Geget (2002) 2 : 77 - 84

Original Article

Soluble Adhesion Molecules in Children with Glomerulopathy

Ramzi El-Baroudy, Hala Gaafar, Manal Abdel Fattah*, and Sohair Abdel Aziz

Departments of Pediatrics, and Biochemistry, Cairo University, Egypt*

ABSTRACT

Background: The adhesion properties of leukocytes are important for the influx and localization of leukocytes in sites of inflammation. Leukocyte adhesion to kidney cells is an early event in renal inflammation as glomerulonephritis. The adhesion properties of leukocytes are important for the influx and localization of leukocytes in sites of inflammation.

Objectives: To determine whether changes in the levels of soluble adhesion molecules may reflect disease activity in acute poststreptococcal glomerulonephritis (APSGN) or may be related to the degree of renal failure in cases of chronic renal failure (CRF). Also, to find any possible correlation between free radical production in these diseases and the levels of soluble adhesion molecules.

Methods: We measured the levels of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble E-selectin (sE-selectin) in twenty children with acute post-streptococcal glomerulonephritis (APSGN) and twenty children suffering from chronic renal failure (CRF) as well as in ten healthy age matched children as a control group. Serum malondialdehyde (MDA: a marker of lipid peroxidation) was also estimated for all subjects to elicit a possible relation between it and the measured adhesion molecules. The antistreptolysin O titer (ASOT) and complement 3 fixation (C_3) were performed in the APSGN group. The creatinine clearance was estimated in the CRF group. Serum creatinine was measured in all subjects. The sICAM-1, sE-selectin and serum MDA levels were also estimated in eight patients in the CRF group, pre- and post- a 3 hour haemodialysis (HD) session.

Results: The results showed a significant increase in the levels of sICAM-1, sE-selectin and MDA in both the APSGN and CRF groups versus the control group, the increase in adhesion molecules being higher in the APSGN group versus the CRF group. This reflects endothelial activation in the groups of patients studied which is more prominent in the APSGN group indicating that the adhesion molecules are probably more involved in the inflammatory stages of renal disease. A significant positive correlation was found between each of sICAM-1, sE-selectin and MDA versus ASOT in the APSGN group and the serum creatinine level in the CRF group. A significant negative correlation was found between the creatinine clearance and sE-selectin in the CRF group. A non-significant positive correlation was found between each of sICAM-1 and sE-selectin versus the MDA levels. Significant decreases in the levels of sICAM-1 and sE-selectin were detected in the post-HD versus the pre-HD samples while no change was detected in the MDA levels.

Conclusions: Thus we conclude that the serum levels of sICAM-1 and sE-selectin reflect not only inadequate clearance but also enhanced synthesis and/or release of these molecules in both APSGN and CRF. The levels of these molecules as well as MDA reflect disease activity in APSGN and the degree of renal dysfunction in CRF.

INTRODUCTION

One of the most important events in the reaction to all forms of injury is adhesion of leukocytes to endothelium, a prelude to their emigration into the tissues. This process is central to inflammation, atherosclerosis and immune reaction[1]. Leukocyte adhesion to kidney cells is an

early event in renal inflammation as glomerulonephritis[2]. Leukocytes release cytotoxic agents including reactive oxygen species, enzymes and cytokines, which participate in tissue degradation and necrotizing lesions[3]. Endothelial-leukocyte adhesion is governed largely by the interaction of complementary adhesion molecules on endothelia and leukocytes. The most important adhesion molecules are the selectins (E, L and P) and the immunoglobulins: intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and the beta 2 and beta 1 integrins. In vivo studies in experimental animals and humans have suggested a role for these molecules in a number of pathological processes including kidney disease[1]. Besides cell-bound adhesion molecules, soluble forms of adhesion molecules have been detected in the circulating blood. Circulating soluble adhesion molecules appear to be biologically active and raised levels have been reported in a variety of disorders[4].

Because the adhesion properties of leukocytes are important for the influx and localization of leukocytes in sites of inflammation, we measured the levels of soluble ICAM-1 (sICAM-1) and soluble E-selectin (sE-selectin) in children with acute post-streptococcal glomerulonephritis (APSGN) and chronic renal failure (CRF). This was done in a trial to determine whether changes in the levels of these soluble adhesion molecules may reflect disease activity in APSGN or may be related to the degree of renal failure in cases of CRF.

Oxidative stress has been demonstrated in both APSGN[5] and CRF[6]. Culture studies done by Cheng et al., demonstrated that reactive oxygen species (ROS) induced ICAM-1 gene expression in cultured cells

Thus the present study, also aims at finding any possible correlation between free radical production in these diseases and the levels of sICAM-1 and sE-selectin.

SUBJECTS AND METHODS

The present study included twenty children with acute poststreptococcal glomerulonephritis (APSGN), twenty children suffering from chronic renal failure (CRF) and ten healthy children as a control group.

The children with APSGN were diagnosed clinically: presentation with hematuria, puffiness of the eyelids, and signs of hypertension (headache, blurring of vision and/or encephalopathy). Diagnosis was confirmed by estimation of complement 3 fixation (C_3). Patients with a level of C_3 less than 60 mg/dl were diagnosed as APSGN[8] (after excluding lupus nephritis by performing specific tests as antinuclear antibodies and antiDNA antibodies).

The chronic renal failure (CRF) children were all suffering from a parenchymal renal disease. Twelve children had a creatinine level of 10 to 50 ml/min/1.73m^2[9], while eight had a clearence below 10 ml/min/1.73m^2 and were undergoing haemodialysis three times weekly (session = 3 hours).

All patients and controls were subjected to:

a. Thorough history taking and clinical examination. Mean arterial blood pressure was calculated using the equation:

$$MABP = \frac{\text{Systolic} + (2 \times \text{diastolic})}{3}$$

b. Abdominal sonography.

c. Laboratory investigations: The following biochemical parameters were measured.

1. Serum creatinine[10].
2. Serum soluble intercellular adhesion molecule-1 (sICAM-1). This was determined using an enzyme-linked immunosorbent assay (ELISA) kit supplied by R & D systems, Abington, Oxon, UK[11].
3. Serum soluble E-selectin (sE-selectin) levels were evaluated by a quantitative sandwich immunoassay, using ELISA commercial kits supplied by R & D systems, Abington, Oxon, UK[12].
4. Serum Malondialdehyde: (MDA: the degradation product of lipid peroxide and a marker for lipid peroxidation): This was estimated colorimetrically by a method depending on the acid-catalysed thermal decomposition of lipid peroxide to MDA which reacts with thiobarbituric acid to form a colored adduct[13].

In addition creatinine clearance was measured in chronic renal failure patients[14]. sICAM-1, sE-selectin and serum MDA levels were estimated before and after a hemodialysis session in eight patients in the chronic renal failure group.

In patients with APSGN, the following parameters were also estimated:

a) C_3 was measured quantitatively, by radial immunodiffusion using kits provided by the Binding Site (Birmingham, UK)[8].

b) Antistreptolysin O titer (ASOT) in serum was estimated using ASOT latex test supplied by Omega Diagnostic Ltd., Scotland UK)[15].

Data Analysis

Student's t test was used to compare groups for differences in the mean levels of all measured parameters. Pearson's correlation coefficient "r" was used to describe correlations between each of sE-selectin, sICAM and MDA and the other parameters studied. Paired "t" test was used to compare the predialysis and postdialysis levels in the eight CRF patients[16].

RESULTS

The present study showed a significant elevation of sICAM-1, sE-selectin and serum malondialdehyde mean levels in both APSGN and CRF patients, in comparison to the control mean values (Table 1). The increase was significantly higher in the APSGN group versus the CRF group.

A significant positive correlation was detected between each of sICAM-1, sE-selectin and serum MDA levels versus the levels of ASOT in APSGN patients (Table 2). Also a significant positive correlation was found between each of sICAM-1, sE-selectin and serum MDA levels versus the serum creatinine levels in the CRF group of patients. A significant negative correlation was detected between sE-selectin and the creatinine clearance in the CRF group (Table 3).

A non-significant positive correlation was found between each of sICAM-1 and sE-selectin and the MDA levels both in the APSGN group ($r = 0.314$, and $r = 0.358$ respectively) and in the CRF group ($r = 0.337$, and $r = 0.351$ respectively).

As regards the effect of hemodialysis on the levels of adhesion molecules and MDA, significant decreases in sICAM-1 and sE-

Table 1: Levels of various clinical and biochemical parameters studied in APSGN and CRF patients versus the control group

Parameter	Control (No. = 10)	APSGN (No. = 20)	CRF (No. = 20)
Age (years)	6.32 ± 2.03 (a)	6.875 ± 2.56 (a)	7.75 ± 1.4 (a)
MABP	81.499 ± 6.009 (a)	98.251 ± 12.085 (b)	87.998 ± 6.874 (c)
Creatinine clearance (ml/min/1.73 m^2)	94.5 ± 9.559 (a)	-	21.625 ± 5.81(b)
C_3 (mg/dl)	125 ± 8.498 (a)	54.2 ± 17.9 (b)	-
ASOT (todd units)	104 ± 19.5 (a)	584.6 ± 202.15 (b)	-
Serum creatinine (mg/dl)	0.8 ± 0.33 (a)	1 ± 0.323 (a)	4.595 ± 1.95 (b)
sICAM-1 (ng/ml)	228.5 ± 37.22 (a)	413.945 ± 67.295 (b)	319.25 ± 65.419 (c)
sE-selectin (ng/ml)	19.296 ± 1.414 (a)	71.82 ± 20.77 (b)	50.803 ± 17.213 (c)
MDA (nmol/ml)	3.15 ± 0.508 (a)	4.85 ± 0.93 (b)	4.36 ± 0.909 (b)

Levels are expressed as mean ± SD. Different letters (a, b, c) applied to different groups indicate significant difference between them. Same letters indicate no significant difference between groups.

N.B: List of the abbreviations shown in this table and the following tables.

APSGN = acute poststreptococcal glomerulonephritis.
CRF = chronic renal failure.
MABP = mean arterial blood pressure.
ASOT = antistreptolysin O titer.
sICAM-1 = soluble intercellular adhesion molecule 1.
MDA = malondialdehyde.

selectin mean values were detected in the eight patients with CRF studied. However, they did not return to the control values (Tables 1, 4). On the other hand, no significant changes in the mean values of serum MDA was detected in the perdialysis versus the postdialysis levels (Table 4).

Table 2: Correlation coefficient (r) between each of sICAM-1, sE-selectin, and MDA; and each of MABP, C3 and ASOT in APSGN patients (No. = 20)

	MABP	C_3	ASOT
sICAM-1	r = 0.395	r = (-) 0.427	r = 0.72*
sE-selectin	r = 0.288	r = (-)0.425	r = 0.619*
MDA	r = 0.276	r = (-) 0.056	r = 0.593*

* = Significant correlation

Table 3: Correlation coefficient (r) between each of sICAM-1, sE-selectin and MDA; and each of MABP, GFR and serum creatinine in CRF patients (No. = 20)

	MABP	Creatinine clearance	Creatinine
sICAM-1	r = 0.189	r = (-) 0.356	r = 0.566*
sE-selectin	r = 0.399	r = (-) 0.515*	r = 0.585*
S-MDA	r = 0.249	r = (-) 0.287	r = 0.798*

* = Significant correlation

Table 4: Mean values of sICAM-1, sE-selectin and MDA before and after haemodialysis (HD) in CRF patients (No. = 8)

	Before HD (No. = 8)	After HD (No. = 8)	p value
sICAM (ng/ml)	351.875 ± 80.086	286.25 ± 54.494	< 0.05*
sE-selectin (ng/ml)	68.781 ± 11.339	45.775 ± 11.394	< 0.05*
MDA (nmol/ml)	4.675 ± 1.079	4.55 ± 0.893	> 0.05 (NS)

NS = non-significant.
* = Significant
Levels are expressed as mean ± SD.

DISCUSSION

Adhesion molecules are required in several physiological processes, but their altered function and/or expression is associated with the pathogenesis of inflammatory diseases[17]. ICAM-1 and E-selectin are thought to play important roles in leukocyte recruitment to the kidney[18], a prominent feature in most types of human glomerulonephritis (GN)[19].

In the present study, significant elevation in the mean levels of sICAM-1 and sE-selectin was detected in APSGN and CRF patients, the increase being more in the APSGN than in the CRF group. Similar results were reported by Kilis-Pstrusinska et al.[20] who detected increased levels of sICAM and sE-selectin in all types of GN indicating endothelial activation in these cases. An increase in sICAM-1 was also reported by Patey et al.[21]. In the present study, a significant correlation was detected between each of sICAM-1 and sE-selectin versus the ASOT in the APSGN group of patients. This indicates that sICAM-1 and sE-selectin levels reflect disease activity, and thus may be used as markers of disease activity. In accordance with our results, Zwolinska et al.[22] reported that sICAM levels may be used as a clinical marker to assess interstitial lesions in human nephritis and systemic vasculitis as sICAM-1 positively correlated with the grade of interstitial ICAM expression in their study. In another study, an increased intraglomerular ICAM-1 was detected in GN in early biopsies and decreased with time. This suggested that adhesions molecules are probably involved in the inflammatory infiltration in this disease[23]. This may also explain the higher levels of the measured adhesions molecules in APSGN patients versus the CRF in the present study. A strong correlation between expression of ICAM-1 and the immune cells had been previously reported suggesting that these adhesion molecules may be useful markers of activity and that they are involved in recruitment of mononuclear cells in cases of GN. This may render renal cells more susceptible to cell mediated renal injury[23].

As regards the CRF patients, and in accordance with the results of the present study, an increase in the levels of sICAM-1 and sE-selectin was reported indicating endothelial activation in these cases[4, 24]. The increase in this study correlated positively with the serum creatinine levels. The sE-selectin levels correlated negatively with the creatinine clearance. This suggests that the levels of these adhesion molecules correlate with the degree of dysfunction and is predictive of renal disease progression. Bonomini et al.[4] also reported a positive correlation between each of sICAM-1 and sE-selectin and serum creatinine levels. On the other hand Ara et al.[25] found no correlation between serum creatinine and these adhesions molecules suggesting that the mechanism of clearance of these molecules is not renal. It was not precisely known whether inflammation causes disease activity or progression of renal disease via effects on soluble adhesion molecules, or if elevated serum levels of soluble adhesion molecules are merely markers of endothelial activation in renal disease[26]. However, recent studies targeting chemokines and adhesion molecules have shown that inhibiting macrophage accumulation can suppress

progressive renal damage in animal models of GN[19]. A new synthetic selectin blocker was found to block leukocyte infiltration in a rat model of GN, with consequent decrease in tissue damage[27].

As regards the oxidative stress in APSGN and CRF patients, the present study confirmed previous reports demonstrating increased oxidative stress in these patients[5,6,21]. The MDA levels correlated positively with the ASOT in APSGN and with the serum creatinine levels in CRF. Turi et al.[28] also, found a correlation between presence of active glomerular disease and evidence of oxidative changes in the RBCs in APSGN. Oxidative stress was reported to upregulate the expression of adhesion molecules, chemoattractant compounds and cytokines. This stress may also potentiate vasoconstriction due, in part, to the catabolism of nitric oxide[29]. Oxidised low density lipoprotein was reported to induce greater monocyte endothelial cell adhesion[24]. The positive correlation between the adhesion molecules and MDA detected in the present study (although it did not reach significance) may be explained by the above reports[24,29]. It is however, not precisely defined whether the increased oxidative stress increased adhesion molecule expression or whether the adhesion molecules increased leukocyte recruitment and consequently enhancing the oxidative burst of the leukocytes.

In the present study, a decrease in sICAM-1 and sE-selectin was detected in the posthemodialysis (Post HD) sample versus the predialysis levels. However, the values did not return to the control levels. Similar results were reported by Bonomini et al.[4]. Thus the serum levels of these proteins reflect not only inadequate clearance but also enhanced synthesis and/or release.

The MDA mean levels showed no significant changes in the predialysis versus the postdialysis values. This is in accordance with the report of Fiorillo et al.[30]. Another study[6] found evidence of oxidative stress in patients with CRF before HD which increased after HD. They suggested that the procedural factors may be contributing in the oxidative stress after HD.

Thus it can be concluded that sICAM-1 and sE-selectin may be used as markers for disease activity in APSGN and markers for the degree of renal dysfunction in CRF patients. They may possibly play an important role in the pathogenesis of these conditions. Oxidative stress and its role on adhesion molecule production or release requires further studies on a rather larger scale so as to elicit the pathogenesis of the precise relation between them. The role of supplementary antioxidants may be also studied for their possible use as adjuvants in the therapy of these conditions.

REFERENCES

1. **Cotran, R. and Mayadas Norton, T. (1998):** Endothelial adhesion molecules in health and disease. Pathol. Biol. (Paris); 46 : 164-70.
2. **Mené, P.; Fais, S.; Cinotti, G. et al. (1995):** Regulation of monocyte adhesion to cultured human mesangial cells by cytokines and vasoactive agents. Nephrol. Dial. Transpl.; 10 : 481-489.
3. **Malech, H. and Gallin, J. (1988):** Neutrophils in human diseases. N. Engl. J. Med.; 37 : 678-693.
4. **Bonomini, M.; Reale, M.; Santarelli, P. et al.**

(1998): Serum levels of soluble adhesion molecules in chronic renal failure and dialysis patients. Nephron; 79 : 399-407.

5. **Devasena, T.; Lalitha, S. and Padma, K. (2001):** Lipid peroxidation, osmotic fragility and antioxidant status in children with acute poststreptococcal glomerulonephritis. Clin. Chem. Acta; 308 (1-2) : 155-161.
6. **Chugh, S.; Jain, S.; Agrawal, J. and Sharma, A. (2000):** Evaluation of oxidative stress before and after hemodialysis in chronic renal failure. J. Assoc. Physicians India; 48 : 981-4.
7. **Cheng, J.; Wung, B.; Chao, J. and Warg, D. (1998):** Cyclic strain-induced reactive oxygen species is involved in ICAM-1 gene induction in endothelial cells. Hypertension; 31 : 125-130.
8. **Mancini, G. and Carbonara, A. (1965):** Immunochemical quantification of antigens by single radioimmunodiffusion. Immunochem.; 2 : 235-245.
9. **William, E. and Harm, W. (1999):** Overview of chronic renal failure. Pediatric Nephrology 4th ed; p. 1151-1154.
10. **Heinegand, D. and Tiderstorm, K. (1973):** Determination of serum creatinine by a direct colorimetric method. Clin. Chem. Acta; 43: 305.
11. **Rothelin, R.; Mainolfi, E. and Czaikavski, M. (1991):** A form of circulating ICAM-1 in human serum. J. Immunol.; 147 : 3788-3793.
12. **Bevilacqua, M. and Nelson, R. (1993):** Selectins. J. Clin. Invest.; 91 : 379-383.
13. **Satoh, K. (1978):** Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin. Chem. Acta; 90 : 37-43.
14. **Payne, R. (1986):** Creatinine clearance and glomerular filtration rate. Ann. Clin. Biochem.; 23 : 243-249.
15. **Escobar, M. (1991):** Hemolytic assays: complement fixation and antistreptolysin O. In Balows, A.; Hausler, W.J.; Herman, K.L. et al. (eds). Manual of Clinical Microbiology 5th ed. p. 73 Washington DC. American Society for Microbiology.
16. **Armitage, P. and Berry, G. (1987):** Statistically methods in medical research. Oxford, Blackwell scientific publications.
17. **Hankanen, E.; von-Willebrand, E.; Teppo, A. et al. (1998):** Adhesion molecules and urinary tumor necrosis factor-alpha in idiopathic membranous glomerulonephritis. Kidney Int.; 53 : 909-917.
18. **Baran, D.; Vendeville, B.; Ogborn, M. and Katz, N. (2000):** Cell adhesion molecule expression in murine lupus-like nephritis caused by lipopolysaccharide. Nephron; 84 (2) : 167-176.
19. **Nicoklic-Paterosn, D. and Atkins, R. (2001):** The role of macrophages in glomerulonephritis. Nephrol. Dial. Transplant.; 16 (Suppl 5) : 3-7.
20. **Kilis-Pstrusinska, K.; Medynska, A.; Wikiera-Maggot, I. and Zwolinska, D. (2001):** Levels of selected soluble adhesion molecules in blood serum of children with chronic glomerulonephritis. Pol. Merkuriusz Lek.; 10 (58) : 247-249.
21. **Patey, N.; Lesavre, P.; Halbavachs-Meccarelli, L. and Neol, L. (1996):** Adhesion molecules in human crescentic glomerulonephritis. J. Pathol.; 179: 414-420.
22. **Zwolinska, D.; Medynska, A.; Szpymger, and Sczepanskam, M. (2000):** Serum concentration of IL-8, IL-6 and their soluble receptors in children on maintenance hemodialysis. Nephron; 86 (4) : 441-446.
23. **Wagrowska-Danilewicz, M. and Danilewicz, M. (1998):** Intercellular adhesion molecule-1, leukocyte function associated antigen-1 and leukocyte infiltration in human glomerulonephritis. Acta Histochem.; 100 : 201-215.
24. **O'Byrne, D.; Devaraj, S.; Islam, K. et al. (2001):** Low-density lipoprotein-induced monocyte endothelial cell adhesion, soluble cell adhesion molecules, and autoantibodies to oxidized-LDL in chronic renal failure on dialysis therapy. Metabolism; 50 (2) : 207-215.
25. **Ara, J.; Mirapeix, E.; Ascaso, C. et al. (2001):** Circulating soluble adhesion molecules in ANCA-associated vasculitis. Nephrol. Dial. Transplant.; 16 (2) : 276-285.
26. **Stenvinkel, P.; Lindholm, B. and Heimburger, M. (2000):** Elevated serum levels of soluble adhesion molecules predict death in predialysis patients: association with malnutrition, inflammation and cardiovascular disease. Nephrol. Dial. Transplant.; 15 (10) : 1624-30.
27. **Ito, I.; Yuzawa, Y.; Mizuno, M. et al. (2001):** Effects of a new synthetic selectin blocker in an acute thrombotic glomerulonephritis. Am. J. Kd. Dis.; 38 (2) : 267-273.
28. **Turi, S.; Nemeth, I.; Torkos, A. et al. (1997):** Oxidative stress and antioxidant defense mechanism in glomerular diseases. Free Radic. Biol. Med.; 22 : 161-8.
29. **Klahr, S. and Morrissey, J. (2000):** The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease. Kidney Int. Suppl.; 57 (75) : 57-14.
30. **Fiorilo, C.; Oliviero, C.; Rizzute, G. et al. (1998):** Oxidative stress and antioxidant defenses in renal patients receiving regular hemodialysis. Clin. Chem. Lab. Med.; 36 : 149-153.