Urinary Cytokines and Renal Doppler Study in Kawasaki Disease

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Objective To investigate whether renal vasculitis is the sole cause or merely a contributing cause of renal inflammation in Kawasaki disease (KD).

Study design This prospective study in a university medical center in Taiwan enrolled 24 children with KD between June 2004 and November 2005. All patients underwent a technetium-99 m dimercaptosuccinic acid scintigraphy single-photon emission computed tomography scan, the results of which were used to group the patients with KD as with or without renal involvement. Urine samples underwent a cytokine analysis. Renal Doppler ultrasonography was used to evaluate renal vasculitis by measuring the pulsatility index (PI) and resistance index (RI).

Results Ten of the 24 patients (42%) with renal inflammatory foci were the study group; the remainder composed the control group. Urinary interleukin (IL)-6 levels were significantly higher in the study group (496.7 $\pm$ 310.9 vs 115.0 $\pm$ 65.9 ng/g urinary creatinine; $P < .01$), as were PI values (1.85 $\pm$ 0.70 vs 1.44 $\pm$ 0.53; $P < .05$). Urinary IL-6 levels and PI values were significantly ($P < .05$) correlated.

Conclusions Increased urinary IL-6 and elevated renal Doppler measures suggest that immune-mediated vasculitis is one of the mechanisms causing renal inflammation in KD. *(J Pediatr 2010;156:792-7)*.

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The pathologic findings of Kawasaki disease (KD) are systemic vasculitis and a high incidence of inflammatory lesions affecting various organs.[1-5] Vascular involvement in KD is in small and medium-sized blood vessels, particularly the coronary arteries. Thus, there is a possibility of renal involvement, because renal arteries are the same size.[6] Several recent studies have discussed renal involvement in patients with KD.[7-9] We previously used technetium-99 m dimercaptosuccinic acid (DMSA) scintigraphy to analyze the high incidence of renal inflammatory foci associated with KD.[10] Whether the renal inflammation that we found in KD was caused solely by renal vasculitis or whether other immune-mediated mechanisms were also involved is unknown, however.

Renal Doppler ultrasonography provides a high-quality, noninvasive display of the flow in renal blood vessels and enables measurements of flow measures that may be important in several kidney diseases.[11-13] Although a DMSA scan remains the gold standard for detecting renal inflammation, a renal Doppler study is more appropriate for investigating renal vascular disease. In native kidneys, Doppler was used to detect focal ischemic areas in acute inflammation or infarction and to assess perfusion in several renal parenchymal diseases.[14,15] Because KD is considered a vasculitis, it may be interesting to examine the renal Doppler flow pattern in patients with KD with and without renal involvement.

In addition, immune system activation is a central feature of KD. Both serum cytokine levels and urinary cytokine levels have been studied. Several studies[16-18] showed evidence of consistently elevated levels of urinary cytokines (interleukin [IL]-6 and IL-8) in patients with acute childhood KD. An inflammatory process within the renal parenchyma in most of the patients was suggested.[16] Thus, we hypothesize that urinary cytokine levels are correlated with the degree of renal inflammation in patients with KD.

In this study, we investigated the mechanisms of renal inflammation in patients with KD by analyzing urinary cytokine levels and their correlation with renal inflammation in patients with KD, investigating the clinical usefulness of renal Doppler studies in patients with KD, and comparing Doppler flow patterns in patients with KD with and without renal involvement.

Cr	Creatinine
DMSA	Dimercaptosuccinic acid
IL	Interleukin
IVIG	Intravenous immunoglobulin
KD	Kawasaki disease
PI	Pulsatility index
RI	Resistance index
S/D ratio	Systolic/diastolic velocity ratio
SPECT	Single-photon emission computed tomography

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Table I. Initial clinical characteristics, laboratory findings, and image studies of patients with KD with or without renal inflammation

	DMSA group		
	Abnormal (n = 10)	Normal (n = 14)	*P* value
Age, months	15.2 ± 6.5	15.8 ± 11.3	.89
Sex, male/female	8/2	10/4	1.0
Duration of fever before treatment, days	5.7 ± 0.95	5.8 ± 1.37	.87
White blood cell count, n/mm^3	17 280.0 ± 3873	18 671.0 ± 8432	.63
Platelet count, K/mm^3	354.0 ± 86	464.0 ± 188	.10
C-reactive protein, mg/L	107.6 ± 66.9	91.1 ± 38.2	.71
Erythrocyte sedimentation rate, mm/h	61.7 ± 32.9	64.4 ± 31.3	.89
Elevated liver enzymes, n	4	7	.47
Elevated serum creatinine, n	1	0	.42
Pyuria, n	6	3	.09
Hematuria, n	0	2	.49
Proteinuria, n	1	1	1.0
Abnormal renal ultrasound n	2	0	.16
Coronary artery lesion, n	6	2	.03*

*$P < .05$.

Methods

Patient Selection

Children admitted to our hospital with acute febrile KD were considered. KD was defined according to clinical criteria.[19] The exclusion criteria were (1) a previous history of urinary tract infection, (2) coincident congenital urogenital abnormality or uropathy, (3) urinary tract infection between the initial and follow-up scintigram, (4) a space-occupying lesion at renal ultrasound, and (5) positive urine culture results. When a patient's fever had subsided for 48 hours, the patient was discharged from the hospital and followed in the outpatient clinic. The study protocol was approved by our hospital's institutional review board, and written informed consent was obtained from the patients' parents.

Sample Collection

When the diagnosis of KD was made, patients were given high-dose human intravenous immunoglobulin (IVIG) (1 g/kg/day for 2 consecutive days) and a high dose of aspirin (60-80 mg/kg/day) based on current recommendations.[20] Laboratory results, including liver and renal function test results, were collected. A urinary analysis and urine culture also were done to rule out the possibility of urinary tract infection. An echocardiogram was performed to evaluate coronary artery abnormalities. Renal ultrasonography was performed at the time of admission to exclude congenital uropathy and space-occupying lesions. An initial DMSA renal single-photon emission computed tomography (SPECT) scan was done during the first week of hospitalization, as described elsewhere.[10] The results were applied to group the patients with KD into those with and without renal involvement.

The first urine sample was collected on admission and before IVIG therapy. The second sample was collected 1 day after IVIG therapy. The freshly voided urine specimens were centrifuged at 1500 rpm for 5 minutes, and the supernatant was stored in aliquots at -80°C until analysis.

Enzyme-linked immunosorbent assay (ELISA) kits (BioSource International, Camarillo, California) were used, following the manufacturer's directions, to measure the concentrations of cytokines in urine. Urinary cytokine levels were corrected using urinary creatinine (Cr) concentration.

Renal-Pulsed Doppler Ultrasonography Examination

During admission, a renal-pulsed Doppler ultrasonography examination, without sedation, of the hilar renal artery was done with sector transducers at 2.5-7.5 MHz (SONOS 5500; Hewlett-Packard, Les Ulis, France). The child was placed in the supine position. The renal hilar artery was traced by placing the transducer in the dorsolateral area of the flank below the costal arch. The sample volume was placed at the renal hilar artery and optimized under acoustic and optical control. At least 3 satisfactory waveforms were obtained. The systolic/diastolic velocity ratio (S/D ratio), pulsatility index (PI), and resistance index (RI) were then measured using online calculation software. The S/D ratio was calculated as [peak systolic frequency shift] ÷ [peak diastolic frequency shift], PI was calculated as [peak systolic frequency shift - peak diastolic frequency shift] ÷ [mean frequency shift], and RI was calculated as [peak systolic frequency shift – peak diastolic frequency shift] ÷ [peak systolic frequency shift].[21] The average of 3 waveforms was used for later statistical analysis. For the renal-pulsed Doppler ultrasonographic examination, 21 age-matched healthy children also were enrolled as a control group.

Statistical Methods

SPSS 12.0 for Windows (SPSS Institute, Chicago, Illinois) was used to analyze the data collected. The analysis included a χ^2 test for the demographic factors and clinical measurements. Numerical data are reported as mean ± standard deviation. The Student *t* test and the Mann-Whitney *U* test were used to compare the mean differences between both groups. Statistical significance was set at $P < .05$. Simple