ORIGINAL RESEARCH

Soluble Toll-like Receptor 4: A New Player in Subclinical Inflammation and Malnutrition in Hemodialysis Patients

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Objective: Toll-like receptor 4 (TLR4) promotes inflammation in hemodialysis patients (HD). A soluble form of extracellular TLR4 (sTLR4) has been recently characterized, which showed the ability to attenuate TLR4 signalling. In this study, we describe the sTLR4 profile in regular HD patients.

Subjects: In a cross-sectional study we enrolled forty prevalent HD patients (68.2 ± 16.3 years, twenty-five males) with a median dialysis vintage of 41 months. Nineteen patients were undergoing standard bicarbonate HD (BHD) and 21 patients on-line hemodiafiltration (HDF). Ten healthy sex-matched subjects constituted the controls (C).

Intervention: Before and after the HD session, serum was tested for sTLR4 levels by ELISA. Moreover, clinical and biochemical data were collected, including body mass index, albumin, and C-reactive protein (CRP) levels. Body composition was expressed as a 3-compartment model, providing lean tissue index and fat tissue index (FTI).

Main Outcome Measure: Describe the profile of sTLR4 in HD patients, evaluating the correlations among sTLR4 levels and the main clinical characteristics, inflammatory and nutritional parameters.

Results: Patients with subclinical inflammation (i.e., high CRP levels without clinical symptomatology) presented higher sTLR4 levels (0.42 ± 0.25 ng/mL) with respect to both C and not inflamed HD patients (0.23 ± 0.19 ng/mL, $P < .05$). There was a significant direct correlation between predialysis sTLR4 and body mass index, FTI (r = 0.55), and CRP levels (r = 0.52) and inverse correlation with lean tissue index and albumin (r = −0.4). In multivariate analysis, sTLR4 resulted directly associated with FTI ($P = .038$). Notably, sTLR4 levels resulted higher in bicarbonate hemodialysis versus hemodiafiltration (0.37 ± 0.18 vs. 0.19 ± 0.21 ng/mL, $P < .05$).

Conclusions: sTLR4 correlates with inflammatory and nutritional parameters, presenting as a new potential player in modulating subclinical inflammation in HD patients.

Introduction

CHRONIC KIDNEY DISEASE represents a high-risk condition characterized by an elevated morbidity and mortality, mainly for cardiovascular complications.[1]

In particular, patients on hemodialysis (HD) present many clinical and biochemical factors that exponentially increase their risk, including chronic inflammation, immune dysfunction, malnutrition, anemia, and so forth.[2,3] These factors are strictly correlated, leading to the development of complicated clinical conditions, such as the so-called malnutrition-inflammation complex syndrome.[4] Subclinical inflammation might recognize multiple causes, including loss of renal function, vascular access infection, dialysis membrane bioincompatibility and diffusion of endotoxins. It is characterized by an imbalance between proinflammatory and anti-inflammatory conditions, finally leading to the dysregulation of crucial cellular and molecular processes.[5,6]

Numerous molecules have been implicated in the pathogenesis of HD-related inflammation, such as interleukin-1 (IL-1), interleukin-6 (IL-6), Tumor necrosis factor-α (TNF-α), and molecules of Transforming Growth Factor-β (TGF-β) superfamily.[7] Among them, Toll-like receptors (TLRs) play a fundamental role in the innate immune system by triggering proinflammatory pathways in response to exogenous and endogenous stimuli, being implicated in the pathogenesis of acute and chronic disorders.[8] TLR4, the best-characterized TLR, binds to Lipopolysaccharide (LPS) of gram-negative bacterial cell walls.[9] On binding of LPS to TLR4 and its coreceptors CD14 and MD-2, the adaptor protein myeloid differentiation factor 88 (MyD88) is recruited to the Toll/IL-1 receptor domain of the receptor.

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These interactions, by a complex downstream signaling cascade, lead to activation of the Nuclear Factor-*k*B (NF-*κ*B) pathway, resulting in the transcription of many proinflammatory genes, including cytokines, chemokines, and other effectors of the innate immune response.[10] It has been demonstrated that TLR4 activation plays a crucial role in the pathogenesis of many pathological conditions, including renal diseases, such as diabetes nephropathy and sepsis-related acute kidney injury.[11,12] Interestingly, a soluble form of extracellular Toll-like receptor 4 domain (sTLR4) has also been characterized, which has shown the ability to attenuate TLR4 signaling.[13] sTLR4 is a polypeptide of about 70 kDa in human (about 20 KDa in mice), which origin is not fully clear. Indeed, it is generated by alternative splicing of TLR4 mRNA but it is not possible to rule out other mechanisms, such as proteolytic cleavage of the extracellular domain of the membrane form of the TLR4.[14,15] In experimental studies, it has been demonstrated that sTLR4 acts as a potent inhibitor of TLR4-mediated inflammation, blocking the interaction between TLR4 and other coreceptor complexes, especially MD2 and CD14, therefore terminating TLR4 signaling.[13]

This action (with the consequent formation of sTLR4/MD-2 complexes) results in a significant attenuation of LPS induced proinflammatory and migration cytokine production in vitro and in vivo.[16] In humans, sTLR4 serum levels have been found elevated in different conditions, like sepsis and some inflammatory diseases.[17] In the specific setting of HD patients, an increased expression of TLR4 on monocytes and neutrophils has been described.[18,19] Similarly, recent evidence described a higher TLR4 gene and protein expression also in muscle tissue of chronic kidney disease patients.[20]

Therefore, TLR4 is a strong candidate as a mediator of systemic inflammation in HD. However, although TLR4 has been object of intense research activity, the presence and the role of sTLR4 has not been investigated, so far, even if this molecule shows interesting characteristics. So, here we studied this issue, aiming to describe sTLR4 profile in regular HD patients.

Materials and Methods

Forty patients on maintenance HD for at least for 6 months were included in a cross-sectional study. Patients with acute infections, active immunological diseases, immunosuppressive therapy, previous transplantation, or history of malignancies were excluded. All the patients were undergoing thrice-weekly 4-hour HD treatment. Sex-matched healthy subjects were the control group. We evaluated clinical and biochemical data, including age, dialysis modality, dialysis vintage, body mass index (BMI), predialysis serum levels of phosphate, albumin, transferrin, calcium, phosphate, lymphocyte count, and C-reactive protein (CRP). According to CRP levels, the patients were stratified in inflamed (i.e. CRP $\geq$1 mg/dL) and noninflamed subjects (i.e., CRP $<$1 mg/dL). Body composition was studied by Body Composition Monitor (BCM; FMC, Bad Homburg, Germany). Measurements were taken before the start of the HD treatment with the patient calm, supine, and relaxed in the dialysis chair for 2 minutes after the electrodes had been attached to the hand and foot on the same side of the body. The BCM expresses body composition as a 3-compartment model, providing lean tissue index (LTI) and fat tissue index (FTI), whereby LTI and FTI are the respective tissue masses normalized to height squared.[21] Ratios LTI/BMI as well as ratios FTI/BMI were calculated. In each patient, before and after the HD session, serum was withdrawn and tested for sTLR4 levels by a commercial ELISA kit (Abnova, Taipei City, Taiwan) with a lower limit of detection of 0.156 ng/mL.

sTLR4 levels after dialysis were corrected for dialysis-induced changes in blood volume by multiplying CRP after dialysis with the ratio between serum albumin before and after dialysis.[22]

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee. Written informed consent was obtained from each participant prior to enrollment in the study.

Statistical Analysis

Quantitative variables were represented by mean $\pm$ standard deviation or median and interquartile range if they were not normally distributed; qualitative ones by number and percentage. Collected data were compared by means of chi-square test or Fisher's exact test, as appropriate, for categorical variables or by Student *t* test or nonparametric Mann–Whitney test in the case of quantitative variables. Differences among control subjects and HD patients were assessed by analysis of variance. Correlations among sTLR4 and clinical and laboratory variables were analyzed with Spearman-Rho, whereas associations were assessed fitting logistic regression models. All tests were 2-sided, and a P value $<$ .05 was considered statistically significant. Data analysis was performed with STATA statistical package (version:11; Stata Corporation, College Station, 2010, Texas).

Results

Patient Characteristics

Patient clinical characteristics are shown in Table 1.

Mean age was 68.2 $\pm$ 16.3 years, with 25 males, whereas median dialysis vintage was 41 months. Mean BMI was 24.7 $\pm$ 4.8 kg/m^2; 9 patients were diabetic. Nineteen patients (47.5%) were treated with standard bicarbonate hemodialysis (BHD), whereas 21 patients (52.5%) were treated with on-line hemodiafiltration (HDF). Mean predialysis albumin was 3.6 $\pm$ 0.4 g/dL, whereas postdialysis levels were 3.9 $\pm$ 0.4 (P $<$.05). According to CRP levels, 9 (22.5%) resulted inflamed with median CRP levels of 3.6 (1.3-5.2) mg/dL, and 31 were noninflamed with CRP of 0.33 (0.32-0.54) mg/dL. As recorded by BCM, mean