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Full Length Article

Complement activation associated with ADAMTS13 deficiency may contribute to the characteristic glomerular manifestations in Upshaw-Schulman syndrome

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ABSTRACT

Introduction: Upshaw-Schulman syndrome (USS) is a congenital form of thrombotic thrombocytopenic purpura (TTP) associated with loss-of-function mutations in the *ADAMTS13* gene, possibly leading to aberrant complement activation and vascular injury. However, USS is extremely rare, and there have been no systematic studies correlating histopathological severity with local *ADAMTS13* expression and complement activation.

Materials and methods: Here, we compared histopathological features, ADAMTS13 immunoreactivity, and immunoreactivity of complement proteins C4d and C5b-9 among renal biopsy tissues from five USS cases, ten acquired TTP cases, and eleven controls.

Results: Pathological analysis revealed chronic glomerular sclerotic changes in the majority of USS cases (4 of 5), with minor glomerular pathology in the remaining case. In two of these four severe cases, more than half of the glomerular segmental sclerosis area was localized in the perihilar region. The average number of ADAMTS13-positive cells per glomerulus was significantly lower in USS cases than controls ($p < 0.05$). Conversely, C4d staining was significantly more prevalent in the glomerular capillary walls of USS cases than controls ($p < 0.05$), while C5b-9 staining did not differ significantly among groups.

Conclusions: These findings suggest that the severity of glomerular injury in USS is associated with deficient ADAMTS13 expression and local complement activation, particularly in vascular regions with higher endothelial shear stress. We suggest that C4d immunostaining provides evidence for complement-mediated glomerular damage in USS.

1. Introduction

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening blood disorder caused by severely reduced activity of the metalloprotease ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13) [1,2]. There are two TTP subtypes, acquired and hereditary. Acquired TTP is more common and is caused by autoantibodies that block ADAMTS13 activity [3]. Hereditary TTP, also known as Upshaw-Schulman syndrome (USS), results from inherited mutations in *ADAMTS13* that cause severe deficiencies in enzymatic activity (< 10% of wild type) [1,2,4,5]. The incidence of TTP is only 4–10 cases/million people/year, and < 5% of TTP cases are

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classified as USS [6], limiting large-scale studies.

Hepatic stellate cells are a primary site of ADAMTS13 synthesis [7,8]. Expression of ADAMTS13 has been detected at the mRNA and protein levels in cultured renal podocytes, glomerular endothelial cells, and tubular epithelial cells; likewise, ADAMTS13 bioactivity has been demonstrated in these cell types [9–11]. Upshaw-Schulman syndrome is characterized clinically by recurrent episodes of thrombocytopenia and microangiopathic hemolytic anemia responsive to infusions of prophylactic fresh frozen plasma (FFP). Renal dysfunction is a major complication of both USS and acquired TTP, and is manifested histopathologically as thrombotic microangiopathy (TMA) characterized by thrombosis, endothelial cell swelling in small vessels, and glomerular mesangiolysis [12].

Manea et al. reported that signs of acute thrombotic microangiopathy, such as glomerular endothelial swelling and vascular thrombus, are characteristic pathological manifestations of USS [9]. More recently, a causal relationship between TMA and complement activation was proposed in atypical hemolytic uremic syndrome and TTP, including USS [13,14]. However, no study has examined the associations of these pathological features with local ADAMTS13 expression and complement activation. To this end, we conducted simultaneous histopathological examination and immunohistochemical staining for ADAMTS13 and complement proteins in renal biopsy tissues from USS patients and matched controls as well as acquired TTP patients.

2. Materials and methods

2.1. USS patient group

The present study enrolled five cases of USS with available renal biopsy specimens. These biopsy samples were originally obtained from five different hospitals in Japan, and cases 1, 2, 3, and 4 have been previously reported [5,15–18]. In all cases, the diagnosis of USS was based on severely impaired ADAMTS13 activity (< 10% of control) in the absence of ADAMTS13 inhibitors, and mutations in the *ADAMTS13* gene. Patient demographics, laboratory data, and clinical courses are summarized in Table 1 and Fig. 1. Average patient age was 21.2 years (range, 9–40 years) and serum creatinine at the time of renal biopsy was 2.62 mg/dl (range, 0.6–6.16 mg/dl). Serum complement levels in cases 2–5 were within normal limits, while no laboratory data were available for case 1. Proteinuria levels ranged from 0.1 to 0.15 g/dl in all cases with available laboratory data (cases 3–5).

2.2. Control group and acquired TTP cases

The control group consisted of 11 biopsy specimens without severe renal impairment. Six biopsy samples were obtained from patients with mild hematuria or minimal change disease, and histology showed only minor glomerular abnormalities (MGA). Five biopsy samples were obtained from patients with focal segmental glomerulosclerosis with proteinuria. The other one case was obtained from an autopsy case without renal disorders. Eight cases of acquired TTP (three biopsy and five autopsy samples) were also examined. Four cases had proteinuria and hematuria, one case had neither proteinuria nor hematuria, and renal data of three cases were unknown. In control and acquired TTP cases, average ages at the time of renal biopsy were 57.8 years (range, 23–83 years) and 38.9 years (range, 22–66 years), and average serum creatinine levels were 1.15 mg/dl (range, 0.49–2.43 mg/dl) and 3.32 mg/dl (range, 0.78–7.59 mg/dl), respectively.

2.3. Pathological examination

Formalin-fixed, paraffin-embedded tissue sections of renal biopsies were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), periodic acid-methenamine-silver (PAM), and elastica Masson trichrome (EMT). Histological findings of glomerular injury changes and vascular lesions suggestive of TMA were examined. The grade of interstitial fibrosis and tubular atrophy (IF/TA) was evaluated according to the Banff scheme [19]. Arteriosclerosis was evaluated qualitatively according to a three-grade scale (mild, moderate, and severe). The results of electron microscopic examinations and previous immunofluorescence studies (IgA, IgG, IgM, and C3 staining) were retrieved from pathological data records of the original hospitals.

2.4. Immunohistochemistry

Sections were deparaffinized and subjected to antigen retrieval. After blocking endogenous peroxidases, the sections were incubated with primary antibodies overnight at 4 °C, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies. The primary antibodies used in the present study (and suppliers) were as follows: anti-ADAMTS13 (PA5-14339; Thermo Fisher, Rockford, USA; 1:200), anti-C4d (BI-RC4d; Biomedica Gruppe, Vienna, Austria; 1:50), and anti-C5b-9 (M0777; Dako, Carpinteria, CA, USA; 1:100). Renal arteriolar smooth muscle cells from normal autopsy cases served as positive controls for ADAMTS13 immunostaining [20]. Tissues from cases of antibody-mediated allograft rejection were used as positive controls for Cd4 staining. For C5b-9 immunostaining, renal biopsies of patients with membranous nephropathy served as positive controls. The primary antibody incubation step was omitted for all negative controls.

2.5. Quantitative evaluation of immunohistological results

The number of ADAMTS13-positive cells per glomerulus was counted for all visible glomeruli in each biopsy section. In autopsy samples, 20 glomeruli were examined. Average number of ADAMTS13-positive cells per glomerulus was calculated for quantitative comparisons. Glomerular immunopositivity for C4d or C5b-9 was defined as circumferential tuft staining in at least one capillary. The average numbers of C4d- and C5b-9-positive glomerular capillaries were compared among USS, control, and TTP groups. Immunopositivity for C4d and C5b-9 in arterioles was defined as staining along the luminal side of the vessel. Positivity for C4d in peritubular capillaries was defined according to the Banff 2007 criteria [19].

Table 1
Clinical characteristics of USS at the time of renal biopsy.

Case no.	Patient	Age (years)	Sex	Serum creatinine (mg/dl)	Serum C3 (mg/dl)	Serum C4 (mg/dl)	Proteinuria (g/dl)	ADAMTS13 gene mutations	
								Father's origin	Mother's origin
1	C3	11	M	Unknown	Unknown	Unknown	Unknown	c.414 + 1G > A	c.414 + 1G > A
2	Q1	30	M	6.16	97	39.4	Unknown	p.G227R	p.C908Y
3	U3	9	F	0.6	111	15	0.15	c.2259delA	c.2259delA
4	W4	16	F	1.23	85	17	0.1	p.G550R	c.746_987 + 373del1782
5	YY	40	M	2.5	83	30.8	0.1	p.R973Nfs*14	p.G1031D

Serum creatinine (male) 0.5–1.1 mg/dl, (female) 0.4–0.8 mg/dl; Serum C3, 80–140 mg/dl; Serum C4, 10–40 mg/dl; Proteinuria, 0–30 mg/dl.