Circulating adhesion molecules ICAM-1, E-selectin, and von Willebrand factor in Henoch-Schönlein purpura

Oğuz Söylemezoğlu, Nedim Sultan, Turkiz Gursel, Necla Buyan, Enver Hasanoğlu

Abstract

Adhesion molecules play an important part in leucocyte transendothelial migration and thus may provide a useful marker of surface expression at inflammatory sites. In 20 patients with Henoch-Schönlein purpura serum intercellular adhesion molecule 1 (ICAM-1), E-selectin, and plasma von Willebrand factor (vWF) were determined by ELISA during the active and inactive phase of the disease. Twelve healthy children were studied as a control group. Serum ICAM-1 concentrations increased during the active phase of the disease and differed significantly compared with the inactive phase ($p < 0.05$). However ICAM-1 in the active phase did not differ significantly compared with controls ($p = 0.08$). Serum E-selectin concentrations did not differ in the active and inactive phase of the disease. By contrast, vWF increased in the active phase of the disease and differed significantly compared with inactive disease and control groups ($p < 0.01$). Considering the adhesion molecules and vWF, only vWF correlated well with the C reactive protein measurement in the active phase, which is considered a good marker of disease activity. These data suggest that plasma vWF is a good marker of vascular inflammation and endothelial damage. Circulating ICAM-1 might be an additional parameter in some of the patients.

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Keywords: adhesion molecules, Henoch-Schönlein purpura, vasculitis, von Willebrand factor.

Leucocytes are prominent in the vascular inflammation of vasculitic syndromes like polyarteritis nodosa, Kawasaki disease, Wegener's granulomatosis, and Henoch-Schönlein purpura. The upregulation and enhanced expression of cytokine inducible cell surface adhesion molecules on polymorphonuclear leucocytes and on the normally non-adhesive vascular endothelium leads to the adherence of inflammatory cells to the vessel wall, their activation, and subsequent extravasation. The adhesion of leucocytes to endothelium and their transmigration across endothelium is dependent on cell adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin.¹ ICAM-1 is constitutively expressed

on both endothelial and circulating cells including leucocytes and is upregulated by immune activation and inflammation. E-selectin is found only on activated endothelium and binds to granulocytes, monocytes, and some memory T cells.^{2,3} E-selectin appears to be a more specific marker of endothelial cell activation, being produced by activated endothelium. Raised concentrations of soluble forms of ICAM-1, VCAM-1, and E-selectin have been found in the sera of patients with a variety of disorders including Wegener's granulomatosis, systemic lupus erythematosus, Kawasaki disease, and chronic inflammatory liver disease.⁴⁻⁷

High circulating concentrations of von Willebrand factor (vWF) have been also proposed as a marker for endothelial cell damage.⁸ As vWF release is enhanced by damage to endothelial cells, it has been demonstrated that circulating concentrations of vWF are increased in many diseases involving endothelial damage. Increased vWF was reported in different vasculitic syndromes including scleroderma, Kawasaki disease, haemolytic uraemic syndrome, and vasculitis in general.⁹⁻¹¹

Henoch-Schönlein purpura is the most common vasculitis syndrome of childhood and is also described as leucocytoclastic vasculitis. Clinical symptoms are characterised by abdominal pain, rash, arthralgia, and haematuria. The exact cause of Henoch-Schönlein purpura is unknown and several theories concerning the pathogenesis have been proposed. Circulating immune complexes containing IgA are detected in the serum of patients as well as immune complexes containing C3 and IgA are also found in the skin, intestines, and kidneys.^{12,13}

These findings support the view that Henoch-Schönlein purpura is mediated by immune complexes. In recent years, there has been an increased number of studies characterising a spectrum of antineutrophil cytoplasmic antibodies (ANCA) associated with systemic vasculitis syndromes. The role of ANCA in the pathogenesis of Henoch-Schönlein purpura and IgA nephropathy was suggested in one study, however recent studies did not confirm these findings.¹⁴⁻¹⁶

The present study was designed to assess whether increased concentrations of circulating ICAM-1, E-selectin, and vWF are present in patients with Henoch-Schönlein purpura when compared with healthy children. Furthermore, we aimed to determine whether

Department of
Paediatric Nephrology,
Immunology and
Haematology, Gazi
University, Ankara,
Turkey
O Söylemezoğlu
N Sultan
T Gursel
N Buyan
E Hasanoğlu

Correspondence to: Dr Oğuz
Söylemezoğlu, Ata sokak 6/1
Yücetepe, 06580
Ankara, Turkey.

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Table 1 Clinical findings and C reactive protein during patient admission

Patient No	Purpura*	Arthralgia/ arthritis	Abdominal pain	Gastrointestinal bleeding	Haematuria/ proteinuria †	C reactive protein (mg/l)
1	++	-	+	+	-/-	52
2	+++	-	-	-	-/-	25
3	++	+	+	-	-/-	15
4	+++	-	+	+	++/-	40
5	+	+	+	-	+++ / +	47
6	++	-	+	+	+ / +	60
7	+++	-	-	-	- / -	86
8	++	+	+	-	- / -	40
9	+++	-	+	-	- / -	15
10	+	-	-	-	+ / +	15
11	++	+	-	-	- / -	30
12	+	+	+	-	- / -	30
13	++	+ / +	-	-	- / -	25
14	+++	-	+	-	- / -	37
15	++	+ / +	-	-	- / -	110
16	+++	+	+	+	+ / -	75
17	+++	+	+	+	++ / +	150
18	+++	-	+	+	++ / +	190
19	+	-	-	-	+++ / -	18
20	+++	-	+	+	+ / -	34

* The severity of purpura: mild +, moderate ++, severe +++. † Strip test for haematuria and proteinuria: for haematuria (erythrocytes/ μ l), + <25, ++ 25–80, +++ 80–200; for proteinuria (g/l) + >0.30, ++ >100.

measurement of serum concentrations of adhesion molecules might provide an effective method to monitor disease activity in patients with Henoch-Schönlein purpura. To investigate this we studied the circulating concentrations of ICAM-1 and E-selectin and compared the results with plasma vWF and C reactive protein in the patients with Henoch-Schönlein purpura.

Patients and methods

Twenty children (11 boys and nine girls; median age 9.3 years, range 3.0–15.0 years) with Henoch-Schönlein purpura diagnosed according to the American College of Rheumatology criteria¹⁷ were studied. The study included only the patients who had the initial presenting phase of the disease. Palpable purpura, abdominal pain, gastrointestinal bleeding, haematuria, and arthralgia were observed in the active phase of the disease (table 1). Duration of the symptoms varied from two to seven days and none of the patients was taking any drugs at the time of admission. During the follow up period the disease was considered inactive when the patient was symptom free. Leucocytoclastic vasculitis of small vessels was also confirmed in skin biopsy specimens of all six patients who had a biopsy. Serum samples were collected from patients at the time of diagnosis and at the time when the disease was considered inactive based on clinical criteria. Samples were stored at -70°C. Twelve age matched healthy children (median age 9.1 years, range 3.2–14.8 years) were studied as controls. All of the children in the control group were healthy, afebrile, and on no medication during the study. The study was approved by the ethics committee of the university.

ASSAY METHODS

Adhesion molecules

Circulating ICAM-1 and E-selectin concentrations were measured using a commercially available ELISA kit (Bender-Med Systems, Vienna, Austria). Samples were diluted 1:10 for the soluble ICAM-1 assay and 1:5 for the

soluble E-selectin assay. In brief, microtitre wells coated with antihuman soluble ICAM or E-selectin, were incubated with diluted samples, standards, and controls. In the second step horseradish peroxidase conjugated monoclonal antibody against ICAM or E-selectin was added. Unbound enzyme conjugated anti-ICAM-1 or anti-E-selectin was removed by washing and the substrate solution was added to reactive horseradish peroxidase. The reaction was terminated by the addition of sulphuric acid and absorbances were measured at 450 nm. The ICAM-1 or E-selectin concentrations were determined from the standard curve.

vWF

Circulating vWF was assayed by a commercially available ELISA kit (Asserachrom vWF, Diagnostica Stago, France). A microtitre plate coated with specific rabbit antihuman vWF antibodies captures the vWF to be measured in the plasma and then rabbit anti-vWF antibody coupled with peroxidase bind to remaining free antigenic determinants of vWF, forming the 'sandwich'. The bound enzyme peroxidase is then revealed by its activity on the substrate orthophenylenediamine in the presence of hydrogen peroxidase. After stopping the reaction with acid, the plate is read at 492 nm in an ELISA reader. Results are reported as per cent of normal plasma.

Plasma concentrations of C reactive protein were measured by immunoturbidimetry (Boehringer). Serum biochemistry was studied by autoanalyser (Technicon).

STATISTICAL ANALYSIS

The Mann-Whitney U test was used for comparing the healthy children with patients. The significance of difference between patients with active and inactive disease were determined by the paired *t* test. Linear regression analysis was used for correlation studies; *p* values less than 0.05 were considered significant. Data were analysed using SPSS +PC and results are given as mean (SE).

Table 2 Mean (SE) circulating adhesion molecules ICAM-1, E-selectin, vWF, and C reactive protein in Henoch-Schönlein purpura (HSP)

	Active HSP		Inactive HSP		Control
ICAM-1 (ng/ml)	432	(35.2)	295	(2.7)	306.6 (33)
E-selectin (ng/ml)	46.9	(2.5)	43	(2.5)	46.3 (2.5)
vWF (%)	206	(16.5)	127	(10.2)	107 (9.1)
C reactive protein (mg/l)	55	(11)	12	(3)	7 (2.1)

Figure 1 Serum ICAM-1 concentrations in the patient and control groups (HSP = Henoch-Schönlein purpura).

Results

In the patients with active and inactive Henoch-Schönlein purpura mean circulating ICAM-1 concentrations were found to be 432 (35.2) ng/ml and 295 (27) ng/ml respectively (see fig 1). Serum ICAM-1 increased in nine (45%) of the patients during the active phase. In healthy controls, ICAM-1 was found to be 306 (33) ng/ml. The mean serum ICAM-1 concentration during the active phase of the disease was significantly higher when compared with that in inactive disease ($p<0.05$), but serum concentrations of ICAM-1 in the active phase did not differ significantly from those in the healthy controls ($p=0.08$)(table 2).

The mean E-selectin concentrations in patients with Henoch-Schönlein purpura during the active and inactive phase were found to be 46.9 (2.5) ng/ml and 43.8 (2.6) ng/ml respectively. In the control group the E-selectin concentration was 45 (3.8) ng/ml. There was no significant difference between all these three groups regarding the serum E-selectin.

In 16 of 20 patients vWF antigen increased during the active period of the disease with a mean vWF value of 206 (% control) compared with 127.7 (%) in the inactive disease and with 107.0 (%) in the control group (fig 2). There was a significant difference in vWF between those with active and inactive disease ($p<0.01$) and also between those with active disease and controls ($p<0.01$).

C reactive protein was significantly higher in the patients with active disease: it was found to be 55 mg/l during flare up and 12 mg/l in the follow up period of inactive disease.

We attempted to investigate the patients in two subgroups: those with systemic involvement, such as gastrointestinal bleeding and renal involvement, and those without. The serum and plasma concentrations of adhesion molecules ICAM-1, E-selectin, C reactive pro-

Figure 2 Plasma vWF concentrations in the patient and control groups (HSP = Henoch-Schönlein purpura).

tein, and vWF did not differ significantly in these subgroups.

Between all these parameters there was a significant correlation between C reactive protein and vWF ($p<0.05$). No correlation was found between ICAM-1, E-selectin, and C reactive protein and there was also no correlation between adhesion molecules and vWF.

Serum blood urea nitrogen and creatinine were all within normal limits in the patient and control groups.

Discussion

We found increased concentrations (> 400 ng/ml) of circulating ICAM-1 in 45% of the patients with active Henoch-Schönlein purpura. Although the serum ICAM-1 concentrations in this group differed significantly compared with patients with inactive disease, it did not differ significantly when compared with the control group. We found the highest concentrations of ICAM-1 in three patients (699 ng/ml, 688 ng/ml, and 580 ng/ml) who had renal involvement with haematuria and/or proteinuria. On the other hand the highest ICAM-1 concentration (700 ng/ml) was found in a patient who had severe purpura without renal involvement. We did not find increased E-selectin concentrations in either of the patient groups.

In different forms of vasculitis such as systemic lupus erythematosus, Wegener's granulomatosis, Kawasaki disease, and microscopic polyarteritis increased serum concentrations of circulating ICAM-1 and or E-selectin were reported.^{4-7 18} Circulating ICAM-1 was initially expected to be a promising inflammatory activity in different diseases. Although ICAM-1 is widely distributed, E-selectin is specific to endothelial cells and only minimally expressed in the resting state.¹⁹ Jeng *et al* reported higher concentrations of ICAM-1 in patients with systemic vasculitis than in those with cutaneous vasculitis.²⁰ Furukawa *et al* suggested that serum ICAM-1 concentrations were an important immunological parameter for the severity of vascular damage during acute Kawasaki disease but in the same study they found no increment of serum ICAM-1 in the patients with anaphylactoid purpura.²¹

There are conflicting reports regarding serum ICAM-1 concentrations in vasculitic syndromes. John *et al* concluded that circulating ICAM-1 concentrations are not useful to define disease activity to indicate a clinical relapse in vasculitis or as a guideline for immunosuppressive treatment.²² Janssen *et al* also reported that soluble (s) ICAM-1 and E-selectin concentrations did not reflect the disease activity in either systemic lupus erythematosus or vasculitis.²³ Circulating adhesion molecules were found to be higher in normal children than in adults and Nash *et al* showed that sE-selectin, sICAM-1, and sVCAM-1 all fell significantly over the age range in normal children.²⁴ This study highlighted the need for the age matched controls, and the data from our study confirm these findings.

E-selectin is found only on activated endothelium in contrast to other adhesion molecules and can be detected in the blood as a soluble form, shed from the cell membrane, and could be used as an index of severity of vasculitis in inflammatory diseases. In the present study E-selectin concentrations did not differ significantly in the three groups and the data did not suggest E-selectin as a good parameter in Henoch-Schönlein purpura. Various circulating E-selectin concentrations have been reported in different forms of vasculitic syndromes. Nash *et al* found high circulating E-selectin and ICAM-1 concentrations in Kawasaki disease and they also reported increased vWF in these patients.¹⁸ Kim and Lee found higher concentrations of E-selectin in Kawasaki disease but not in patients with Henoch-Schönlein purpura.²⁵ Voskuyl *et al* found increased concentrations of circulating ICAM-1 and ICAM-3 but not E-selectin in patients with rheumatoid vasculitis.²⁶ Some of these studies supported our findings and suggested that circulating adhesion molecule expression is more prominent in the medium sized vasculitis. Bradley *et al* demonstrated increased expression of ICAM-1 and E-selectin in the skin biopsy specimens of vasculitis patients of different forms including Henoch-Schönlein purpura and neutrophil infiltration was only found in the presence of E-selectin. Expression of E-selectin was thought to persist in the skin and act as a homing receptor for neutrophils and lymphocytes.²⁷

We found higher concentrations of vWF in the acute phase of the disease that was significantly different from the inactive disease and control groups and correlated well with the high C reactive protein. Sixteen of 20 patients had increased concentrations of vWF in the active phase. The other four patients who had normal concentrations of vWF had no signs of systemic involvement and a mild form of vasculitis was restricted to the skin. vWF is an adhesive multimeric glycoprotein synthesised by megakaryocytes and endothelial cells isolated from large and small veins, capillaries, the aorta, and arteries.^{28, 29} Nash *et al* reported high concentrations of vWF in febrile patients with infectious disease and they suggested high vWF were mainly due to the acute phase reaction.¹⁸

In the present study we did not have another control group with febrile children to compare with the patient group. In recent years various authors have reported an increase of vWF in the plasma of different vasculitic syndromes.^{9, 30} Increased vWF concentrations are regarded as a reliable index of vascular damage and high concentrations of vWF have been proposed to be a specific marker for endothelial damage.^{31, 32}

In conclusion, we have shown that plasma vWF antigen concentration increased in children with active Henoch-Schönlein purpura and these raised values most likely reflect increased amounts of vWF. However vWF concentrations are an indirect measurement of the endothelial cell damage; high concentrations can therefore reflect the activation or damage. The study indicated the importance of monitoring vWF concentrations as a useful marker of the severity of vasculitis. Our data also revealed that although some patients may have high concentrations of ICAM-1, monitoring of circulating ICAM-1 and E-selectin is not as useful as vWF in indicating disease activity and severity in Henoch-Schönlein purpura. Detection of adhesion molecules in the damaged vessels may shed light on the pathogenesis of the disease.

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