Soluble adhesion molecules in children and young adults with chronic renal failure treated conservatively

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Abstract

Purpose: Chronic renal failure (CRF) patients present with signs of immunodeficiency, such as increased incidence of infections. Cell adhesion molecules, determining leukocyte migration, may be responsible for the impaired immune response. The aim of the study was to measure soluble (s) vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), P-selectin and L-selectin levels in sera of CRF children and young adults.

Material and methods: The evaluation of adhesion molecule concentrations by ELISA was performed on 15 patients with serum creatinine levels below 265.2 μmol/l (gr. I), 15 patients with serum creatinine levels above 265.2 μmol/l (gr. II) and 15 controls.

Results: sVCAM-1, sICAM-1 and sP-selectin concentrations were elevated in both groups vs controls, whereas sL-selectin levels were decreased in all CRF patients. Mean sVCAM-1 and sICAM-1 values in gr. I and gr. II were comparable. sL-selectin and sP-selectin mean values in gr. II were lower than in gr. I. sICAM-1 correlated with haemoglobin and erythrocyte count in both groups and with haematocrit and serum urea – in gr. I.

Conclusions: Enhanced (sVCAM-1, sICAM-1, sP-selectin) and diminished (sL-selectin) adhesion molecule concentrations in both groups show a state of immunologic imbalance, already present in early stages of CRF. Differences in sL-selectin concentrations between gr. I and II imply a progressive character of CRF-related leukocyte dysfunction. sICAM-1 correlation with anaemia markers may suggest the connection between this molecule and the CRF-related disorders.

Key words: soluble adhesion molecules, immunodeficiency, chronic renal failure, children, young adults.

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Received 15.03.2004 Accepted 10.08.2004

Introduction

Chronic renal failure (CRF) and its treatment are associated with complex impairment of the immune system. CRF patients present with acquired immunodeficiency, which is clinically manifested by prolonged skin allograft survival, anergy in cutaneous delayed – type hypersensitivity tests, dysregulated response to vaccinations and increased incidence of infections [1-3]. The latter may result from the insufficient migration of immunocompetent cells, possibly leading to the impaired defence against pathogens.

Integrins, selectins (E-selectin, L-selectin and P-selectin) and cell adhesion molecules from immunoglobulin superfamily, like intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), take part in the subsequent stages of adhesion cascade, determining leukocyte migration to the sites of inflammation or tissue damage [4-7]. Activation of the membrane-bound adhesion molecules, observed during this process, results in their proteolytical shedding from cells into the circulation [8,9]. These soluble forms appear to be biologically active and influence leukocyte attachment to the vascular endothelium, thus playing a crucial role in effective immune response [10,11].

There are studies concerning the immune status of adults with uraemia, but hardly any have dealt with the children population [1-3,12-19]. The aim of the present study was therefore to evaluate serum levels of soluble (s) adhesion molecules: sICAM-1, sVCAM-1, sP-selectin and sL-selectin, in children and young adults with chronic renal failure treated

conservatively. The analysis of selectins, initiating leukocyte rolling, and immunoglobulin superfamily members, responsible for adhesion and diapedesis, was aimed at evaluating the subsequent stages of adhesion cascade. The choice of particular adhesins depended also on the variety of their membrane form location and possible evaluation of endothelial (sICAM-1, sVCAM-1), leukocyte (sL-selectin) and platelet (sP-selectin) activity. We also searched for relationship between circulating adhesin levels and markers of renal function impairment as well as CRF-related complications like anaemia or disturbances in calcium-phosphate metabolism.

Material and methods

The study included 30 children and young adults (15 boys and 15 girls) with CRF, treated conservatively in 2 Departments of Paediatric Nephrology in Wroclaw and Zabrze. Their age ranged from 18 months to 22 years (mean 10.5 years). Mean time of therapy, since the diagnosis of CRF has been established, was 5.5 years (range 3 months – 14 years). Primary diseases causing CRF were: reflux nephropathy (n=11), chronic glomerulonephritis (n=4), urinary tract obstruction (n=3), neurogenic bladder (n=3), amyloidosis (n=2), polycystic kidney disease (n=2), haemolytic uraemic syndrome (n=1), cystynosis (n=1), dysplasia (n=1) and unknown (n=2). CRF patients were divided into two subgroups based on the extent of renal function impairment. Group I included 15 children (9 girls, 6 boys) aged 18 months – 18 years (mean 11.5 years) with mean serum creatinine level 185.6 ± 44.2 μmol/l (range 106.0 μmol/l – 265.2 μmol/l). Group II consisted of 15 children (6 girls, 9 boys) aged 20 months – 22 years (mean 10.1 years) with mean serum creatinine level 512.72 μmol/l ± 256.36 μmol/l (range 265.2 μmol/l – 1281.8 μmol/l). In both groups urinary tract abnormalities were the dominant cause of CRF. The glomerular filtration rate (GFR) was estimated by the Schwartz formula. GFR ranged from 50 to 15 ml/1.73m^2/min in gr. I and was less than 15 ml/1.73m^2/min in gr. II. The control group contained 15 children (10 boys, 5 girls, mean age 11.5 years) with a diagnosis of urinary tract abnormalities or urolithiasis, with normal kidney function. Detailed characteristics of examined children are shown in *Tab. 1*. When necessary, phosphate binders and vitamin D analogue supplementation was applied. None of the patients showed clinical evidence of infection, had malignancies, received recombinant erythropoetin, took antibiotics, or immunosuppressive therapy. Informed consent was obtained from the subjects and their parents.

In each patient, sICAM-1, sVCAM-1, sP-selectin and sL--selectin plasma levels were evaluated. Blood for the examination was drawn from peripheral vein during routine control. Samples were centrifuged at 4°C, 2000 g , for 10 minutes, then serum was stored at -20°C until assay. Serum concentrations of adhesion molecules were determined by commercially available ELISA kits (R&D Systems, Inc., Minneapolis, Minn., USA) on Statfax 2100 (Analco). Each sample was measured in duplicate and the arithmetic mean was considered as a final result. Results were calculated by reference to standard curves. Limits of detection, intra- and interassay variations for adhesion molecules were as follows: sVCAM-1 – 2 ng/ml, 3.5%, 7%; sICAM-1 – 0.35 ng/ml, 3.5%, 7.5%; sP-selectin – 0.5 ng/ml, 4%, 8%; sL-selectin – 0.3 ng/ml, 4%, 8%.

The following investigations were also carried out: renal function evaluation (serum urea and creatinine levels), haematocrit, haemoglobin concentration, peripheral blood erythrocytes, leukocyte, platelet and lymphocyte count, serum total proteins, total cholesterol, triglicerides, calcium, inorganic phosphate.

Results are expressed as mean values ± SD. Differences between all groups were evaluated by using non-parametric tests (Kruskall-Wallis, Mann-Whitney U). Correlations between variables were evaluated by Spearman's correlation coefficient. Statistical analysis was performed using the package Statistica 5.0 (StatSoft). A p value < 0.05 was considered significant.

Results

Baseline laboratory test results are shown in *Tab. 1*.

Serum sVCAM-1 levels

The concentrations of sVCAM-1 were increased in all CRF patients vs controls, irrespective of their serum creatinine levels (*Tab. 2*). No significant difference between sVCAM-1 concentrations in group I and group II was observed. The levels of sVCAM-1 correlated with granulocyte count ($r = 0.58$; $p < 0.05$) in group I, with lymphocyte count ($r = -0.57$; $p<0.05$) and platelet count ($r = -0.53$; $p<0.05$) – in group II.

Serum sICAM-1 levels

The concentrations of sICAM-1 were elevated in CRF patients from both groups, when compared with controls (*Tab. 2*). There were no significant differences between sICAM-1 concentrations in group I and group II. Strong linear correlations were found between sICAM-1 levels and serum concentrations of: haemoglobin ($r = -0.83$; $p < 0.001$), haematocrit ($r = -0.86$; $p < 0.001$), erythrocyte count ($r = -0.82$; $p < 0.001$), urea ($r = 0.70$; $p < 0.01$) and granulocyte count ($r = -0.71$; $p < 0.01$) – in group I; haemoglobin ($r = -0.66$; $p < 0.01$) and erythrocyte count ($r = -0.53$; $p < 0.05$) – in group II.

Serum sP-selectin levels

The levels of sP-selectin were elevated in all CRF patients, when compared with controls (*Tab. 2*). sP-selectin concentrations in group I were significantly higher than in group II. The levels of sP-selectin correlated with alkaline phosphatase activity ($r = 0.74$; $p < 0.001$) in group I and with lymphocyte count ($r = 0.59$; $p < 0.05$) in group II.

Serum sL-selectin levels

The levels of sL-selectin were decreased in all CRF patients vs controls, irrespective of their serum creatinine levels (*Tab. 2*). Additionally, sL-selectin concentrations in group II were significantly lower than in group I. No correlation was found between sL-selectin and biochemical markers in any of the analyzed groups.

Table 1. **CRF patients characteristics and baseline laboratory test results presented as mean ± SD**

Results	I GROUP n = 15	II GROUP n = 15
Age (years)	11.5 ± 4.4	10.1 ± 5.7
CRF duration (months)	72 ± 53.8	63.8 ± 53.8
Haematocrit (%)	36.3 ± 6.0	29.8 ± 3.7
Haemoglobin (g/dl)	12.4 ± 2.1	10.0 ± 1.3
Erythrocytes (T/l)	4.3 ± 0.6	3.4 ± 0.5
Leukocytes (G/l)	8.1 ± 2.5	6.7 ± 2.0
Neutrophils (%)	57.2 ± 9.0	55.5 ± 11.6
Lymphocytes (%)	34.1 ± 7.7	37.9 ± 12.7
Trombocythes (G/l)	309.1 ± 94.9	297.1 ± 107.6
Sodium (mmol/l)	142.1 ± 4.7	140.9 ± 3.8
Potassium (mmol/l)	4.4 ± 0.8	4.5 ± 0.9
Calcium (mmol/l)	2.25 ± 0.5	2.38 ± 0.4
Phosphate (mmol/l)	1.49 ± 0.52	1.71 ± 0.46
Urea (mmol/l)	85.8 ± 43.5	132.0 ± 47.0
Creatinine (μmol/l)	186.3 ± 40.7	511.0 ± 256.4
Total proteins (g/l)	73.2 ± 12.7	70.7 ± 0.9
Total cholesterole (mmol/l)	5.59 ± 1.7	5.51 ± 1.3
Triglicerydes (mmol/l)	21.2 ± 13.7	16.2 ± 8.9

Table 2. **Mean serum sVCAM-1, sICAM-1, sP-selectin and sL-selectin concentrations in examined groups**

Parameter [ng/ml] Mean ± SD	Control group n = 15	CRF – group I n = 15 serum creatinine <265.2 μ mol/l	CRF – group II n = 15 serum creatinine >265.2 μ mol/l	Differences between controls, gr. I and gr. II (Kruskall-Wallis test)	Differences between controls and gr. I (Mann-Whitney U test)	Differences between controls and gr. II (Mann-Whitney U test)	Differences between gr. I and gr. II (Mann-Whitney U test)
sVCAM-1	625.00 ± 80.00	1442.00 ± 711.03	1478.67 ± 559.76	p = 0.0000	p = 0.0000	p = 0.0000	p = 0.6600
sICAM-1	231.60 ± 60.87	334.93 ± 109.56	348.67 ± 82.27	p = 0.0006	p = 0.0042	p = 0.0002	p = 0.7400
sP-selectin	93.13 ± 14.59	189.33 ± 61.13	140.13 ± 55.28	p = 0.0002	p = 0.0000	p = 0.0238	p = 0.0279
sL-selectin	6492.66 ± 1914.45	1982.67 ± 391.51	1618.00 ± 484.93	p = 0.0000	p = 0.0000	p = 0.0000	p = 0.0327

Discussion

Deleterious effects of uraemia may change various components of immune system. In our study, the analysis of soluble cell adhesion molecules in children with chronic renal failure treated conservatively revealed the elevation of sVCAM-1, sICAM-1 and sP-selectin serum concentrations. Bonomini et al. [20] also found increased serum levels of sICAM-1, sVCAM-1, sE-selectin and sP-selectin in adult patients with CRF on conservative treatment and on maintenance dialysis. In the predialysis group circulating adhesion molecule levels correlated positively with serum creatinine levels. Likewise, Mrowka et al. [21] described the increased concentrations of sICAM-1 and sVCAM-1, strongly correlating with serum creatinine levels, in patients with CRF treated conservatively, as well as in the dialyzed ones. In both studies, the authors pointed out impaired excretory kidney function and inefficient elimination of molecules as the main causes of their accumulation. In our patients, we only noticed the relationship between sICAM-1 and serum urea levels in the group of patients with serum creatinine concentration below 265,2 μmol/l. Ara et al. [22] did not confirm the association between sICAM-1, sVCAM-1, sE-selectin, or sP-selectin and serum creatinine levels in CRF patients either. Taking into account the fact that sICAM-1 and sVCAM-1 concentrations in our study did not increase as renal insufficiency progressed, we may speculate that various overlapping mechanisms may be responsible for elevated concentrations of those molecules. It was documented that the activity of pro-inflammatory cytokines (e.g. TNF-α) is enhanced in uraemia [23,24]. Pigott et al. [9] revealed the elevation of sICAM-1 and sVCAM-1 concentrations due to stimulation by TNF-α. Thus, the increased levels of sVCAM-1 and sICAM-1 in our patients may result from TNF-α stimulation. Correlations observed between sICAM-1 and markers of anaemia need to be further investigated.

sP-selectin, elevated in all our patients, can be derived from both platelets and endothelium. The study by Gamble et al. [25] revealed that sP-selectin inhibits the adhesion of neutrophils, previously activated with TNF-α, to the endothelium. That reaction weakens the effectiveness of further leukocyte migration. The latter was also confirmed by Nagata et al. [26], who observed the inhibition of leukocyte superoxide anion production in the presence of recombinant, circulating P-selectin. Our study [27] showed that the higher serum creatinine concentration, the more severe peroxidative damage in CRF children. Taking into account these results and our

observations, we hypothesized that enhanced concentrations of sP-selectin may be regarded as an indicator, reflecting disordered leukocyte-endothelial adhesion and impaired production of active oxygen compounds in uraemia.

Among soluble adhesion molecules, sL-selectin was the only one to show decreased serum concentrations in adults with CRF [22,28]. Our results confirmed diminished levels of sL-selectin also in children. Dou et al. [28] explained this phenomenon by impaired granulocyte function in uraemia, which resulted in reduced adhesion molecule synthesis. Schleiffenbaum et al. [11] and Kawabata et al. [29] compared the expression of membrane receptors for L-selectin with the levels of its soluble form. They emphasized that the presence of sL-selectin in serum is a consequence of its release from the surface of activated leukocytes. Schleiffenbaum et al. [11] also showed that sL-selectin binds to L-selectin counterreceptors on endothelium, thus inhibiting the adhesion of leukocytes to the endothelium. Therefore, lowered 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 reflect the impairment of leukocyte function and migration, that may be, at least in part, responsible for increased incidence of infections in CRF children. Moreover, our examination revealed that sL-selectin levels in patients with serum creatinine levels above 265.2 μmol/l were lower than those in patients with the values below 265,2 μmol/l. Nonetheless, sL-selectin concentrations decrease as renal function deteriorates, thus showing progressive granulocyte impairment. In contrast, Dou et al. [28] did not observe correlation between sL-selectin concentration and expression of L-selectin on granulocytes. They suspected that the altered balance between sL-selectin synthesis and elimination may be responsible for its reduced activity in patients with CRF.

In conclusion, enhanced (sICAM-1, sVCAM-1, sP--selectin) and simultaneously diminished (sL-selectin) adhesion molecule concentrations document a state of imbalance, already appearing in children and young adults with mild CRF. Differences in sL-selectin levels between examined groups show the progressive character of leukocyte dysfunction in CRF patients. Relationships between sICAM-1 and markers of anaemia suggest the role of this molecule in disturbances observed in CRF patients. However, the latter needs further detailed investigation.

Acknowledgements

The part of this work was presented at the ERA-EDTA Congress in Copenhagen in 2002.

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