High-flux and Low-flux Dialysis Membranes and Levels of Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 in Children With Chronic Kidney Failure

Happy K Sawires,[1] Waleed A Mohamed,[2] Mona F Schaalan[3]

[1]Department of Pediatrics, Cairo University, Cairo, Egypt
[2]Department of Chemistry, Cairo University, Cairo, Egypt
[3]Department of Pharmacy, Misr International University, Cairo, Egypt

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Introduction. During hemodialysis, the expression of different adhesion molecules changes, thus serving as markers of biocompatibility of dialysis membranes. Our aim was to investigate whether low-flux and high-flux dialysis membranes have different effects on the concentration of adhesion molecules and their association with leukocytes and pro-inflammatory cytokines.
Materials and Methods. We enrolled 80 pediatric patients on hemodialysis. Baseline levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were measured. The patients were classified into 2 groups to use either low-flux filters or high-flux filters for 3 months. At the end of the 3 months, predialysis samples were obtained for measurement of ICAM-1, VCAM-1, TNF-α and interleukin-1. Post-dialysis samples were collected for measurement of CBC, ICAM-1, VCAM-1, TNF-α, and interleukin-1. Forty volunteers were involved as a control group.
Results. Both TNF- α and IL-1 were higher in the patients compared to the control group ($P < .001$). Compared to the control group, there was a significant increase in ICAM-1 and VCAM-1 ($P < .001$) in both groups predialysis and postdialysis. The postdialysis increments of ICAM-1 with the high-flux membranes were significantly less compared to the low-flux membranes ($P < .001$). Serum ICAM-1 and VCAM-1 significantly correlated with TNF-α and interleukin-1 in all groups.
Conclusions. The postdialysis increments of the adhesion molecules are due to the effect of dialysis membranes, which is less with the use of high-flux filters.

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INTRODUCTION

An effective immune response depends on leukocytes migration to the site of inflammation. The membrane bound forms of selectins (E-selectin, L-selectin, and P-selectin) and molecules of the immunoglobulin superfamily, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), take part in a process of leukocyte migration called the "adhesion cascade.[1,2]" In vitro studies have suggested that targeting interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) can be used to regulate ICAM-1 and VCAM-1.[3]

During hemodialysis, the leukocytes in the circulation are activated and a number of pro-inflammatory cytokines are secreted, including IL-1 and TNF-α.[4] The expression of different adhesion molecules changes during hemodialysis sessions,

thus serving as markers of biocompatibility of dialysis membranes.[5] However, investigation of soluble adhesion molecules shed proteolytically from cells into the circulation has given contradictory results in hemodialysis patients.[6]

In this study, we evaluated serum levels of the two soluble adhesion molecules (ICAM-1 and VCAM-1) and two other pro-inflammatory cytokines (TNF-α and IL-1) in patients with end-stage renal disease (ESRD) on maintenance hemodialysis. Our aim was to investigate whether low-flux and high-flux dialysis membranes have different effects on the concentration of these adhesion molecules and whether there is any relationship between circulating adhesion levels and leukocytes or pro-inflammatory cytokines.

MATERIALS AND METHODS

In this prospective nonrandomized study, we enrolled 80 pediatric patients with ESRD in the Center of Pediatric Nephrology and Transplantation of Cairo University Children's Hospital. The study protocol was approved by the local ethics committee, and informed written consent was obtained from the parents of the patients. The study was conducted through a 3 months' duration. Inclusion criterion was pediatric age ($\leq$ 13 years) when diagnosed with ESRD (glomerular filtration rate, < 15 mL/min/1.73 m^2). All of the included patients were clinically stable and free of active infection during the study period (clinically and as evidenced by C-reactive protein). None of our patients had received antibiotic, anti-inflammatory, or corticosteroid medications during the study. It should also be noted that there was no need for albumin supplementation to either groups. Patients with acute kidney injury or with unsatisfactory vascular access affecting dialysis adequacy were excluded from the study.

At the beginning of the study, predialysis blood samples were taken for measurements of ICAM-1 and VCAM-1 as baseline values. Then, the patients were assigned into 2 groups (40 patients for each group which were haphazardly selected). According to our unit protocol, all patients were on 3-hour sessions of dialysis, 3 times per week, using Fresinius 4008B hemodialysis machine. Patients in the 1st group used low-flux polysulfone filters (Fresinius F4 and F5, according to patients' surface area), pediatric lines using sodium bicarbonate-based dialysis solution, and heparin for a 3 months' duration. Patients in the 2nd group used high-flux polysulfone filters (Fresinius F40 or F50, according to patient's surface areas) using the same base and anticoagulant for 3 months without changing any of the other dialysis prescription parameters (except for ultrafiltration to reach their ideal dry weight as needed). At the end of the 3 months, midweek predialysis arterial samples were obtained for measurement of albumin, C-reactive protein, ICAM-1, VCAM-1, TNF-α, and IL-1. Postdialysis venous samples were collected for measurement of complete blood count, ICAM-1, VCAM-1, TNF-α, and IL-1.

Forty volunteers (age- and sex-matched who came for routine checkup in outpatient clinic) were involved in our study for measurement of ICAM-1, VCAM-1, TNF-α, and IL-1 as a control group.

Hemodialysis

Hemodialysis machines with volumetric control (Fresenius Medical Care 4008B and 4008S, Homburg, Germany) were used. The standard dialysis bath consisted of sodium, 140 mEq/L; potassium, 2 mEq/L; calcium, 3 mEq/L; and bicarbonate, 35 mEq/L. The ultrafiltration rate was programmed to reach the patient's optimal dry weight defined as the postdialysis body weight below which the patients developed symptomatic hypotension or muscle cramps in the absence of edema. Heparin was used for anticoagulation. Equilibrated Kt/V used to measure dialysis adequacy.

Serum Biochemical Pramaeters

A fasting blood sample of 5 mL was obtained. The samples were allowed to clot at room temperature for 30 minutes at 4°C at 300xg. Sera were stored frozen at -20°C for ICAM-1, VCAM-1, TNF-α, and IL-1 assay. They were assayed by commercial human ICAM-1, VCAM-1, TNF-α, and IL-1 enzyme-linked immunosorbent assay kits from Thermo Fisher Scientific Inc (Barrington, Il, USA; Cat. No ELHs, ICAM-1, VCAM-1, TNF-α, and IL-1). This assay is a sandwich enzyme immunoassay based on concurrent capture of ICAM-1, VCAM-1, TNF-α, and IL-1 molecules from the samples to the wells of a microtitre plate coated with a monoclonal anti human antibodies and a binding of a second biotinylated monoclonal antihuman antibody to the captured molecules. The amount of ICAM-1, VCAM-1, TNF-α, and IL-1 detected in each sample

is measured by increasing the absorbance which is directly proportional to the concentration of the sample.

Statistical Analyses

Quantitative data were presented as mean ± standard deviation values. Qualitative (Categorical) data were presented as frequencies and percentages. Postdialysis increments of ICAM-1 and VCAM-1 were calculated as the difference between predialysis and postdialysis levels of these adhesion molecules. The paired *t* test was used to study changes in ICAM-1, VCAM-1, TNF-α, and IL-1 in each group. The independent *t* test was used to compare ICAM-1, VCAM-1, TNF-α, and IL-1 in different groups. The Mann-Whitney test was used to compare the increments of adhesion molecules. The 1-way analysis of variance test was used to compare results in different groups and control group. The Pearson correlation coefficient was used to determine significant correlations between quantitative data. The significance level was set at $P < .05$. Statistical analyses were performed with the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA).

RESULTS

Sixteen patients were excluded from the study because of unsatisfactory vascular access. Characteristics of the patients are shown in Table 1. There was no significant change in the equilibrated Kt/V between the two groups (1.56 ± 0.33 versus 1.56 ± 0.29; $P = .61$). Also, there was no significant change in serum albumin ($P = .15$). C-reactive protein was negative in all of the studied patients. The mean age of the control group was 8.7 ± 3.2 years. They were 26 (65%) males and 14 (35%) females. The mean serum creatinine in control group was 0.5 ± 0.32 mg/dL.

Serum ICAM-1 and VCAM-1 levels were 107.80 ± 30.17 pg/mL and 228.30 ± 62.70 pg/mL, respectively, in the control group. Serum levels of TNF-α and IL-1 were 7.35 ± 1.87 pg/mL and 20.63 ± 4.74 pg/mL, respectively, in this group. Serum levels of thses markers in the two studied groups are shown in Table 2 and Figures 1 and 2. Both TNF-α and IL-1 were significantly higher in ESRD patients of both studied groups compared to control group ($P < .001$). Also, both TNF-α and IL-1 were significantly higher postdialysis compared

Table 1. Characteristics of Children on Hemodialysis*

Characteristic	Low-flux Dialysis	High-flux Dialysis
Number of patients	40	40
Mean age, y	9.7 ± 2.4	10.7 ± 2.78
Age range, y	5 to 13	4 to 13
Sex		
Male	22 (55.0)	23 (57.5)
Female	18 (45.0)	17 (42.5)
Mean time on dialysis, y	4.01 ± 1.19	3.34 ± 1.02
Time on dialysis range, y	1 to 6	2 to 6
Cause of kidney failure		
Unknown	14 (35.0)	15 (37.5)
Obstructive uropathy	9 (22.5)	11 (27.5)
Glomerulonephritis	5 (12.5)	3 (7.5)
Nephronophthitis	3 (7.5)	3 (7.5)
Cystinosis	2 (5.0)	0
Chronic interstitial nephritis	2 (5.0)	2 (5.0)
Bardet-Biedl syndrome	0	2 (5.0)
Others	5 (12.5)	4 (10.0)
Predialysis studies		
Mean serum urea, mg/dL	151.2 ± 12.2	138.6 ± 8.2
Mean serum creatinine, mg/dL	8.97 ± 0.42	8.70 ± 0.46
Mean serum albumin, mg/dL	3.17 ± 0.18	3.12 ± 0.12
Postdialysis studies		
Mean hemoglobin, g/dL	10.2 ± 2.2	10.4 ± 2.7
Mean leukocytes, × 10^9/L	7.74 ± 3.11	7.65 ± 2.71
Mean neutrophils , %	52.85 ± 9.37	50.92 ± 8.21
Mean lymphocytes, %	42.85 ± 8.17	33.12 ± 7.06

*Values in parentheses are percentages.

Table 2. Serum Biochemical Parameters*

Parameter	Low-flux Dialysis	High-flux Dialysis	*P*
ICAM-1, pg/mL			
Baseline	850.30 ± 144.45	833.42 ± 160.30	.75
Predialysis	876.95 ± 154.30	770.20 ± 163.03	.001
Postdialysis	936.80 ± 157.73	814.58 ± 160.40	< .001
Postdialysis increments	59.84 ± 24.66	44.38 ± 7.01	< .001
VCAM-1, pg/mL			
Baseline	1777.62 ± 294.05	1784.90 ± 320.57	.39
Predialysis	1820.28 ± 311.31	1580.70 ± 350.19	< .001
Postdialysis	1952.15 ± 343.69	1764.32 ± 292.97	.005
Postdialysis increments	131.87 ± 101.19	183.62 ± 163.50	.15
TNF-α, pg/mL			
Predialysis	176.32 ± 26.44	152.25 ± 34.11	.001
Postdialysis	201.60 ± 35.02	159.92 ± 25.38	< .001
IL-1, pg/mL			
Predialysis	1784.90 ± 320.57	1764.32 ± 292.97	< .001
Postdialysis	1580.70 ± 350.19	183.62 ± 163.50	< .001

*ICAM-1 indicates intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; TNF-α, tumor necrosis factor- α; and IL-1, interleukin-1.

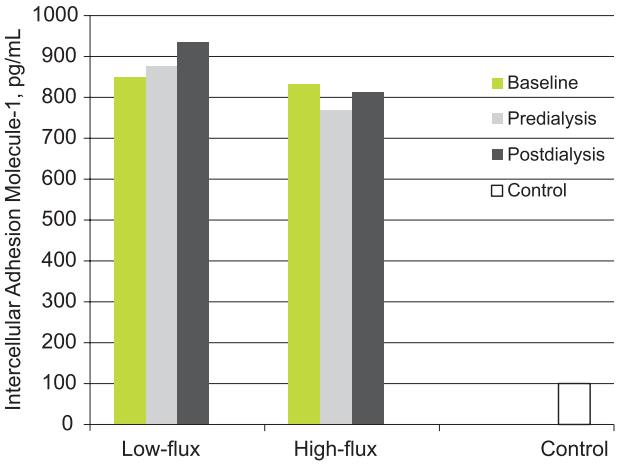

Figure 1. Mean levels of intercellular adhesion molecule-1 in patients using low-flux and high-flux membranes compared to control group.

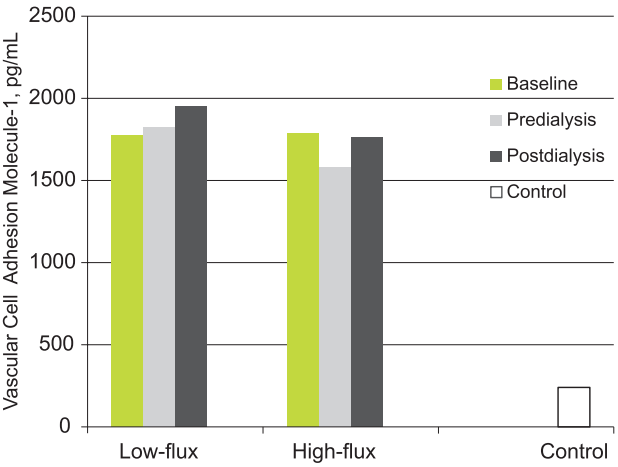

Figure 2. mean levels of vascular cell adhesion molecule-1(VCAM-1) in patients using low- and high-flux membranes compared to control group.

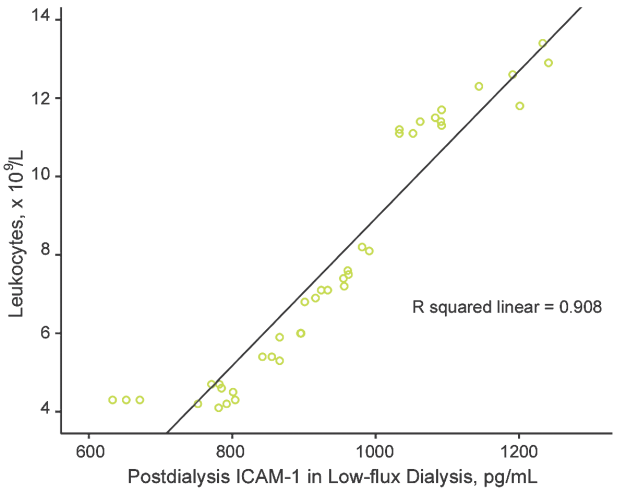

Figure 3. Correlation between postdialysis intercellular adhesion molecule-1 (ICAM-1) and leukocyte count in low-flux dialysis group.

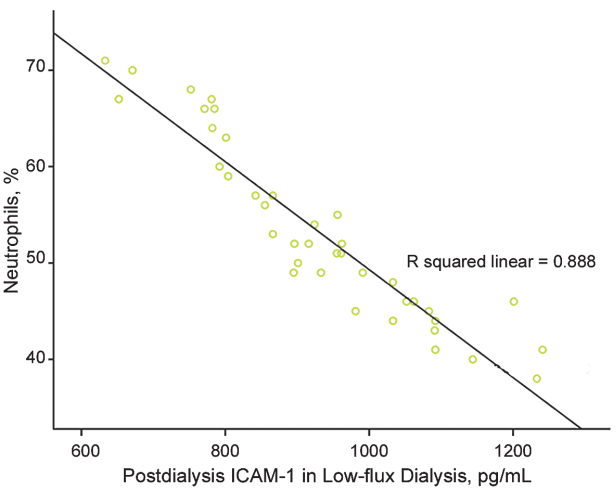

Figure 4. Correlation between post-dialysis intercellular adhesion molecule-1 (ICAM-1) and neutrophils in low-flux dialysis group.

to predialysis levels in dialysis groups ($P < .001$ and $P < .001$, respectively).

Serum Intercellular Adhesion Molecule-1

Compared to the control group, there was a significant increase in ICAM-1 level in hemodialysis patients ($P < .001$) in both groups at the start of the study, predialysis, and postdialysis. There was no significant change in serum ICAM-1 levels at the start and end of the study ($P = .36$) in the low-flux group. On the other hand, there was a significant decline of predialysis ICAM-1 at the end of study when compared with its value before start of the study in the high-flux group ($P = .04$).

There was a statistically significant linear correlation between postdialysis ICAM-1 and leukocyte count (positive correlation) and neutrophils (negative correlation) in the low-flux group (Figures 3 and 4), but not in the high-flux group. On the other hand, there was no significant correlation between postdialysis ICAM-1 and lymphocytes in either of the groups.

Serum ICAM-1 was significantly correlated with TNF-α in all groups (low-flux predialysis: $r = 0.844$, $P < .001$; low-flux postdialysis: $r = 0.973$, $P < .001$; high-flux predialysis: $r = 0.972$, $P < .001$; and high-flux postdialysis: $r = 0.913$, $P < .001$) and IL-1 in all groups (low-flux predialysis: $r = 0.789$, $P < .001$; low-flux postdialysis: $r = 0.756$, $P < .001$; high-flux predialysis: $r = 0.737$, $P < .001$; and high-flux postdialysis: $r = 0.719$, $P < .001$).

Serum Vascular Cell Adhesion Molecule-1

When compared with the control group, there was a significant increase in VCAM-1 in hemodialysis patients ($P < .001$) in both groups at start of the study, predialysis, and postdialysis. There was no significant change in serum VCAM-1 levels at the start and end of the study ($P = .57$) in the low-flux group. On the other hand, there was a significant decline of predialysis VCAM-1 at the end of study when compared with its value before start of the study in the high-flux group ($P = .006$).

Also, there was a significant linear correlation between postdialysis VCAM -1 and leukocyte count (positive correlation) and neutrophils (negative correlation) in the low-flux group (Figure 5 and 6), but not in the high-flux group. There was no significant correlation between postdialysis VCAM-1 and lymphocytes in either of the groups.

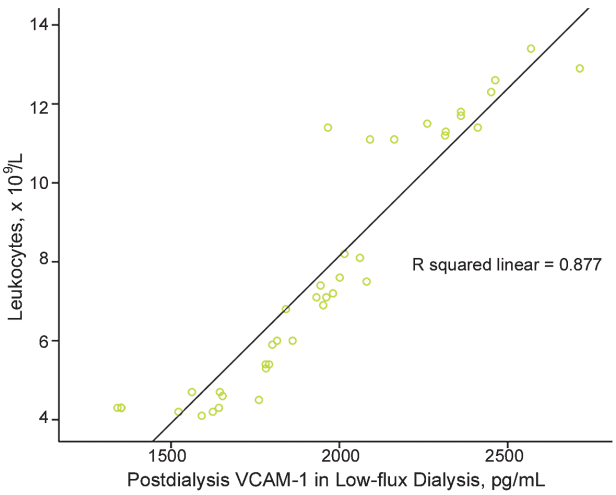

Figure 5. Correlation between post-dialysis vascular cell adhesion molecule-1 (VCAM-1) and leukocyte count in low-flux dialysis group.

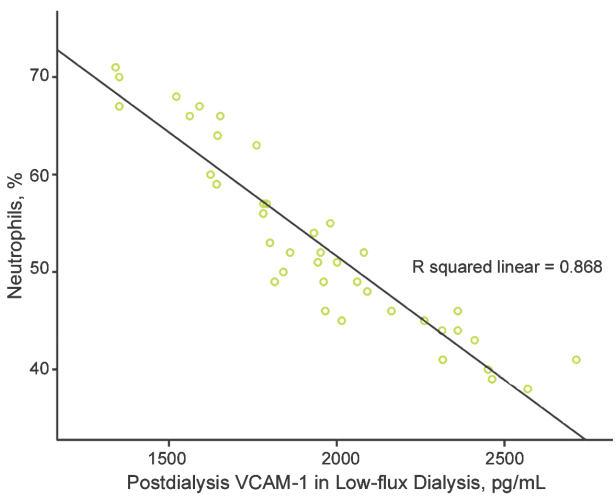

Figure 6. Correlation between post-dialysis vascular cell adhesion molecule-1 (VCAM-1) and neutrophils in low-flux dialysis group.

Serum VCAM-1 was correlated with TNF-α in all groups (low-flux predialysis: $r = 0.878$, $P < .001$; low-flux postdialysis: $r = 0.994$, $P < .001$; high-flux predialysis: $r = 0.928$, $P < .001$; and high-flux postdialysis: $r = 0.805$, $P < .001$) and IL-1 in all groups (low-flux predialysis: $r = 0.888$, $P < .001$; low-flux postdialysis: $r = 0.747$, $P < .001$; high-flux predialysis: $r = 0.704$, $P < .001$; and high-flux postdialysis: $r = 0.734$, $P < .001$).

DISCUSSION

Predialysis elevation of serum levels of ICAM-1 and VCAM-1 in our results may be attributed to decreased elimination by the impaired kidney as the kidney plays an important role in their catabolism.[1] Another explanation was proposed by Pigott and coworkers[7] who showed that elevated serum VCAM-1 levels could be induced by TNF-α-activated endothelial cells. Many authors hypothesize that one of the possible causes of increased predialysis levels of these adhesion molecules may be TNF-α activation, the intensity of which is not dependent on the type of hemodialysis membrane used.[8,9] The last explanation is in agreement with our study, as we found positive correlation between both ICAM-1 and VCAM-1 with proinflammatory cytokines (TNF-α and IL-1) either predialysis or postdialysis and irrespective of the dialysis membrane used. Dialysis membranes, being of non-self-nature, come to contact with various effector systems of blood. Various humoral and cellular inflammatory reaction cascades can be triggered and can explain elevated postdialysis levels of ICAM-1 and VCAM-1 in our results.[10] Another explanation for postdialysis elevation of the adhesion molecules is the effect of ultrafiltration which leads to hemoconcentration of these adhesion molecules.

There are a lot of contradictory results about the levels of these adhesion molecules in hemodialysis patients.[1,11,12] Most of these studies were done in adult hemodialysis patients. Those who supported that some of these adhesion molecules were not significantly elevated attributed that to bounding of these adhesion molecules to dialysis membranes. In children, the membrane areas of dialyzers are smaller than those used in adults. Therefore, adsorption of adhesion molecules may be less pronounced.

Although both dialysis membranes were of the same polysulfone nature, our results showed significant decline in predialysis serum levels of ICAM-1 and VCAM-1 after use of high-flux membrane. This appears to be different from Papayianni and colleagues who found that the type of dialysis membrane used does not influence circulating predialysis levels of these molecules, and they concluded that other factors may be responsible for the observed alterations.[13] However, they compared modified cellulose and polysulfone dialysis membranes. In our study, we compared low-flux and high-flux polysulfone membranes. High-flux membranes, being highly permeable, are efficient in removal of both small nonprotein bound and middle uremic toxins molecules, while low-flux membranes do not remove middle molecule toxins.[14,15] Although ICAM-1 and VCAM-1 are two members of immunoglobulin-like supergene family of adhesion molecules with molecular weight 90 kDa to 110 kDa, by lowering level of TNF-α (one of middle molecule uremic toxins responsible for high predialysis levels of adhesion molecules), high-flux membranes is successful in lowering, to an extent, the levels of these adhesion molecules.

Increments of ICAM-1 after use of high-flux filters were significantly less than such increments after use of low-flux filters. On the other hand, there was no significant difference in increments of VCAM-1 between both filters. This could be explained by fact that VCAM-1, in contrast to ICAM-1, is not found in leukocytes and hence would not be expected to be shed on leukocyte-membrane interactions, and as previously mentioned increased levels of these molecules may be regarded as a marker of endothelial cell activation.[14] However it must be emphasized that, apart from leukocytes and endothelial cells, various other cell types including fibroblasts and smooth muscle cells have been shown to be cellular sources for these molecules. It is obvious that the identification of the cells responsible for adhesion molecules release during hemodialysis will considerably aid the interpretation of the above results.

The linear correlation of ICAM-1 and neutrophils is well understood as this adhesion molecule serves as a counter-receptor for neutrophil β_2 integrin molecules, leading to high-avidity neutrophils spreading and the start of trans-endothelial migration.[16] Our study is in agreement with Musial and colleagues who found linear correlations between ICAM-1 and VCAM-1 and granulocytes in chronic renal failure patients.[1] We found that this correlation was only significant in low-flux but not in high-flux patients. We can postulate that the granulocytes are less influential by changes in these adhesion molecules with high-flux membranes. Why this correlation was found only in low-flux and not high-flux membranes needs to be elucidated.

In spite of the advantages found in the present study such as the presence of control group some limitations are present such as the need of measurements of adhesion molecules in patients with chronic renal failure under conservative management and ESRD patients with peritoneal dialysis to compare results.

CONCLUSIONS

Serum levels of ICAM-1 and VCAM-1 are higher in hemodialysis patients compared with controls. The postdialysis increments of ICAM-1 are due to the effect of dialysis membranes which is less with the use of high-flux filters. Elevated levels of these adhesion molecules are associated with neutrophils activation among low-flux and not high-flux users.

CONFLICT OF INTEREST

None declared.

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Correspondence to:
Happy K Sawires, MD
Department of Pediatrics, Cairo University, Cairo, Egypt
E-mail: happy7_kd@yahoo.com

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