ORIGINAL ARTICLE

Plasma and urinary soluble adhesion molecule expression is increased during first documented acute pyelonephritis

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Background: The degree of inflammatory reaction and leucocyte trafficking during acute pyelonephritis has been related to the risk of developing renal parenchymal scarring. Adhesion molecules play a central role in leucocyte recruitment during inflammation.

Aims: (1) To determine whether circulating and urinary concentrations of E-selectin and intercellular adhesion molecule 1 (ICAM-1) were abnormal during first documented acute pyelonephritis; (2) to investigate whether circulating or urinary concentrations were predictive for the development of abnormalities on DMSA imaging.

Methods: Plasma and urine samples were collected from 40 children with a first episode of acute pyelonephritis within one week of infection (acute sample) and at six weeks (late sample). Control samples were collected from 21 healthy age matched controls and 18 age matched controls with febrile illness not secondary to urinary tract infection.

Results: Plasma and urinary sE-selectin were higher in acute samples (median 176.3 ng/ml and 0.12 ng/mmol respectively) compared with late (97.8 ng/ml and 0.029 ng/mmol) and both control (65.6 ng/ml and 0 ng/mmol) and febrile control (urine 0 ng/mmol) samples. Plasma sICAM-1 was higher in acute samples (428 ng/ml) than controls (365.2 ng/ml), and acute sICAM-1 urine concentrations were higher than febrile control concentrations (3.2 *v* 0.7 ng/mmol). No correlations were detected between sE-selectin or sICAM-1 and acute or late DMSA scan changes.

Conclusion: Plasma and urinary sE-selectin and sICAM-1 are significantly increased during acute pyelonephritis, though no correlation exists between the presence of high plasma or urine concentrations and DMSA scan changes, both during acute infection and six weeks post-infection.

Acute pyelonephritis is a common bacterial infection of childhood. Renal parenchymal scarring is the most important long term complication of infection, and is responsible for up to 24% of children entering end stage renal failure programmes worldwide.[1–4] Established risk factors for the development of renal parenchymal scarring following acute pyelonephritis include the presence of vesicoureteric reflux (VUR), recurrent infection, delayed treatment, and young age at the time of infection.[5 6] More recently, findings in experimental animals have shown that the intensity of the inflammatory reaction within the renal parenchyma following infection may also be inversely related to the risk of scarring.[7 8] Where the number of neutrophils infiltrating the renal parenchyma is reduced by the use of agents such as colchicine (which impairs neutrophil function), cyclophosphamide (which induces neutropenia), or cobra venom (which reduces complement mediated chemotaxis), persistence of bacteria in the parenchyma is increased and the risk of scarring reduced.[9–11]

Adhesion to vascular endothelial cells is necessary for inflammatory cells to exit the circulation and infiltrate the surrounding tissue,[12 13] a process mediated by cell adhesion molecules. The sequential steps of rolling, strong adhesion, and diapedesis are mediated by the selectins, particularly E-selectin, intercellular adhesion molecule 1 (ICAM-1), and platelet endothelial cell adhesion molecule 1 (PECAM-1) respectively. Increased expression of ICAM-1 and E-selectin has previously been shown in the kidney in pyelonephritis and different glomerulonephritides by immunohistochemistry.[14–17] In addition to membrane bound adhesion molecules, soluble variants have been detected in the circulation and in other body fluids including sputum, bronchial secretions, cerebrospinal fluid, and urine.[18–21] The origin and functions of these soluble adhesion molecules have not been clearly defined, though they may represent shedding of the membrane bound form by activated endothelium and mononuclear cells, or may represent an alternatively spliced protein variant.[18] Raised circulating concentrations of soluble ICAM-1 (sICAM-1) and soluble E-selectin (sE-selectin) have been reported in meningococcal disease (where sICAM-1 concentrations were shown to be predictive of outcome), neonatal septicaemia, and chronic renal failure.[22–24] Increased concentrations of both sICAM-1[25] and sE-selectin[26] have been reported in the plasma of children with established reflux nephropathy, though in both studies children were investigated when free from infection. No previous study has investigated urinary and plasma soluble adhesion molecule concentrations during acute pyelonephritis.

The aims of this study were: (1) to determine whether circulating and urinary concentrations of E-selectin and ICAM-1 were abnormal during first documented acute pyelonephritis; and (2) to investigate whether circulating or urinary concentrations were predictive for the development of abnormalities on dimercaptosuccinic acid (DMSA) imaging.

SUBJECTS AND METHODS

Children admitted to hospital with a first episode of acute pyelonephritis were recruited into the study. To be diagnosed

Abbreviations: CRP, C reactive protein; ICAM-1, intercellular adhesion molecule 1; MCUG, micturating cystourethrogram; PECAM-1, platelet endothelial cell adhesion molecule 1; sICAM-1, soluble ICAM-1; VUR, vesicoureteric reflux

as having acute pyelonephritis, children had to be acutely unwell with a temperature of at least 38°C, an increased C reactive protein (CRP) or erythrocyte sedimentation rate, and a pure growth of $>10^5$ organisms/ml on a clean catch, catheter, or suprapubic sample of urine. Children with any previous history of urinary tract infection or urological problems, including obstructive uropathy, vesicoureteric reflux, and neuropathic bladder were excluded. Age matched healthy children who were admitted to hospital for minor general surgical procedures served as one set of control subjects. A second set of controls (febrile control group) consisted of children seen in the acute admissions unit of our institution with febrile illness, in whom urinary tract infection was subsequently excluded as the source of infection. The purpose of this second group was to ensure the specificity of any difference in adhesion molecule concentrations that might be detected between children with acute pyelonephritis and the healthy control group.

The study was approved by the Salford and Trafford Local Research Ethical Committee. Full informed consent was obtained from the participant's parents or legal guardian.

Imaging

All children underwent an acute DMSA scan within the first week after presentation, and those with an abnormal scan had a repeat scan performed at least six weeks later. DMSA scans were interpreted as being abnormal if there was an area of reduced uptake of isotope, if the renal contour was abnormal, or if there was a disparity of more than 10% in split function between the left and the right kidneys. All children less than 2 years of age and children with an abnormal second DMSA scan additionally underwent a micturating cystourethrogram (MCUG) in keeping with our routine clinical policy to determine the presence or absence of VUR.

Measurement of sE-selectin and sICAM-1 in plasma and urine

Plasma (EDTA anticoagulated blood) and urine were collected from all patients within one week of presentation at the time of their initial DMSA scan (acute sample). Those patients undergoing second DMSA scans had a second blood and urine sample collected at the time of this scan (late sample). Control subjects had a urine sample collected preoperatively and a single blood sample collected immediately after the induction of general anaesthesia prior to any surgical or other intervention (control sample). The febrile control subjects had a urine sample collected at the time of their assessment in the acute admissions unit. Febrile control subjects were subsequently excluded if the source of the fever was determined to be a urinary tract infection. Samples were stored at −80°C and batched until the assay was performed. sE-selectin and sICAM-1 were measured in plasma and urine samples by enzyme linked immunosorbent assay (ELISA; R&D Systems, Abingdon, UK). Plasma samples were diluted 1/20 in assay buffer and urine samples measured undiluted. All assays were run in duplicate. To control for interplate variation, a biological sample of known concentration was included in all assays as well as a plasma sample from one single patient. The lower limits of detection of the assays were 0.1 ng/ml for sE-selectin and 0.35 ng/ml for sICAM. Values below the lower limit of detection for both proteins were taken as being negative. In order to correct for varying urinary concentrations, urinary concentrations were expressed as a ratio of adhesion molecule/mmol creatinine.

Statistics

Plasma and urinary sE-selectin and sICAM-1 concentrations were not all normally distributed in the study population, hence all values are expressed as median and range. Differences between the two groups were evaluated by the Mann–Whitney U test, or Kruskal–Wallis test where there were more than two groups. Differences between paired samples were assessed by the Wilcoxon rank test, and categorical variables by the χ^2 test. A p value of <0.05 was taken as being statistically significant.

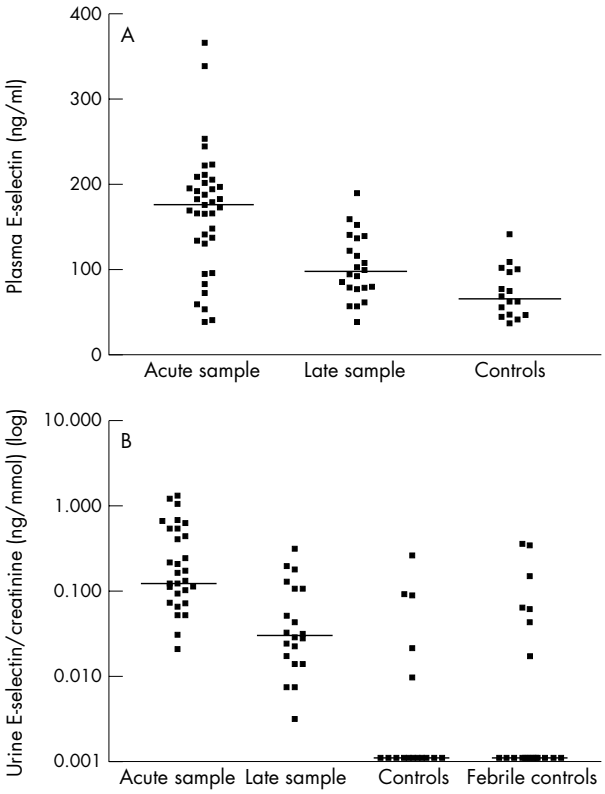

Figure 1 Plasma (A) and urine (B) sE-selectin levels in children with acute pyelonephritis (acute and late samples), healthy controls, and febrile controls. Horizontal bars denote median values. Plasma acute samples *v* late samples p = 0.003 and acute samples *v* controls p<0.0001. Urine acute samples *v* late samples p = 0.002 and acute samples *v* controls p = 0.0003 and febrile controls p 0.0014.

RESULTS

Characteristics of the study population

Forty children with acute pyelonephritis, 21 afebrile healthy controls, and 18 febrile controls were recruited. The median age (range) of patients was 1.7 years (0.1–10.8), controls 2.3 years (0.16–10), and febrile controls 2.7 years (0.4–12) (NS). All children with pyelonephritis had normal predicted glomerular filtration rates calculated using the Schwartz formula.[27] Two patients had moderate proteinuria at the time of urine sampling.

DMSA and MCUG findings

Thirty (75%) of the 40 patients had abnormal acute DMSA scans. Six of these 30 patients had persisting abnormalities on the late scan and were therefore classified as being at significant risk for the development of renal parenchymal scarring (RPS). Four children did not complete the study, hence they could not be classified. Twenty seven children underwent an MCUG; 11 were found to have VUR.

Plasma and urinary sE-selectin concentrations

Plasma sE-selectin concentrations were significantly increased in the acute samples (median 176.3 ng/ml) compared with both late (97.8 ng/ml, p = 0.003) and control samples (65.6 ng/ml, $p < 0.0001$) (fig 1A). Urinary sE-selectin/creatinine ratios in the acute sample were similarly significantly increased (0.12 ng/mmol) compared with late (0.029

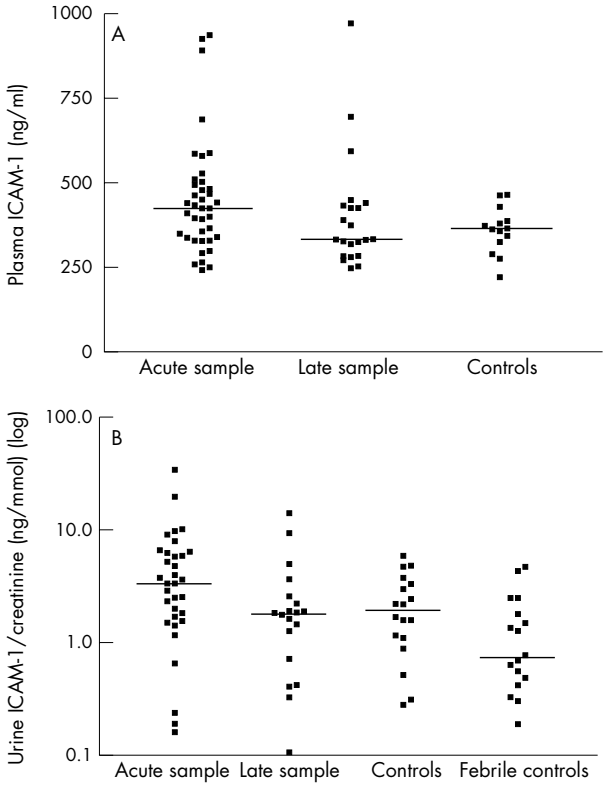

Figure 2 Plasma (A) and urine (B) sICAM-1 levels in children with acute pyelonephritis (acute and late samples), healthy controls, and febrile controls. Horizontal bars denotes median values. Plasma acute samples *v* late samples p = 0.049. Urine acute samples *v* febrile control samples p = 0.0009.

ng/mmol, p = 0.002), control (0 ng/mmol, p = 0.0003), and febrile control samples (0 ng/mmol, p = 0.0014) (fig 1B). Urinary sE-selectin was detectable in 87.5% of children with pyelonephritis compared with 25% of controls ($p < 0.0001$).

Plasma and urinary sICAM-1 concentrations

Plasma sICAM-1 concentrations were significantly increased in the acute sample (median concentration 428.0 ng/ml) compared with control samples (365.2 ng/ml, p = 0.049), though there was no difference detected between the acute and late samples (333.4 ng/ml, p = 0.13; fig 2A). Urinary sICAM-1 concentrations were higher in the acute sample (3.2 ng/mmol) than in febrile controls (0.7 ng/mmol, p = 0.0009), though no other differences were detected between the patient and control groups.

Relation between DMSA changes, clinical parameters, and plasma and urinary soluble adhesion molecules

There was no correlation detected between the concentrations of plasma or urinary E-selectin or ICAM-1 and the presence or absence of changes on either the acute or the late DMSA scan. No correlation was detected between urinary sE-selectin or sICAM-1 concentrations and urinary white cell count, peripheral blood white cell count, CRP, or body temperature.

DISCUSSION

This is the first report of increased soluble cell adhesion molecule expression in children with acute pyelonephritis. Concentrations of sE-selectin were significantly increased in the plasma and urine at presentation, falling, though remaining significantly increased at six weeks post-infection compared with healthy control subjects. sICAM-1 concentrations were also significantly increased in acute plasma and urine samples. The magnitude of elevation observed was consistent with values in previous reports of children with meningococcal and neonatal septicemia,[22 23] and the plasma concentrations in our control subjects were similar to previously reported control values.[22]

Two previous studies by the same research group have investigated soluble adhesion molecule expression in children with VUR, with and without reflux nephropathy.[25 26] Children were studied when clinically well, with no evidence of acute urinary tract infection. These studies only reported circulating serum concentrations, and found raised sE-selectin and sICAM-1 in children with VUR compared with healthy controls. Higher concentrations of both proteins were detected in children with parenchymal scarring in comparison with those with VUR and no scarring, though for ICAM-1 this was only in children under 2 years of age. The authors postulated that the higher circulating adhesion molecule concentrations in children with reflux nephropathy reflected increased tissue damage as a result of a progressive inflammatory response in the renal parenchyma. The detection of higher ICAM-1 concentrations in children with VUR with no evidence of nephropathy compared with control subjects is, however, more difficult to explain.

In this study, children were investigated during their first episode of acute pyelonephritis, and urine samples were collected in addition to plasma samples. Adhesion molecule concentrations fell over the six weeks between the acute and late samples, providing further evidence for significant up-regulation at the time of acute infection. We did not, however, document a fall in sE-selectin below concentrations in control subjects, possibly in keeping with the findings of Kobayashi and colleagues.[26] Furthermore, we could not detect any correlation between either sE-selectin or sICAM-1 concentrations and the presence or absence of either VUR or acute or late DMSA changes.

The functional significance of soluble cell adhesion molecules in plasma and urine has not been fully elucidated. It has been suggested that soluble molecules may block leucocyte adhesion by binding to ligands on leucocytes, thus functioning as competitive inhibitors of the membrane bound forms.[20] The binding and clearance of sICAM-1 may be enhanced by the activation of CD11a and CD11b on leucocytes during inflammation, thus resulting in paradoxically low circulating concentrations.[20] There may therefore be factors independent of pathological changes in the kidney that influence circulating concentrations; this may explain the lack of correlation between tissue expression and plasma concentration of ICAM-1 that was previously reported in patients with IgA nephropathy and other glomerulonephritides.[28 29]

The presence of sE-selectin in the urine during acute pyelonephritis has not been reported previously. Renal epithelial cells do not constitutively express E-selectin in vivo or in vitro; however, activation by tumour necrosis factor α and other cytokines up-regulates expression in glomerular endothelial cells.[16] Urinary sE-selectin was detectable in more than 85% of children with pyelonephritis in this study, compared with 25% of control subjects. The source in normal individuals is likely to be predominantly from glomerular filtration. It is at present unclear as to whether the increased urinary sE-selectin detected represents increased filtration of the raised circulating component, or reflects direct excretion of locally produced protein into the urine. All of the children in this study had normal GFRs, thus eliminating any effect of impaired renal function on the raised concentrations seen in the children with pyelonephritis.

Raised concentrations of urinary sICAM-1 have been previously reported in patients with bladder carcinoma, renal allograft rejection, and adults with renal allograft dysfunction caused by pyelonephritis.[30 31] We detected raised circulating plasma concentrations in children during the acute stages of pyelonephritis, but both plasma and urine sICAM-1 were

readily detectable in virtually all of the patients and controls. The origin of urinary ICAM-1 is unknown, though possible sources include glomerular filtration and/or shedding from proximal tubular epithelial cells.[28] Epithelial cells isolated from freshly voided urine from healthy controls have been shown to stain for ICAM-1,[32] and a good correlation has been shown between tubular expression of ICAM-1 and urinary concentrations of sICAM-1.

While the widespread presence of sICAM-1 in the urine of healthy individuals limits its diagnostic utility, we have presented preliminary evidence that the detection of sE-selectin in the urine may be a sensitive marker of acute pyelonephritis.

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