PLASMA P-SELECTIN IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME CAUSED BY ESCHERICHIA COLI O157 : H7

HIDEKAZU KAMITSUJI, KAZUMA NONAMI, NAOKO ISHIKAWA, TOMOHIKO MURAKAMI
AKIFUMI NAKAYAMA*, YAYOI UMEKI* and MITSURU NAKAJIMA**

Department of Pediatrics, Nara Prefectural Nara Hospital
**Central Clinical Laboratory*
***Department of Pediatrics, Nara Medical University*
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Abstract: Microvascular thrombosis in the kidney plays an important role in the pathogenesis of hemolytic uremic syndrome (HUS). To evaluate the pathophysiological significance of the plasma levels of P-selectin, which is releared from activated or damaged endthelial cells, we measured the plasma concentrations of this protein in children with HUS associated with verotoxin-producing Escherichia coli (VTEC). In the acute phase of HUS, plasma levels of P-selectin were significantly higher than those in non-HUS or healthy controls, and returned to normal range in the recovery phase. The elevated levels of plasma P-selectin were found to correlate with an increase in soluble thrombomodulin and thrombin antithrombin III complex, implying an association between platelet activation, endothelial cell injury and activation of the coagulation cascade. However, there was no definite correlation between P-selectin levels and platelet counts or serum creatinine levels. From these findings, we concluded that the elevation of elevated circulating P-selectin levels in HUS associated with VTEC infection is related to endothelial cell injury and platelet activation as a primary event, and its measurement may be helpful for an early diagnosis of this disease. (奈医誌. J. Nara Med. Ass. **50**, 515～523, 1999)

Key words : hemolytic uremic syndrome, verotoxin, P-selectin

INTRODUCTION

Hemolytic uremic syndrome (HUS) is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Among the various causative factors, verotoxingenic Escherichia coli (VTEC) strains, especially E. coli O157, are responsible for the majority of gastroenterocolitis-associated HUS illnesses in children in Japan[1]. In HUS associated with VTEC infection, injured endothelial cells induced by enhanced production of inflammatory mediators, such as tumor necrotic factor-α or interleukins produced upon stimulation with bacterial lipopolysaccharide (LPS) and/or verotoxin (VT), provoke platelet activation and progression of microvascular thrombosis. This process is considered to be a main causative factor in the development of this disease[2-4].

Recently, Johnston et al.[5] developed a monoclonal antibody against P-selectin, that is an integral membrane glycoprotein stored in the secretory granules of platelets and endothelial cells and rapidly redistributes to the cell surface in response to various physiological or

pathological stimuli[6]. By using the enzyme-linked immunosorbent assay (ELISA), P-selectin is found to be present in the circulation of healthy children and elevated in the of patients with thrombotic thrombocytopenic purpura (TTP) or HUS[7]. However, it is still uncertain whether plasma P-selectin levels reflect platelet activation or endothelial cell damage.

In this study, to investigate the pathophysiological significance of circulating P-selectin in VTEC associated with HUS, we measured its plasma concentrations by quantitative ELISA in children with HUS associated with VTEC infection or non-HUS illnesses. Concomitantly, we also measured the plasma concentrations of von Willebrand factor (vWF) or soluble thrombomodulin (sTM) as a clinical indicator of endothelial cell injury[8], and thrombin antithrombin III complex (TAT) as that of thrombin activity[9], and these measurements were compared with the clinical findings.

PATIENTS AND METHODS

Patients

The subjects were 26 children (13 females and 13 males, a mean age of 6.5±5.2 years), with abdominal pain and diarrhea. In all subjects, infection with E. coli O157 : H7 was identified by stool culture or significant antibody titers to the O157 lipopolysaccharide (LPS). Titers higher than 1 : 160 were serologically positive[10]. Detection of verotoxin (VT) genes from stool samples was performed in 17 children by polymerase chain reaction (PCR), using two pairs of VT-1- and VT-2- specific oligonucleotide primers, and an internal control. Among the 26 children, 13 were diagnosed as having HUS. HUS was defined as the presence of hemolytic anemia (hemoglobin <10 g/dl), thrombocytopenia (platelet count $<10\times10^4/mm^3$), and acute renal failure (GFR <75 ml/min by Schwarz formula[11]). The acute phase refers to the presence of all three categories and the recovery phase describes the improvement of hemolytic anemia (hemoglobin >10 g/dl), thrombocytopenia (platelet counts $>10\times10^4/mm^3$) and acute renal failure (GFR >75 ml/min). Ten healthy children (5 females and 5 males, with a mean age of 9.8±3.4 years) served as controls.

Methods

Plasma was obtained from blood collected in sodium citrate 0.129 M(1 volume for 9 volumes of blood) and stored at −80℃ until used. Plasma P-selectin concentration was measured using a GMP-140 EIA kit (Takara, Kyoto, Japan). The sensitivity of the ELISA for plasma P-selectin was 10 ng/ml. Plasma sTM concentration was quantified with a one-step sandwich enzyme immunoassay (EIA) Kit (Fuji Chemical Industries, Takaoka, Japan). The plasma concentrations of the vWF antigen and TAT were quantified with the respective EIA Kit (vWF : Roche Diagnostics KK., Tokyo, Japan ; TAT : Sinotest, Sinotest KK, Tokyo).

Statistical analysis

Values were expressed as the mean±SEM. The data from different groups were compared using the Wilcoxon rank sum test. Linear regression was calculated by Spearman's correlation test. Values of $p<0.05$ were considered significant.

RESULTS

Clinical characteristics

Characteristics of patients and control subjects are shown in Table 1. Pathogenic E. coli

O157 : H7 was identified in all 26 children by stool cultures or a significant antibody titer to lipopolysaccharide. Detection of the VT gene from stool samples was noted in 17 patients. Both VT-1 and VT-2 were detected in 16 children, whereas VT-2 was detected only in 1. Among the 26 children who presented bloody diarrhea with abdominal pain, 13 developed HUS, and peritoneal dialysis was performed on 7 of them. Encephalopathy clinically identified as such by seizure was observed in 2. There was no difference in VT gene analysis between the non-HUS and the HUS groups.

Table 1. Patient characteristics

	Controls	HUS	Non-HUS
No.	10	13	13
M/F	5 / 5	8 / 5	5 / 8
Age (y)	9.8±3.4	6.4±5.5	6.8±5.7
O-157 (+) and/or LPS (+)	N.D.	13	13
VT 1	N.D.	7 (+) 6 (N.D.)	9 (+) 1 (−) 3 (N.D.)
VT 2	N.D.	7 (+) 6 (N.D.)	10(+) 3 (N.D.)
Dialysis		7 /13	0 /13
Encephalopathy		2 /13	0 /13

N.D. : Not done

Table 2. Platelet counts and plasma concentrations of P-selectin in children with E. coli O157 : H7 infection and the controls

	HUS		Non-HUS	Control subjects
	Acute stage	Recovery stage		
Platelet counts ($\times 10^4/mm^3$)	n=13	n=11	n=13	n=10
Median	6.44±2.3*	28.5±11.1	32.0±9.3	28.8±3.8
Range	3.4~10.0	13.0~50.0	15.0~48.0	21.0~36.0
P-selectin (ng/ml)	n=13	n=11	n=13	n=10
Median	215.3±116.0*	112.1±42.3	96.0±17.1	75.8±13.9
Range	83.0~495.0	33.0~170.0	66.0~118.0	55.0~98.0

* $p<0.005$ vs non-HUS or control subjects

Platelet counts, serum creatinine and plasma P-selectin levels

Platelet counts in patients with the acute phase of HUS were significantly lower than those in non-HUS or control subjects ($p<0.005$), and returned to normal range in the recovery stage. Plasma levels of P-selectin in patients with the acute phase of HUS were significantly higher than those in non-HUS or control subjects ($p<0.005$). Serum creatinine levels showed a similar tendency ($p<0.005$) (Table 2). However, no significant differences in platelet counts, plasma P-selectin and serum creatinine levels were found between patients with non-HUS and those in healthy controls. In patients with HUS, plasma P-selectin levels were not correlated with platelet counts and serum creatinine levels (Fig. 1). Changes of platelet counts, serum creatinine and plasma P-selectin levels in 6 patients with HUS are shown in Fig. 2. HUS developed between 2 and 5 days after the onset of gastroenterocolitis. Elevated levels of plasma

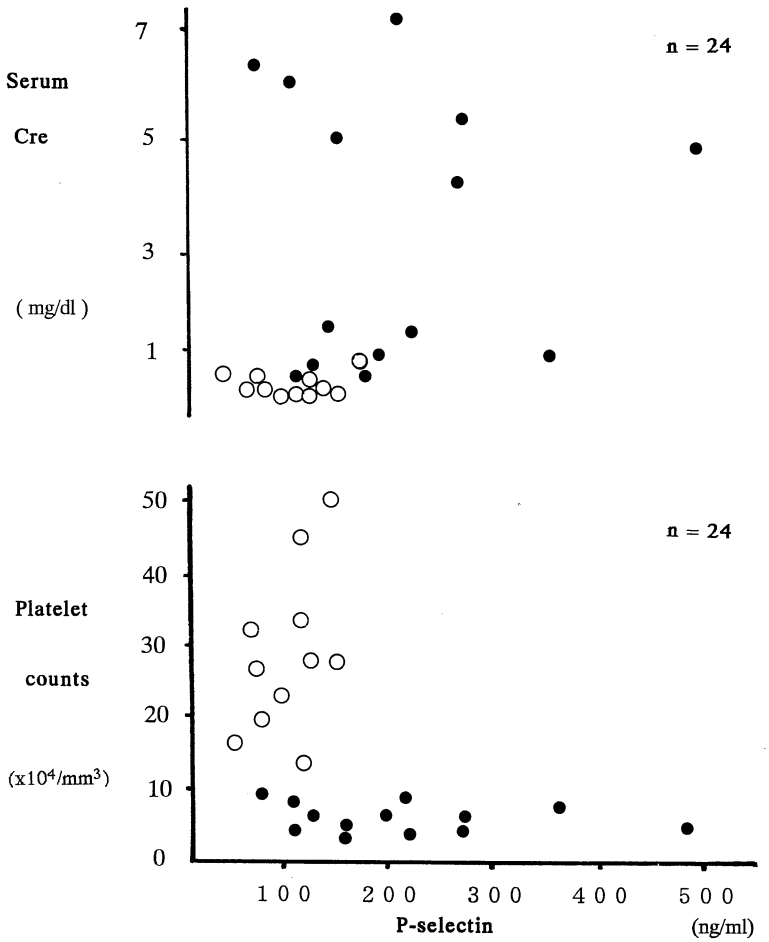

Fig. 1. Relationship between plasma P-selectin, serum creatinine and platelet counts in HUS patients.
● : Acute phase, ○ : Recovery phase

P-selectin were noted within 2 weeks after the onset and returned to normal range in the 3rd week. No patients underwent plasma P-selectin measurement before the onset of HUS. A similar pattern was also observed in the change of platelet couunts. However, a fall in elevated serum creatinine levels was delayed one or two weeks, compared with P-selectin levels.

Correlation of plasma P-selectin levels with vWF, sTM, TAT or serum creatinine

High concentrations of vWF, sTM or TAT were observed in patients with an acute stage of HUS when compared to those of non-HUS subjects (Table 3). In these patients, plasma P-selectin levels showed a positive correlation with sTM ($r=0.581$, $p<0.01$) or TAT ($r=0.534$,

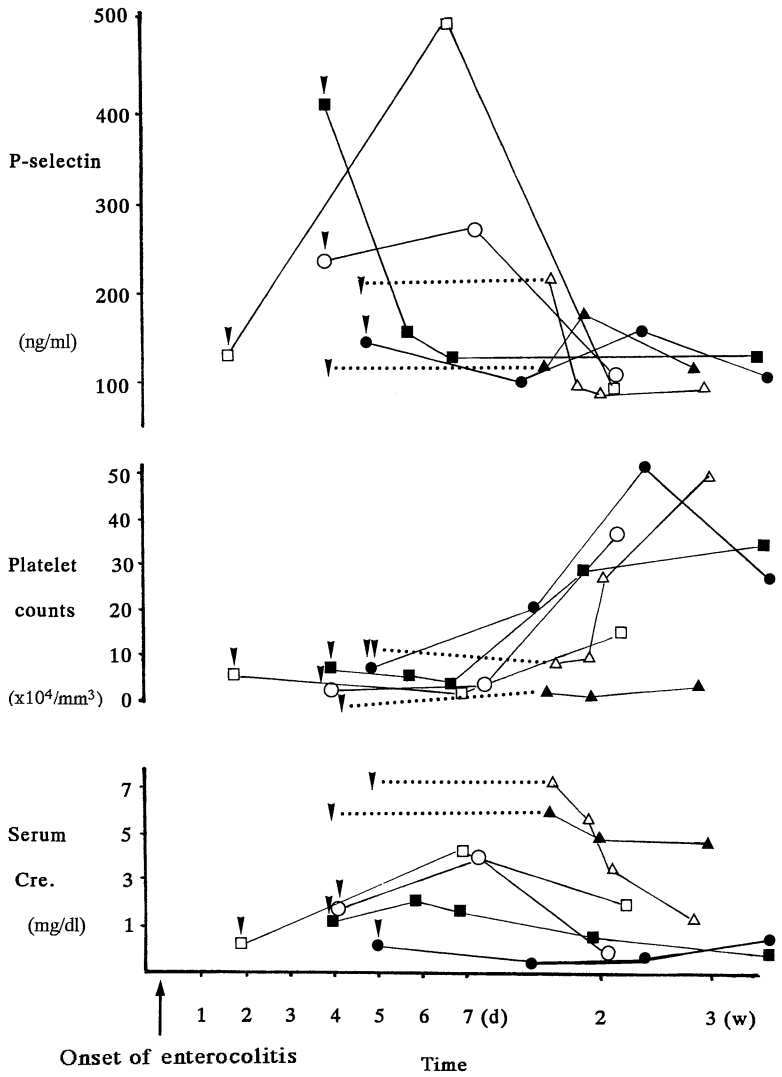

Fig. 2. Changes of plasma P-selectin, platelet counts and serum creatinine levels in 6 patients with HUS associated with VTEC infection.
▼ : Outbreak of HUS

Table 3. Plasma concentrations of von Willebrand factor (vWF), soluble thrombomodulin (sTM) and thrombin antithrombin III complex (TAT) in children with E. coli O157 : H7 infection and the controls

	HUS	Non-HUS	Control subjects
vWF (%)	n=9	n=9	n=10
Median	175.8±62.4	82.8±33.2	90.0±16.1
Range	118.0~267.0	44.0~160.0	65.0~120.0
sTM (TU/ml)	n=11	n=12	n=10
Median	8.0±4.0*	2.1±0.47	1.58±0.4
Range	4.0~15.9	1.5~2.9	1.0~2.5
TAT (ng/ml)	n=12	n=11	n=10
Median	23.1±20.8*	1.8±1.3	1.09±0.4
Range	3.3~60.0	0.9~3.1	0.7~1.8
S-Cre (mg/dl)	n=13	n=13	n=10
Median	3.2±2.4*	0.37±0.1	0.35±0.07
Range	0.6~7.0	0.2~0.6	0.3~0.5

* $p<0.005$ vs non-HUS or control subjects

$p<0.05$) (Fig. 3), but not with vWF. In addition, vWF levels did not show a definite correlation with platelet counts. The sTM was correlated with serum creatinine ($r=0.792$, $p<0.001$).

DISCUSSION

In this study, the patients with HUS associated with VTEC infection revealed significantly higher levels of plasma P-selectin when compared with non-HUS or healthy control subjects. Elevation of plasma P-selectin levels noted predominantly during the 1 st and 2 nd week after the onset of gastro-enterocolitis, and returned to normal after complete remission.

In patients with hemodialysis using a regenerated cellulose membrane, the plasma levels of P-selectin were shown to reflect the platelet activation, based on a comparison study with other platelet related materials[12]. However, there was no definite correlation between plasma P-selectin levels and platelet counts in patients with HUS associated with VTEC infection. A positive correlation was noted between P-selectin levels and sTM. Although the concentration of plasma sTM seemed to depend on renal function[13,14], these findings suggest that the increase in plasma P-selectin levels mainly resulted from injured endothelial cells during the active stage of this HUS rather than platelet activation.

In patients with HUS associated VTEC infection, P-selectin levels did not reflect the renal function determined by serum creatinine, and the decreased renal fuunction in these patients persisted over a longer time compared with platelet counts or P-selectin levels. As to the

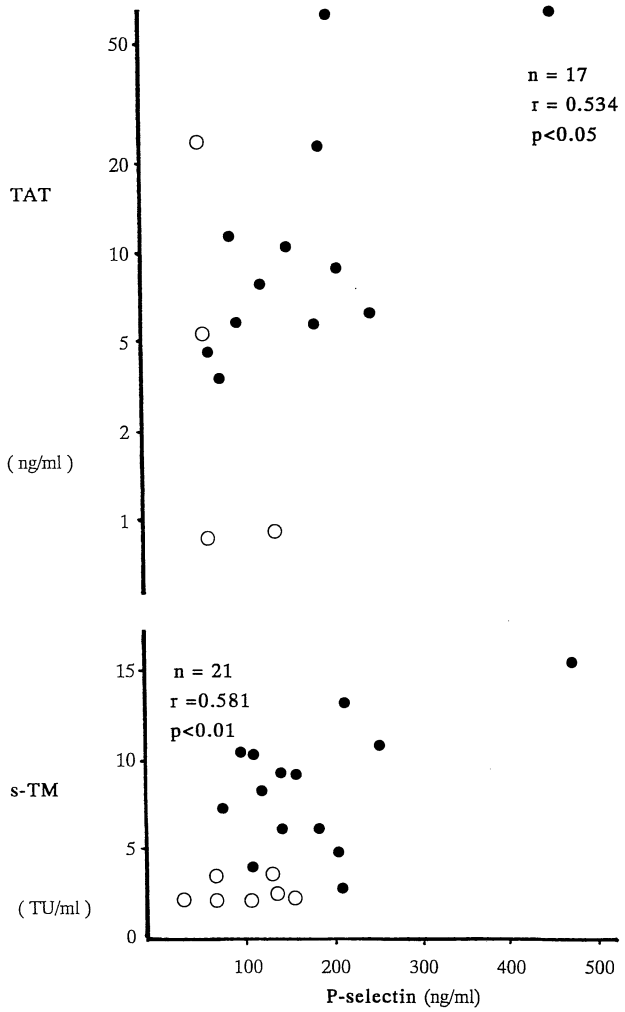

Fig. 3. Relationship between P-selectin and thrombin antithrombin III complex (TAT) or soluble thrombomodulin (sTM) in HUS patients.
● : Acute phase, ○ : Recovery phase

microvascular disorder of the kidney, several studies have shown the involvement of a reduction in a tissue factor pathway inhibitor[15], an enhanced plasminogen activator inhibitor[16], and reduced bioavailability of prostaglandin I_2 (PGI_2)[17]. These factors, together with platelet aggregation, may therefore contribute to sustaining the microangiopathic process by decreasing endothelial thromboresistance and stabilizing microvascular thombi, which would sesult renal damage.

Plasma TAT, which is a marker of thrombin activity in circulating blood[9], was also elevated in the active phase in HUS associated with VTEC infection. The plasma levels of TAT showed a positive correlation with P-selectin levels but not with serum creatinine. These findings

suggested that endothelial injury concomitantly with platelet aggregation may contribute to the enhancement of thrombin activity in circulating blood.

The plasma concentration of vWF, which is stored in the Weibel-Pallade bodies of endothelial cells and released into circulation by injured vascular endothelium[18,19], was increased in HUS patients and correlated well with sTM levels. Although the vWF contributes to the formation of microvascular thrombi by binding to platelet receptors, the increase of the vWF antigen did not correlate with P-selectin, TAT or platelet counts. Further analyses including multimer assessment of vWF are needed to clarify these phenomena.

A limitation of this study was a lack of information on P-selectin expression in damaged organs due to the difficulty of obtaining tissues at the acute stage of HUS under the conditions of bleeding tendency with thrombocytopenia. The establishment of an animal model for HUS associated with VTEC infection may clarify our findings.

From these findings, we concluded that plasma P-selectin in HUS associated with VTEC infection may reflect the microangiopathic state in the active phase, and the serial measurement of this protein may be of value for diagnosing the early phase of this HUS.

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