. Inadequate HD and malnutrition may be additional factors determining the development of AS plaques in HD patients. As expected, SBP, DBP, MAP and PP were higher in AS-positive compared to AS-negative patients. The presence of AS was not affected by the duration of HD. Serum levels of the anti-inflammatory cytokine IL-10 were significantly decreased in AS-positive patients. Additionally, the inverse correlation found between hs-CRP and IL-10 may be evidence of the limitation of the anti-inflammatory response in atherosclerotic uremic patients. The small number of cases is the most important restriction of our study and our results need to be validated in larger series. An important question that remains to be answered is which is the main triggering factor for AS: increased inflammation or decreased anti-inflammation?

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