

Relationship between the Soluble F11 Receptor and Markers of Inflammation in Hemodialysis Patients

Moro O. Salifu, Quirine Kolff, Preethi Murty, Dhiren M. Haria, Mahamadu Zimpa, Muhammad Shakeel, Howon Lee, Elizabeth Kornecki, and Anna Babinska

Background: The human F11 receptor (F11R) is an important cell adhesion molecule implicated in inflammatory thrombosis. We hypothesize that serum levels of the soluble released form of F11R (sF11R) are elevated in dialysis patients since these patients have higher cardiovascular disease burdens than the general population. In this study, we examined whether sF11R levels were elevated in hemodialysis (HD) patients and correlated with known inflammatory cytokines.

Methods: We used new and standard enzyme-linked immunosorbent assay techniques to measure levels of sF11R, as well as high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor α (TNF- α), interleukin-6 (IL-6), and interleukin-10 (IL-10), in a cross section of 52 HD patients and compared these with 15 healthy controls.

Results: The mean age of the patients was 56 ± 17.3 years; 60% were female, and 36% had diabetes mellitus. Serum levels of sF11R, hs-CRP, TNF- α , IL-6, and IL-10 were all significantly higher in patients than in control sera ($p < .05$). Within the patient group, there was a significant positive correlation between sF11R and TNF- α ($r = .41, p = .003$), IL-10 ($r = .32, p = .023$), and IL-6 ($r = .32, p = .023$), whereas hs-CRP showed no significant correlation ($r = -.27, p = .052$).

Conclusion: We conclude that the sF11R level is elevated in HD patients and correlates with known markers of cardiovascular disease. sF11R may be a novel cardiovascular risk marker, and longitudinal studies are needed to better assess its relationship with cardiovascular disease morbidity and mortality in this population.

Key words: hemodialysis, F11 receptor, junctional adhesion molecule A, tumor necrosis factor, interleukin-6, cardiovascular disease

Cardiovascular disease (CVD) remains the major cause of morbidity and mortality in patients with kidney failure.^{1–3} A number of inflammatory cytokines, including high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor α (TNF- α), have been shown to be important markers of CVD, thereby linking the pathogenesis of atherosclerosis to chronic

inflammation.^{4–6} It is now clear that platelets play a crucial role in this inflammatory process of atherosclerosis, initiated by platelet-endothelial membrane interactions via several receptors.^{7,8} Our laboratory has characterized and cloned the human F11 receptor (F11R), an integral platelet-membrane protein, structurally identified as a cell adhesion molecule and a member of the immunoglobulin superfamily. The F11R, residing on chromosome 1q21.2–q21.3, is the human ortholog of a murine protein found in the tight junctions of the vascular endothelium junctional adhesion molecule A (JAM-A).⁹

The adhesion of platelets to the endothelium precedes atherosclerotic plaque invasion by monocytes, making it the initial step in thrombus formation.¹⁰ Blockage of platelet adhesion to the vessel wall at the very initial stage of atherogenesis in apolipoprotein E-deficient (apoE^{-/-}) mice decreased the formation of atherosclerotic plaques in carotid arteries.⁸ A recent study using double knockouts of JAM-A^{-/-} and apoE^{-/-} genes provided information for the critical

From the Department of Medicine (M.O.S., Q.K., P.M., D.M.H., M.Z., M.S., H.L.), Division of Nephrology, and Department of Anatomy and Cell Biology (E.K., A.B.), SUNY Downstate Medical Center, Brooklyn, NY.

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Address correspondence to: Dr. Moro O. Salifu, SUNY Downstate Medical Center, 450 Clarkson Avenue, Box 52, Brooklyn, NY 11203; e-mail: moro@salifu.net.

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role of F11R/JAM-A in lesion formation in atherosclerosis-prone mice.¹¹

Human platelets constitutively express $8,067 \pm 1,307$ F11R molecules on their membrane surface.¹² When platelet activation is initiated by a functional monoclonal antibody MAbF11 or by physiologic agonists such as collagen or thrombin, the F11R becomes phosphorylated, triggering signaling events, resulting in cytoskeletal reorganization, cell adhesion, granular secretion, and aggregation.^{13,14} We have identified, sequenced, and cloned the human gene for F11R and demonstrated that F11R plays a critical role in platelet adhesion to cytokine-inflamed endothelial cells.^{9,15,16}

The soluble form of F11R (sF11R) is thought to be an extracellular portion of the F11R molecule, shed from platelets and/or the endothelium by an enzymatic cleavage pathway, comparable to shedding of the IL-6R and other membrane proteins.¹⁷ We hypothesize that high serum levels of sF11R represent up-regulation of the F11R itself and thus may serve as a potential marker for atherosclerotic disease. Our laboratory has developed a sensitive enzyme-linked immunosorbent assay (ELISA) for the measurement of serum sF11R levels.

With our ability to detect and assay sF11R levels, this cross-sectional study was designed to determine whether sF11R was elevated in the patient population compared with normal healthy subjects. Furthermore, we examined whether significant correlations exist between sF11R and known markers for CVD in hemodialysis patients as this population has a high risk for the development of CVD. This is the first report on sF11R levels in patients and its association with CVD.

Materials and Methods

Patient Population

The study was approved by the Institutional Review Board of SUNY Downstate Medical Center. Fifty-two stable outpatients on hemodialysis between 6:00 am and 3:00 pm, on hemodialysis for at least 3 months with no evidence of acute illness requiring hospitalization in the preceding 3 months prior to the study, consented to participate in this study. Each patient received 4 hours of hemodialysis, three times a week, through a native fistula or arteriovenous graft. They also received recombinant erythropoietin 80 to 200 IU/kg on each dialysis, depending on the hemoglobin levels. Patients were excluded if they were taking drugs known to inhibit platelet function, such as aspirin, clopidogrel, and H1 receptor blockers. Each patient received heparin as an anticoagulant before each dialysis session. Twenty-six healthy adult

controls with no known medical diseases volunteered to participate in the study. We excluded volunteers with elevated hs-CRP above 3 mg/L, suggestive of undiagnosed acute or chronic illness. Data from the remaining 15 controls were used in the final analysis.

Data Collection

Patient data included demographics (age, gender, race), cause of kidney failure, hypertension, diabetes, and CVD. CVD was defined as the composite end point of myocardial infarction, congestive heart failure, angina, ischemic heart disease, peripheral vascular disease, and stroke. Laboratory data taken from the patient medical record included blood urea nitrogen, serum creatinine, platelet count, hemoglobin, and urea reduction ratio. Control data included age and gender.

Sample Collection

All blood samples were collected between 6:00 am and 3:00 pm. Whole blood (10 mL) from patients was collected in serum separator tubes through venous access before the start of the hemodialysis. Whole blood (10 mL) from control subjects was obtained by venipuncture of the antecubital vein. All samples were allowed to clot and retract and were centrifuged for 10 minutes at 1,100 rpm. Serum was aliquoted and stored at -20°C until analysis.

Analytic Methods

Commercial ELISAs were used to measure IL-6 (Pierce/Endogen, Rockford, IL), IL-10 (Pierce/Endogen), hs-CRP (DAKO, Carpinteria, CA), and TNF- α (R&D Systems, Inc, Minneapolis, MN). The sF11R levels were determined using an ELISA technique developed in our laboratory using the F11R antibody M.Ab.F11. The F11R recombinant protein, antibodies, and reagents for the F11R ELISA now are commercially available from R&D Systems and HyCult biotechnology (Uden, Netherlands). The standard curve was prepared using recombinant human JAM-1/Fc (0.1 pg/mL to 200 ng/mL). The capturing F11R monoclonal antibody M.Ab.F11 (10 $\mu\text{g/mL}$) was coated overnight at 4°C onto microplate wells and blocked with phosphate-buffered saline 1% bovine serum albumin, followed by a 2-hour incubation at 37°C with 100 μL standard solutions of sF11R protein or nondiluted or twofold diluted serum samples, followed by incubation for 2 hours at 37°C with a detection biotinylated human F11R antibody (200 ng/mL). The diluted streptavidin-horseradish peroxidase (1:400) was applied and incubated for 30 minutes at room temperature followed by 100 μL of substrate solution for 30 minutes and 50 μL of the stop

Table 1 Demographics and Clinical Characteristics of Patients (*N* = 52)

Characteristic	Result
Age (yr)	56.0 ± 17.3
Gender (%)	
Male	40.0
Female	60.0
African Americans (%)	98.0
Comorbidities (%)	
Hypertensive	73.0
Diabetes	36.0
Cardiovascular disease	36.5
Other cause of renal failure	12.4
Hemoglobin, g/dL	11.7 ± 1.8
Platelet count × 10 ³ /μL	211 ± 57.7
Urea reduction ratio (%)	71.6 ± 8.6

solution. Optical density measurements were taken at 450 nm using a microplate reader (Bio-Rad Laboratories model 650, Hercules, CA). This assay system is most accurate in the concentration range of 0.1 pg to 200 ng/mL.

Statistical Methods

Descriptive statistics were used to detail the demographics and clinical characteristics of the patients. Data that did not follow a normal distribution were analyzed by Mann-Whitney *U* test or Spearman correlations. Normally distributed data were analyzed using independent *t*-test and Pearson correlations. All means are represented with ± standard error of the mean (SEM). Linear regression analysis was used to determine if any of the cytokines measured were predictive of sF11R levels. SPSS, version 13.0 for Windows (SPSS Inc, Chicago, IL), was used for statistical analysis. A two-tailed *p* value less than .05 was considered statistically significant.

Results

The patient population examined in this study, as shown in Table 1, consisted of 52 patients with a mean age of 56 ± 17.3 years; 60% were female, and 98% were African Americans. One patient was Asian in

origin. Comorbidities included diabetes mellitus (36%), hypertension (73%), and other causes of renal disease (12.4%). Patients meeting the composite cardiovascular end point of myocardial infarction, angina, congestive heart failure, peripheral vascular disease, and stroke comprised 36.5%. Fifteen healthy volunteers (mean age 40 ± 11.7 years; 40% female; five black, Caucasian, and Asian each) served as controls. There were no significant differences in mean cytokine levels between these volunteer groups.

Table 2 details the levels of sF11R measured in healthy and patient populations. We observed that sF11R levels were significantly higher in the patient population than in the control group (137.7 ± 16.0 vs 30.8 ± 8.5 pg/mL; *p* = .001). Also, all other cytokine levels were significantly higher in the patient group compared with controls: hs-CRP, 4.7 ± 0.4 versus 0.9 ± 0.2 mg/dL, *p* = .001; IL-6, 68.5 ± 17.0 versus 6.5 ± 2 pg/mL, *p* = .001; IL-10, 5.8 ± 1.7 versus 0.9 ± 0.2 pg/mL, *p* = .001; and TNF-α, 12.9 ± 5.4 versus 0.5 ± 0.3 pg/mL, *p* = .044. Using control median values (sF11R = 23.4 pg/mL, hs-CRP = 0.61 pg/mL, IL-10 = 0.86 pg/mL, IL-6 = 3.85 pg/mL, and TNF-α = 0 pg/mL) as cutoff points, 98% of patients had sF11R levels above the median for control sF11R. Similarly, 94% of patients had hs-CRP, 67% had IL-10, 90% had IL-6, and 54% had TNF-α levels above their control medians.

As shown in Figure 1, significant positive correlations were found between sF11R and TNF-α levels (*r* = .41, *p* = .003) and between sF11R and IL-6 (*r* = .32, *p* = .023). We also observed that IL-10 levels correlated with sF11R (*r* = .32, *p* = .023). No significant correlation between hs-CRP and F11R was observed (*r* = -.27, *p* = .052).

Given that sF11R level is in part due to circulating platelets, we calculated the sF11R to platelet count ratio and determined the correlation of this ratio with other markers of inflammation. The sF11R to platelet ratio correlated significantly with IL-6 (*r* = .37, *p* = .007) and TNF-α (*r* = .51, *p* = .001). The correlation of the ratio with IL-6 and TNF-α was much stronger than with sF11R alone.

Table 2 Mean (± SEM) Serum Levels of Soluble F11 Receptor and Known Inflammatory Cytokines

Cytokine	Patients (n = 52)	Controls (n = 15)	<i>p</i> Value
sF11R, pg/mL	137.7 ± 16.6	30.8 ± 8.5	.001
hs-CRP, mg/L	4.7 ± 0.4	0.9 ± 0.2	.001
TNF-α, pg/mL	12.9 ± 5.4	0.5 ± 0.3	.044
IL-6, pg/mL	68.5 ± 17.0	6.5 ± 2.0	.001
IL-10, pg/mL	5.8 ± 1.7	0.9 ± 0.3	.003

hs-CRP = high-sensitivity C-reactive protein; IL = interleukin; sF11R = soluble F11 receptor; TNF = tumor necrosis factor.

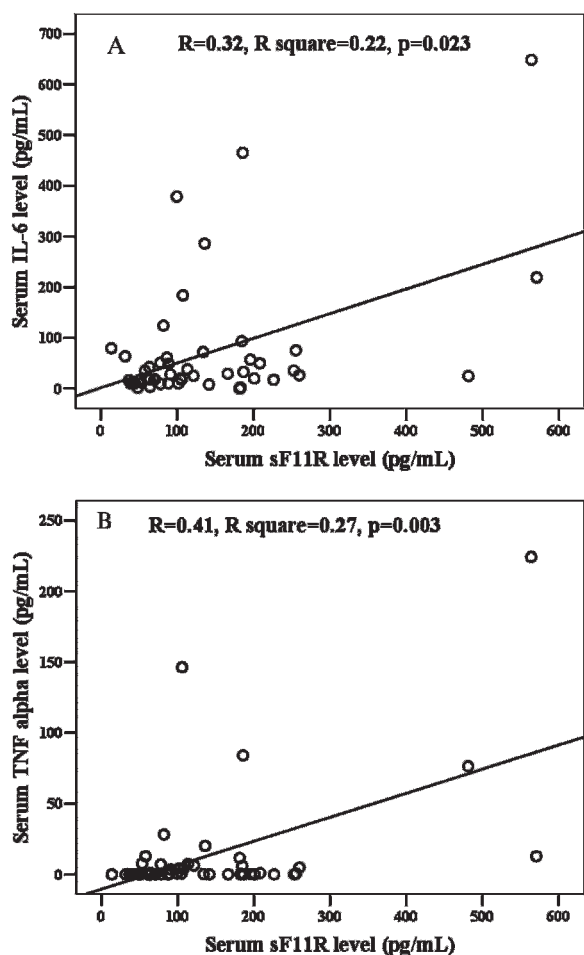


Figure 1 A, Correlation between serum soluble F11 receptor (sF11R) and interleukin-6 (IL-6) ($r = .32$, $r^2 = .22$, $p = .023$) levels in hemodialysis patients. B, Correlation between serum sF11R and tumor necrosis factor α (TNF- α) ($r = .41$, $r^2 = .27$, $p = .003$) in hemodialysis patients.

Linear regression analysis showed that only TNF- α ($p = .04$) was predictive of serum sF11R levels ($r^2 = .30$). No significant differences in sF11R levels were found between patients with and without diabetes, hypertension, and CVD in this patient population. There was also no significant correlation between sF11R and the other inflammatory markers with diabetes, hypertension, and CVD.

Discussion

This is the first cross-sectional analysis of sF11R in hemodialysis and predominantly African American patients showing a marked increase in serum levels of sF11R and moderate correlation with known inflammatory cytokines. Although these results do not suggest causality and the study was not powered to

detect significant differences in sF11R in patients with and without CVD per se, the results suggest a link between sF11R and known markers of CVD that requires further examination.

There is accumulating evidence that F11R is involved in CVD. Platelet involvement in plaque formation is one of the key factors in the development of atherosclerotic lesions.^{7,8,10} The activation of the F11R appears to trigger two pathways: the first includes crosslinking of the F11R to the Fc γ RII and plays a role in the aggregation of platelets but not adhesion.¹³ The second pathway involves the binding of the constitutively expressed F11R of platelets to the up-regulated F11R located on the luminal surface of the inflamed endothelium through homophilic interactions.¹⁶ Activation of F11R has been found to cause sensitization of platelets, increasing the platelet reactivity to circulating platelet receptor agonists such as adenosine diphosphate and thrombin. Subsequently, following the adhesion of platelets to the inflamed endothelium through F11R, subthreshold concentrations of naturally circulating agonists would be expected to cause stable buildup of platelet plaques, thereby contributing to atherosclerosis.¹³

TNF- α has been found to contribute significantly to the presence of carotid plaque formation¹⁸ and is correlated with high mortality rates in hemodialysis patients.¹⁹ A recent in vitro study showed that incubation of murine endothelial cells with TNF- α caused an increase in the expression of cell surface F11R.²⁰ A plausible explanation for the correlation between sF11R and TNF- α levels in the circulation of patients might be found in the transcription factor nuclear factor κ B (NF- κ B) located in the promoter region of the F11R gene.^{9,14} NF- κ B is known to activate gene transcription after TNF- α stimulation.^{21,22} Thus, high levels of TNF- α would be expected to increase the transcription of the F11R gene, thereby increasing the expression of F11R on the luminal surface of the inflamed endothelium. The newly synthesized F11R on the inflamed endothelium would cause enhanced platelet adhesion to these cells. With chronic inflammation, proteolysis of the F11R by enzymes secreted from platelets and inflammatory cells would release the external portion of the integral F11R, as the soluble form sF11R, into the circulation of patients.

Our study showed that both hs-CRP and IL-6 were significantly elevated in our patient population, consistent with previous observations showing that both cytokines are elevated and highly predictive of cardiovascular risk in hemodialysis patients.^{23,24} C-reactive protein has been shown to promote platelet

adhesion to endothelial cells,²⁵ suggesting a possible link between CRP and sF11R, although such a correlation was not observed in this study. A larger study would be needed to further evaluate the relationship between CRP and sF11R and of the relationship between CVD morbidity and mortality.

Our small sample size limits our ability to extend this investigation to more rigorous analysis of the role of F11R in CVD. Such a study would require a prospective longitudinal design including other racial groups to determine if baseline sF11R values predict future cardiovascular events. sF11R may be elevated in dialysis patients because of decreased renal clearance; however, the positive correlation with IL-6 and TNF- α suggests that sF11R may be an inflammatory marker.

We conclude that sF11R is elevated in dialysis patients and correlates with known markers of CVD. This soluble factor may be a novel inflammatory cardiovascular risk marker. Further research is under way to better assess the relationship between sF11R, other markers of inflammation, and CVD in a larger population of these patients and in patients with chronic kidney disease not on dialysis, as well as in patients after kidney transplantation.

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