

## Original Article

### Soluble Intracellular Adhesion Molecule 1 (sICAM-1) in Post-Streptococcal Acute Glomerulonephritis.

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#### ABSTRACT

**Background:** Glomerulonephritis is associated with infiltration of inflammatory cells such as polymorphonuclear leukocytes, macrophages, and T or B-lymphocytes. This is closely related to the expression of cellular adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1). In human diseases in which unchecked inflammation contributes to the pathogenesis of the disease process, soluble forms of adhesion molecules including ICAM-1 are elevated.

**Objectives:** To study the changes in sICAM-1 that occur in post-streptococcal acute glomerulonephritis (PSAGN).

**Methods:** sICAM-1 levels were measured (by ELISA) in 25 children with PSAGN during their initial presentation. Diagnosis of PSAGN was based on the presence of haematuria, transient hypocomplementaemia and positive laboratory evidences of recent streptococcal infection. Patients were compared to 15 healthy children of matched age and sex.

**Results:** Patients in the acute attack had significantly higher levels of sICAM-1 when compared to control [median (IQR) = 45.17 (40.5-49.5) vs 32.5 (26.5-33.4), ng/ml,  $p < 0.0001$ ]. sICAM-1 levels correlated significantly with serum C3 ( $r = -0.58$ ,  $p = 0.002$ ), serum creatinine ( $r = 0.46$ ,  $p = 0.02$ ), and the severity of both systolic and diastolic hypertension ( $r = 0.64$ ,  $p = 0.001$  and  $r = 0.65$ ,  $p < 0.0001$ , respectively). No significant correlation was detected between sICAM-1 levels and the severity of proteinuria, haematuria, or oedema. Out of the studied 25 cases; 15 cases were reassessed 3 months after normalization of their complement level and a significant drop in sICAM-1 was found ( $p = 0.001$ ).

**Conclusions:** These results suggest that ICAM-1 may have a pathophysiologic role in PSAGN and that sICAM-1 may be used as a marker for the severity of the disease.

#### INTRODUCTION

Glomerulonephritis is associated with infiltration of inflammatory cells such as polymorphonuclear leukocytes, macrophages, and T or B-lymphocytes<sup>(1,2)</sup>. The initial phases of neutrophil recruitment into any inflammatory focus are dependent on neutrophil adhesion to vascular endothelium<sup>(3,4)</sup>. Leukocyte adhesion is an early event for the subsequent migration into extravascular tissues<sup>(5)</sup>. This is closely related to the expression of cellular adhesion molecules, such as intercellular

adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1, and major histocompatibility complex class II on glomerular capillary endothelial cells, mesangial cells, or tubular epithelial cells<sup>(6)</sup>. In vivo, ICAM-1 is normally expressed on renal vascular endothelium<sup>(7)</sup>. The soluble form of ICAM-1 (sICAM-1) contains most of the extracellular region of ICAM-1 and also retains its ability to bind to the ligand, lymphocyte function associated molecule 1 (LFA-1)<sup>(8)</sup>. In human diseases in which unchecked inflammation contributes to the

pathogenesis of the disease process, soluble forms of adhesion molecules including ICAM-1 are elevated. These elevations have been interpreted as indicating a role for the molecules in the pathogenesis of inflammation, as well as indicating the magnitude of the inflammatory response<sup>(9)</sup>.

### AIM OF THE WORK

The aim of this work is to study the changes of sICAM-1 that occur in PSAGN and their potential significance.

### PATIENTS AND METHODS

Twenty-five Egyptian children with diagnosis of PSAGN were included in the study. They were 11 males and 14 females with their ages ranging from 2.5 to 13 years [median (IQR) = 5 (3.25-7.25) years]. These patients were recruited consecutively from the Pediatric Nephrology Unit in Mansoura University Children's Hospital, Egypt, in the period from January 1999 to March 1999. Diagnosis of acute PSAGN depended on the presence of haematuria, transient hypocomplementaemia and positive laboratory evidence of recent streptococcal infection. Twenty patients (80%) had history of throat infection 2-3 weeks before the onset of the illness. The remaining five patients (20%) had history of impetigo. Sixteen patients (64%) were hypertensive and nine patients (36%) had normal blood pressure. Two patients developed hypertensive encephalopathy and only one patient developed hypertensive heart failure. Out of the 25 studied cases, 15 patients were reassessed 3 months after normalization of their serum complement levels; they were 3 males and 12 females with their ages

ranging from 2.5 to 13 years (median 5 years, IQR = 3-7.5). The control group included 15 healthy children of matched age and sex.

**Clinical assessment:** The severity of edema was graded on a scale from 1-4, no edema: 1, puffy eye lids: 2, mild edema in the lower limb: 3, and massive edema: 4. The severity of hypertension was also graded on a scale from 1-3, no hypertension: 1, non-severe: 2 and severe, 3<sup>(10)</sup>.

**sICAM-1 Assays:** Sera were obtained and stored at -20°C, they were analyzed by solid-phase enzyme-linked immunosorbent assay based on the double sandwich method using anti-ICAM-1 murine monoclonal antibodies (Medgnix EASIA). The concentration of sICAM-1 is calculated after reading the absorbance on spectrophotometer at wavelength 450 nm. The minimum detectable serum level for the assay was 0.5 ng/ml.

**Urinalysis:** Haematuria was arbitrarily graded on a scale from 0 to 4 by light microscopy: negative: 0, 1-5/visual field: 1, 6-20: 2, 21-50: 3 and > 50/visual field: 4.

Urinary protein was quantitated by the quantitative turbidimetric method (Stanbio Laboratory; Texas)<sup>(11)</sup>.

Serum creatinine was measured by the Jaffe reaction<sup>(12)</sup>.

**Statistics:** Data were analyzed by the SPSS 8.0 for windows. Data showed non-parametric distribution by Kolmogorov Smirnov test. Data were expressed as median and interquartile rang (IQR). Tests used included Mann-Whitney U-Wilcoxon W test; Spearman Correlation test; Kendall's tau\_b test; and Wilcoxon Signed Ranks test.

## RESULTS

Patients in the acute attack had significantly higher levels of sICAM-1 when compared to control [median (IQR) = 45.17 (40.5-49.5) vs 32.5 (26.5-33.4) ng/ml,  $p < 0.0001$ ]; [Table 1]. sICAM-1 levels correlated negatively with serum C3 ( $r = -0.58$ ,  $p = 0.002$ ), positively with serum creatinine ( $r = 0.46$ ,  $p = 0.02$ ), and the severity of both systolic (SH) and diastolic hypertension (DH) ( $r = 0.64$ ,  $p = 0.001$  and  $r = 0.65$ ,  $p < 0.0001$  respectively). No significant correlation was detected between sICAM-1 levels and the severity of proteinuria, haematuria,

or edema; [Table 2]. Hypertensive patients had significantly elevated sICAM-1 when compared to those with normal blood pressure [median (IQR) = 48.05 (45.3-69.1) vs 40 (37.6-40.9) ng/ml,  $p < 0.0001$ ]; [Table 3]. sICAM-1 levels in patients who recovered after 3 months were not significantly different from control [median = 35, IQR = (28-36) vs 32.5 IQR = (26.5-33.4) ng/ml,  $p = 0.25$ ]; [Table 1]. Follow up of serum sICAM-1 in reassessed cases after 3 months revealed a significant drop ( $p = 0.001$ ); [Table 4].

**Table 1: Comparison of sICAM-1 levels (ng/ml) between PSAGN and control<sup>a</sup>**

PSAGN <sup>a</sup> (n = 25)	PSAGN <sup>b</sup> (n = 15)	Control (n = 15)	p*
45.17 (40.5-49.5)	35 (28-36)	32.5 (26.5-33.4)	p1 < 0.0001* p2 = 0.25

Values are expressed as median (IQR)

PSAGN<sup>a</sup> = initial presentation

P1 = PSAGN<sup>a</sup> vs control

\* Mann-Whitney U test

PSAGN<sup>b</sup> = 3 months later

P2 = PSAGN<sup>b</sup> vs control

**Table 2: Correlation between sICAM-1 levels and some parameters in patients with PSAGN during the acute attack (n = 25).**

Parameter	sICAM-1	
	r	p
<b>C3<sup>a</sup></b>	-0.58	0.002*
<b>Creatinine<sup>a</sup></b>	0.46	0.02*
<b>Systolic hypertension<sup>b</sup></b>	0.64	0.001*
<b>Diastolic hypertension<sup>b</sup></b>	0.65	< 0.0001*
<b>Proteinuria<sup>a</sup></b>	0.25	0.21
<b>Haematuria<sup>b</sup></b>	0.04	0.78
<b>Edema<sup>b</sup></b>	-0.06	0.7

<sup>a</sup> Spearman correlation test

<sup>b</sup> Kendall's tau\_b correlation test

**Table 3: Comparison of sICAM-1 levels (ng/ml) between hypertensive and normotensive PSAGN<sup>a</sup>**

Hypertensive (n = 16)	Normotensive (n = 9)	p
48.05 (45.3-69.1)	40 (37.6-40.9)	< 0.0001*

Values are expressed as median (IQR)

<sup>a</sup> Mann-Whitney U test

**Table 4: Comparison of sICAM-1 Levels during activity of disease and after improvement<sup>a</sup>**

+ve Ranks	-ve Ranks	Ties	p
Active < Improved	Active > Improved	Active = Improved	
0	15	0	0.001*

<sup>a</sup> Wilcoxon Matched Pairs Signed Ranks Test

## DISCUSSION

Glomerular ICAM-1 expression is upregulated in a variety of renal diseases including acute renal failure<sup>(13)</sup> and proliferative glomerulonephritis<sup>(14)</sup>. Intense mesangial ICAM-1 expression is found in mild stages of IgA nephropathy, Schoenlein-Henoch syndrome<sup>(13)</sup>, idiopathic membranous glomerulonephritis<sup>(15)</sup>, and in focal segmental glomerulosclerosis<sup>(6)</sup>. Endothelial expression of ICAM-1 is known to facilitate leukocyte infiltration, which ultimately induces various glomerular injuries<sup>(16)</sup>. In vitro ICAM-1 expression is dependent on Tumor Necrosis Factor (TNF) and is sustained as long as TNF remains in the culture medium of endothelial cells<sup>(17)</sup>. Anti-ICAM-1 therapy was established to have therapeutic effects on initiation and

progression of experimental crescentic glomerulonephritis<sup>(18,19)</sup>.

The soluble forms of cellular adhesion molecules including ICAM-1 have been recognized as in vivo markers of the presence of inflammatory mediators. Both de novo synthesis of sICAM-1 from lymphocytes and other cells and the proteolytic molecules originating from membrane binding ICAM-1 is suspected as the origins of sICAM-1<sup>(8)</sup>. Our results revealed a significant increased production sICAM-1 in acute phase of PSAGN when compared to control; on resolution of the disease activity sICAM-1 dropped significantly. Moreover; during the initial presentation; sICAM-1 correlated negatively with serum C3 level. The increase of sICAM-1 noticed in patients at the initial presentation

and its normalization after recovery of the disease is probably related to changes in systemic immunological status in the patients during the acute phase of the illness.

During the initial presentation sICAM-1 correlated positively with serum creatinine and degree of both systolic hypertension and diastolic hypertension. Moreover, hypertensive patients had higher sICAM-1 levels when compared to normotensive patients. These findings suggest that sICAM-1 may be an indicator of the severity of the disease. On the other hand, no significant correlation was found between sICAM-1 and either of the severity of haematuria, proteinuria or edema. Although a variety of studies have incriminated cellular adhesion molecules as important mediators in glomerular injury, yet the exact role of these molecules - if any - in induction of proteinuria, haematuria is still unclear. In experimental glomerulonephritis, glomerular ICAM-1 expression is related to PMN infiltration, which is subsequently followed by proteinuria. Early treatment with monoclonal antibodies to

ICAM-1 significantly prevents proteinuria but does not prevent PMN infiltration in the glomeruli<sup>(20)</sup>. In PASGN, intraglomerular ICAM-1 expression was significantly increased and correlated with the degree of proteinuria. Interstitial ICAM-1 expression was also increased and correlated with serum creatinine level<sup>(21)</sup>.

The potential significance of measuring sICAM-1 in renal diseases is still unclear. sICAM-1 level is significantly elevated in both undialyzed patients with chronic renal failure and in patients on peritoneal dialysis and haemodialysis. This probably reflects inadequate clearance as well as enhanced synthesis and release<sup>(22)</sup>. sICAM-1 is also elevated in patients with acute rejection of transplanted grafts<sup>(23)</sup>. sICAM-1 may be an indicator of the activity of human nephritis with systemic vasculitides such as anaphylactoid purpura nephritis and lupus nephritis<sup>(24)</sup>. Plasma sICAM-1 levels are elevated in ANCA positive renal vasculitis<sup>(25)</sup>. Based on the current study results, sICAM-1 may be similarly used to assess the immunological activity and severity of PSAGN.

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