ORIGINAL ARTICLE

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Soluble adhesion molecules in children and young adults on chronic hemodialysis

Abstract Children on chronic hemodialysis (HD) present with impaired immunity that may result from disturbances in leukocyte migration, caused by changes in expression of adhesion molecules on endothelium and immunocompetent cells. However, it is still not clear whether the type of dialyzer or a single dialysis session influences the concentrations of soluble adhesion molecules in these patients. We evaluated by ELISA serum levels of soluble (s) VCAM-1, ICAM-1, L-selectin, and P-selectin in 22 patients on cuprophane HD (CU), 8 on polysulfone HD (PS), 10 on vitamin E-modified cellulose HD (VE), and 15 controls. In all HD patients, sVCAM-1 levels were elevated compared with controls and were higher in CU than in VE. The sICAM-1 concentrations were increased in VE compared with controls, but remained unchanged in CU and PS. The sL-selectin levels were reduced in all HD patients. The mean values of sP-selectin were comparable in CU, PS, and controls. The lowest levels were observed in VE. In CU patients, sVCAM-1, sICAM-1, and sP-selectin concentrations rose after HD. A single PS session had no impact on adhesion molecules, whereas a VE session increased the level of sVCAM-1. The type of dialysis membrane may change the profile of adhesion molecule concentrations, thus influencing the immune system of a child on HD. The increase in levels of adhesion molecules in the course of a single HD session, which was pronounced in CU and VE patients, suggests poor biocompatibility of these dialyzers.

Keywords Adhesion molecules · Biocompatibility · Hemodialysis · Cuprophane · Polysulfone · Vitamin E-coated membrane

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Introduction

Leukocyte migration to sites of inflammation plays a pivotal role in an effective immune response [1]. This process is based on leukocyte attachment to endothelium, mediated by adhesion molecules [2]. Integrins, selectins (E-selectin, L-selectin, and P-selectin), and molecules from the immunoglobulin superfamily (e.g., ICAM-1, VCAM-1) determine subsequent stages of the adhesion cascade [2]. The latter depends on the activity of cytokines and chemotactic agents [1]. Tumor necrosis factor (TNF)-α and interleukin-1 promote binding of leukocytes to endothelial cells by increasing the expression of adhesion molecules and causing their proteolytic shedding from cells into the circulation [3, 4]. However, leukocyte-endothelium binding may be inhibited by soluble forms of adhesion molecules that preserve their activity [5]. Kawabata et al. [6] have shown that high concentrations of sL-selectin inhibit low-affinity binding of leukocytes to endothelium. Moreover, sP-selectin can bind to neutrophils preactivated with TNF-α and inhibit their adhesion to endothelium [7].

There have been reports of changes in levels of integrin and selectin during hemodialysis (HD) sessions on various dialyzers [6, 8, 9]. However, the current literature on soluble adhesion molecules in HD patients is contradictory [10, 11, 12, 13, 14]. Moreover, none of the reports have concerned children or young adults.

In this study, we evaluated the serum levels of four soluble adhesion molecules (sVCAM-1, sICAM-1, sL-selectin, sP-selectin) in patients on maintenance HD treated with cuprophane (CU), polysulfone (PS), or vitamin E (VE)-modified cellulose membranes. The

investigation of selectins, which initiate leukocyte rolling, and immunoglobulin superfamily members, responsible for adhesion and diapedesis, was aimed at analyzing the subsequent stages of the adhesion cascade. The choice of particular adhesins also depended on the location of their membrane-bound form and ability to evaluate endothelial (sICAM-1, sVCAM-1), leukocyte (sL-selectin), and platelet (sP-selectin) activity. Our aim was to analyze whether the type of dialyzer used or a single dialysis session might influence adhesion molecule concentrations and adhesion cascade functioning, thus modifying the immunological profile of children and young adults on chronic HD.

Materials and methods

Children and young adults on maintenance HD were divided into three groups: 22 patients (11 girls, 11 boys, mean age 17.5 years, range 11.5–20.5 years) on CU membrane HD (mean duration of therapy 2 years, range 1 month to 8 years), 8 patients (5 girls, 3 boys, mean age 18 years, range 16.5–20 years) on PS membrane HD (mean duration of therapy 2.3 years, range 1.5–3 years), and 10 patients (5 girls, 5 boys, mean age 19 years, range 13–21.5 years) on VE-modified cellulose membrane HD (mean duration of therapy 7 years, range 1.8–14 years). The factors causing chronic renal failure were chronic glomerulonephritis (36%), chronic pyelonephritis (34%), and urinary tract malformations (30%). The control group consisted of 15 patients (7 girls, 8 boys, mean age 15 years, range 12–18 years) with a diagnosis of urinary tract abnormalities or urolithiasis, with normal kidney function. HD sessions (4–5 h) were performed 3 times a week, using bicarbonate dialysate. The membrane area was between 1.0 m^2 and 1.6 m^2. The dialyzers were not reused. All patients were on a stable anticoagulation regimen using low molecular weight heparin. None of the patients showed clinical evidence of infection, had malignancies, took antibiotics, corticosteroids, or immunosuppressive therapy. Informed consent was obtained from the subjects and their parents, if necessary.

Blood samples were drawn from the efferent line of the first-use dialyzer before starting a HD session, 15 min after the beginning of HD (at the time of maximal leukocyte activation), and at the end of the session. In controls, blood was drawn from a peripheral vein. Samples were centrifuged at 4°C for 10 min, and then serum samples were stored at −20°C until assay. Serum concentrations of sVCAM-1, sICAM-1, sL-selectin, and sP-selectin were evaluated by commercially available ELISA kits (R and D Systems, Minneapolis, Minn., USA). Each sample was measured in duplicate and the arithmetic mean was considered as a final result. Standards (recombinant human sVCAM-1, sICAM-1, sL-selectin, and sP-

selectin) and serum samples were transferred to 96-well microplates coated with murine monoclonal antibodies to human sVCAM-1, sICAM-1, sL-selectin, and sP-selectin, respectively. The wells were first incubated with a sheep polyclonal antibody to recombinant human antigen, then with the appropriate substrate (tetramethylbenzidine). The reaction was stopped with acid solution, then the absorbance was measured at 450 nm with the correction wavelength set at 620 nm. Measurements were performed according to the manufacturer's instructions. Results were calculated by reference to standard curves. Limits of detection for adhesins were as follows: sVCAM-1 2 ng/ml, sICAM-1 0.35 ng/ml, sL-selectin 0.3 ng/ml, and sP-selectin 0.5 ng/ml.

Results are expressed as mean values±SD. Differences between all groups as well as between adhesin values during HD sessions were evaluated by using non-parametric tests (Kruskall–Wallis, Friedman, Mann-Whitney U, Wilcoxon). Statistical analysis was performed using the package Statistica 5.0. A P value <0.05 was considered significant.

Results

Serum sVCAM-1 levels

In all groups sVCAM-1 concentrations were higher than in controls ($P<0.00001$) and mean values in CU were higher than in VE patients ($P<0.01$) (Table 1). There was a significant increase in sVCAM-1 levels during a single HD session with a CU membrane ($P<0.05$ after 15 min of HD vs. before HD, $P<0.02$ after HD vs. before HD and after HD vs. after 15 min of HD) (Fig. 1). A single session of VE membrane dialysis increased the level of sVCAM-1 ($P<0.01$ before HD vs. after HD). PS membrane dialysis had no influence on sVCAM-1 levels (Fig. 1, Table 2).

Serum sICAM-1 levels

Mean sICAM-1 values (Table 1) in PS and in CU patients did not differ from those observed in controls, whereas concentrations detected in VE patients were significantly higher ($P<0.01$). Mean values of sICAM-1 increased during a single HD session with a CU membrane ($P<0.02$ after HD vs. before HD). No differences in sICAM-1 levels during sessions with PS and VE membranes were observed (Fig. 2, Table 2).

Table 1 Serum sVCAM-1, sICAM-1, sP-selectin, and sL-selectin concentrations in patients before hemodialysis (HD) (*CU* cuprophane, *PS* polysulfone, *VE* vitamin E)

Parameter (ng/ml) Mean±SD	Control group n=15	Values before single HD session			Differences assessed by Kruskal-Wallis test between:	
		CU patients n=22	PS patients n=8	VE patients n=10	control group, CU, PS, and VE patients	CU, PS, and VE patients
sVCAM-1	625.00±80.00	1,849.90±366.00*2, *4	1,566.25±397.48*2	1,578.00±718.80*2	P=0.0000	P=0.0426
sICAM-1	231.60±60.87	291.18±93.16	309.00±136.49	401.60±209.79*1	P=0.0135	P=0.1155
sP-selectin	93.13±14.59	114.72±52.54*4	116.25±32.04*5	70.70±13.36*1	P=0.0036	P=0.0065
sL-selectin	6492.66±1,914.45	1,210.90±317.69*3	1,547.50±328.14*3	1,237.00±130.30*3	P=0.0000	P=0.068

Mann-Whitney U-test results: *1 $P<0.01$, *2 $P<0.00001$, *3 $P<0.000001$ vs. controls; *4 $P<0.01$ CU vs. VE; *5 $P<0.01$ PS vs. VE
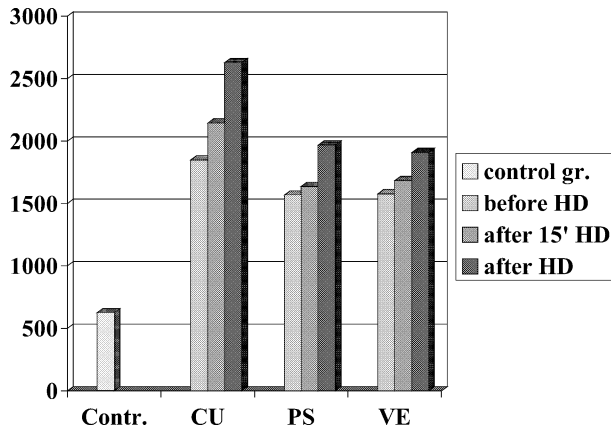
Fig. 1 Serum sVCAM-1 concentrations in patients before hemodialysis (HD), after 15 min of HD, and at the end of HD. Differences assessed by Friedman's test. Cuprophane (CU) $P=0.0009$, polysulfone (PS) $P=0.093$, vitamin E-coated (VE) $P=0.0048$
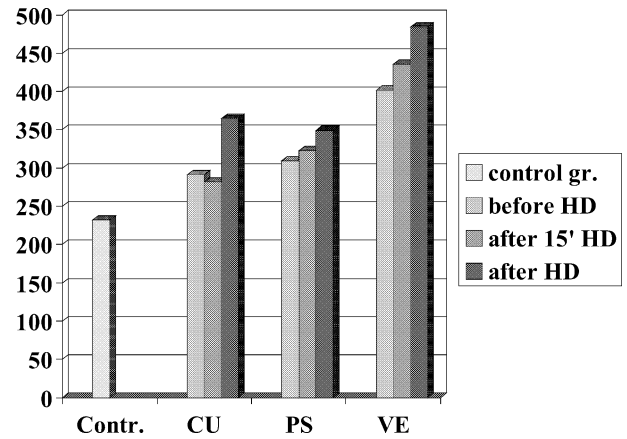

Fig. 2 Serum sICAM-1 concentrations in patients before HD, after 15 min of HD, and at the end of HD. Differences assessed by Friedman's test. CU $P=0.0187$, PS $P=0.342$, VE $P=0.074$

Serum sP-selectin (soluble CD62P) levels

Mean sP-selectin levels (Table 1) in CU and PS patients were comparable with those observed in controls and were decreased in VE patients ($P<0.01$). The levels in the VE group were also lower than those in CU and in PS patients ($P<0.01$) (Table 1). A single session of CU dialysis increased the levels of sP-selectin ($P<0.02$ after HD vs. after 15 min of HD), whereas PS and VE sessions did not change sP-selectin levels (Fig. 3, Table 2).
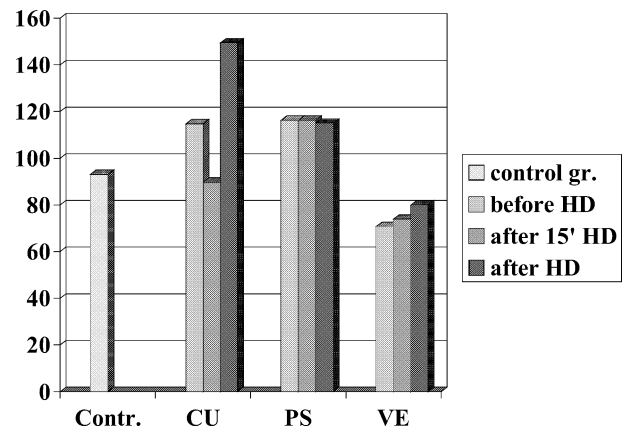
Serum sL-selectin levels

In all HD patients mean sL-selectin values were lower than in controls (Table 1). There were no differences between the patients dialyzed on different membranes.

Fig. 3 Serum sP-selectin concentrations in patients before HD, after 15 min of HD, and at the end of HD. Differences assessed by Friedman's test. CU $P=0.009$, PS $P=0.875$, VE $P=0.097$

Table 2 Serum sVCAM-1, sICAM-1, sP-selectin, and sL-selectin concentrations in patients before HD (B), after 15 min of HD (M), and at the end of dialysis (E)

| Parameter (ng/ml)	B	M	E	Friedman's test
CU patients				
Mean±SD				
sVCAM-1	1849.90±366.00	2144.28±415.94	2628.00±422.07	$P=0.0009$
sICAM-1	291.18±93.16	281.14±96.03	364.70±151.04	$P=0.0187$
sP-selectin	114.72±52.54	89.71±36.97	149.40±112.61	$P=0.009$
sL-selectin	1210.90±317.69	1240.71±315.06	1276.00±281.30	$P=0.947$
PS patients				
Mean±SD				
sVCAM-1	1566.25±397.48	1635.62±609.27	1968.12±540.72	$P=0.093$
sICAM-1	309.00±136.49	322.50±129.71	349.00±187.41	$P=0.324$
sP-selectin	116.25±32.04	116.25±27.22	115.00±41.05	$P=0.875$
sL-selectin	1547.50±328.14	1520.00 ±396.41	1566.25±272.23	$P=1.0$
VE patients				
Mean±SD				
sVCAM-1	1578.00±718.80	1683.33±733.41	1908.33±703.51	$P=0.0048$
sICAM-1	401.60±209.79	435.55±224.33	484.60±221.74	$P=0.074$
sP-selectin	70.70±13.36	73.88±17.56	80.00±14.59	$P=0.097$
sL-selectin	1237.00±130.30	1246.66±127.47	1291.00±167.09	$P=0.895$
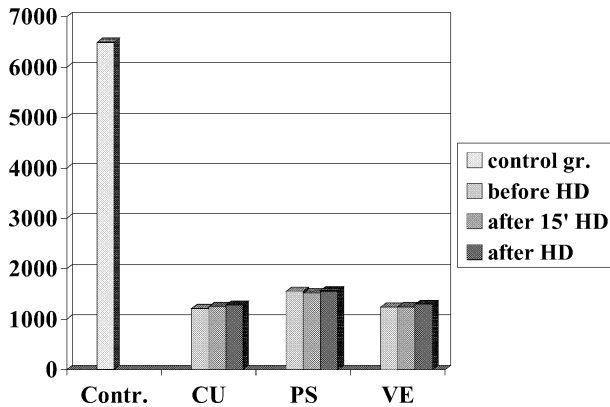
Fig. 4 Serum sL-selectin concentrations in patients before HD, after 15 min of HD, and at the end of HD. Differences assessed by Friedman's test. CU $P=0.947$, PS $P=1.0$, VE $P=0.895$

The levels of sL-selectin remained unchanged during HD sessions performed on all dialyzers evaluated in this study (Fig. 4, Table 2).

Discussion

Our study revealed a significant increase in sVCAM-1 concentrations in all HD patients, irrespective of the dialyzer used, thus supporting previous investigations [11, 12, 13]. Mean levels of adhesion molecules in subjects dialyzed on CU membranes were higher than those in patients dialyzed on VE membranes. Moreover, both CU and VE sessions increased the levels of sVCAM-1, thus suggesting poor biocompatibility of both types of membranes compared with PS. These results were confirmed by Mrowka et al. [12]. Rabb et al. [13] observed a significant decrease of sVCAM-1 levels after a 3-h HD session on new CU dialyzers and suggested the adsorption of adhesin onto the membrane as a possible mechanism. However, our measurements were performed after at least 4 h of dialysis, so possible detachment of bound adhesin cannot be excluded. Moreover, the membrane areas of dialyzers in young patients are smaller than those used in adults. Therefore, adsorption of adhesion molecules may be less pronounced. Finally, pronounced differences between the three groups and susceptibility to contact with membranes during a single HD session suggest that high levels of sVCAM-1 do not simply mirror activation, but respond to different stimuli. Firstly, sVCAM-1 elevation in all patients may be due to decreased elimination by the impaired kidney. Secondly, Pigott et al. [3] showed the induction of elevated sVCAM-1 levels by TNF-α-activated endothelial cells, while our investigation revealed increased TNF-α concentrations in children on HD [15]. Therefore, we hypothesize that one of the possible causes of increased predialysis sVCAM-1 levels may be TNF-α activation, the intensity of which does not depend on the type of HD membrane used [15, 16, 17].

sICAM-1 levels were unchanged, compared with controls, in patients treated with either CU or PS dialyzers, and elevated in patients dialyzed on VE membranes. The latter is a new finding, whereas other authors have reported either increased [11, 13, 14] or unchanged [12] sICAM-1 concentrations in adults on CU HD. These discrepancies may result from the susceptibility of the immune system of a child to blood-membrane contact, generating cell hyporeactivity due to chronic stimulation. sICAM-1 is also a marker of endothelial activation that could be more intense in adults on HD than in young patients. However, adhesion molecule concentrations failed to distinguish between different membrane types. These results were confirmed by Bonomini et al. [11] and Mrowka et al. [12]. The sICAM-1 increase during HD with CU membrane may be due to bioincompatibility of this type of membrane.

Significantly decreased sL-selectin levels were detected in all our patients. Schleiffenbaum et al. [5] showed that sL-selectin is shed from activated leukocytes and binds to L-selectin counter-receptors on endothelium, thus inhibiting leukocyte adhesion to endothelium. Therefore, decreased sL-selectin concentrations result from either leukocyte dysfunction and failure to shed sL-selectin, or from sL-selectin binding to endothelial receptors. Consequently, decreased sL-selectin concentrations may reflect leukocyte function and impairment of migration that may be, at least in part, responsible for the increased incidence of infections in HD patients.

Although baseline sL-selectin concentrations in patients treated with PS membranes were higher than those in children on CU or VE dialyzers, this difference did not reach the statistical significance. However, this result suggests that PS, due to its greater biocompatibility, impairs leukocyte adhesion to a lesser extent than cellulose or CU membrane. We did not observe the CU-related sL-selectin elevation during HD described by other authors [6, 9]. In our patients the sL-selectin concentration was stable during HD sessions, regardless of the membrane type. Our findings support the hypothesis of granulocyte hyporesponsiveness due to repetitive stimulation by contact with bioincompatible cellulose membranes [9], which may be the reason for the lack of variation of sL-selectin levels in the course of HD.

The levels of sP-selectin in CU and PS patients did not differ from those in controls, suggesting cell hyporeactivity due to repetitive stimulation by blood-membrane contact in children on HD. In contrast, Bonomini et al. [11] detected elevated sP-selectin concentrations both in adult patients on dialysis with CU and synthetic membranes. However, when we compared the values in CU and PS children, no significant difference was noticed. VE patients presented with significantly decreased sP-selectin concentrations compared with CU and PS patients. The analysis of our results is difficult because this is the first study on soluble adhesion molecules in patients on VE-modified membranes. Firstly, VE-coated dialyzers reduce neutrophil activation during HD and weaken the effects of the oxidative burst [18, 19]. Secondly, Nagata et

al. [20] showed that sP-selectin can bind to P-selectin counter-receptors on platelets, thus inhibiting formation of platelet-leukocyte aggregates and leukocyte superoxide production. Consequently, low sP-selectin levels in VE patients may result from their binding to platelet receptors and reflect the decreased production of reactive oxygen species in this group. Interestingly, adhesin levels in VE patients were lower than in controls, which suggests the possible bioincompatibility of this dialyzer. In contrast to Bonomimi et al. [11], we did not observe a change in sP-selectin concentrations during CU HD. However, there was an increase in the adhesin level when the values measured 15 min after the beginning of the session and after HD were considered. These results confirm those obtained by Stuard et al. [21], who detected CD62P overexpression on leukocytes during cellulose HD, and suggest poor biocompatibility of this type of membrane. However, the latter should be confirmed by the study of a larger group of patients.

In conclusion, elevated (sVCAM-1, sICAM-1) or lowered (sL-selectin, sP-selectin), compared with controls, adhesin levels in patients on maintenance HD, may illustrate deficiency and activation of immunocompetent cells and impairment of the adhesion cascade, which may be partially responsible for the dysregulation of the immune response in HD patients. Changes in concentrations of adhesion molecules during a single HD session depend on the type of membrane used. Our results show a big difference between the dialyzers examined, suggesting the greatest biocompatibility in the case of CU dialyzers and, surprisingly, rather poor biocompatibility of VE-coated membranes. However, the relationships between soluble adhesins and dialyzer membrane surfaces require further detailed investigation.

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