

Endothelial dysfunction in adult patients with a history of Kawasaki disease

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Abstract To assess the existence of endothelial dysfunction and the possibility of the early onset of atherosclerosis in the chronic stage of Kawasaki disease (KD), we examined endothelial function in adult patients late after the onset of KD. We evaluated two age-matched groups: 35 adult KD patients (KD group) (mean age, 27.0 years; mean interval time, 24.1 years), and 36 healthy adults (control group). To assess vascular endothelial function, flow-mediated dilatation (%FMD) of the brachial artery and urinary nitrites and nitrates (NOx) were examined. We also measured adhesion molecules and several coagulation-fibrinolysis markers. In addition, we measured high-sensitive C-reactive protein (hs-CRP) as a chronic inflammatory marker, and brachial-ankle pulse wave velocity (baPWV) as a marker for arterial stiffness. % FMD was significantly reduced in the KD group when compared with that of the control group (KD group, $10.4 \pm 2.6\%$; control group, $14.4 \pm 3.2\%$, $p < 0.05$), particularly in patients with coronary artery lesions. Thrombin-antithrombin III complex values were higher in the KD group,

although no significant differences were observed in the other markers for endothelial function. Hs-CRP was significantly elevated only in the patients with coronary aneurysms. Furthermore, in the male KD patients, the baPWV values were significantly higher than those in the control subjects. This study revealed that the adult patients with a history of KD had systemic vascular endothelial dysfunction, and also suggested that a history of KD was possibly one of the risk factors for early onset of atherosclerosis.

Keywords Kawasaki disease · Adult · Vascular endothelial function · High-sensitive C-reactive protein · Atherosclerosis

Abbreviations

KD	kawasaki disease
CALs	coronary artery lesions
%FMD	Flow-mediated dilatation
ISDN	Isosorbide dinitrite
%EID	Endothelium-independent dilatation
SBP	systemic blood pressure
DBP	diastolic blood pressure
Cre	Creatinine
NOx	Nitrites and nitrates
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble vascular adhesion molecule-1
TAT	Thrombin-antithrombin III complex
tPA PAI-1	Tissue plasminogen activator-plasminogen activator inhibitor-1 complex
vWV	Von Willebrand factor
hs-CRP	high-sensitive C-reactive protein
BMI	body mass index
TC	total cholesterol
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
TG	Triglyceride

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Introduction

Kawasaki disease (KD) is an acute febrile disorder and is characterized by systemic vasculitis that affects infants and young children. In the acute stage, coronary aneurysm is a serious complication because it can lead to stenotic lesions or myocardial ischemia [12]. Recently, the possibilities of the existence of endothelial damage and the early onset of atherosclerosis in KD patients even during the chronic stage have been indicated. Impaired coronary arteries, at the sites of both aneurysms and regressed aneurysms, have been substantiated by the intracoronary injection of acetylcholine and by the intravascular ultrasound images [8, 10]. Hamaoka et al. demonstrated that in patients with KD, coronary flow reserve was reduced in the coronary aneurysms and stenotic lesions [7]. Furthermore, other reports showed that systemic endothelial dysfunction occurred late after the onset of KD by measuring flow-mediated dilatation [5, 9, 11]. However, clinical evidence underlying the incidence of this endothelial dysfunction remains unclear at present. Additionally, these examinations were investigated mainly in children or adolescents. Because endothelial dysfunction is closely related to the risk factors for atherosclerosis, it is necessary to longitudinally evaluate systemic endothelial function in adult KD patients.

Therefore, to evaluate whether endothelial dysfunction could occur in the chronic stage of KD, particularly in adult patients with KD, we examined endothelial function in adult patients late after the onset of KD.

Methods

Study subjects

This study comprised 35 adult subjects referred to as the KD group (age 20.0–35.0 years; mean \pm SD 27.0 \pm 4.2 years) and 36 age-matched subjects referred to as the control group. All subjects in both groups were non-smokers (defined as individuals who had never smoked) and had no medical history of hypertension, hyperglycemia, or hypercholesterolemia. We questioned the female subjects about menstrual cycle and excluded those in menstrual phase because it is known that serum estradiol level is low during this time. The KD group was followed in the University Hospital, Kyoto Prefectural University of Medicine (interval time 24.1 \pm 4.5 years). The control subjects were healthy volunteers—doctors, nurses and medical students—who willingly participated in this study. We classified the KD patients into three groups: group A, comprising nine patients with coronary artery lesions (CALs); group B, comprising six patients with transient CALs; and group C, comprising 20 patients without CALs.

Of the nine patients with CAL, eight continued to receive antiplatelet drugs and/or anticoagulants. This study was approved by the ethics committee of our institution. Informed consent for the research protocol was obtained from all subjects.

Study protocol

The subjects were advised against consuming food rich in vitamins C and E and polyphenol, and against performing heavy exercise on the day before the examination.

We obtained the past history of all the subjects, performed a physical examination, and examined the electrocardiograms at rest and after exercise and echocardiograms of the subjects. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and glucose were evaluated at study entry. We calculated TC/HDL-C by dividing TC by HDL-cholesterol.

For the study protocol, we measured the following items as the markers for endothelial function: soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular adhesion molecule-1 (sVCAM-1) as endothelial cell injury markers; thrombin-antithrombin III complex (TAT), tissue plasminogen activator-plasminogen activator inhibitor-1 complex (tPA PAI-1), and von Willebrand factor (vWF) as coagulation-fibrinolysis markers; and NO_x (NO₂⁻ and NO₃⁻), the metabolic products of nitric oxide, which are the major endothelium-derived vasodilating mediators [25]. Furthermore, we measured flow-mediated endothelium-dependent vasodilatation, that is, the change in the diameter of the brachial artery, based on the release of nitric oxide induced by reactive hyperaemia [2].

High-sensitive CRP (hs-CRP) was evaluated as a powerful marker of vascular wall inflammation to examine the effect of chronic inflammation on endothelial dysfunction in patients late after the onset of KD [1]. We also measured the brachial-ankle pulse wave velocity (baPWV) for assessing arterial stiffness [17, 24].

All examinations were carried out in the afternoon.

Brachial artery study

Endothelium-dependent and endothelium-independent dilatation of the brachial artery was measured using high-resolution ultrasonography with a 12.0-MHz linear-array transducer (Vivid 3; GE Medical Systems, USA) as previously described [2]. Briefly, after the subjects lay at rest in the supine position for 10 min in a temperature-controlled room (25°C), a longitudinal section of the right brachial artery was scanned 2–5 cm above the right elbow. The stimulus for flow-mediated dilatation is provided by reactive hyperaemia with a cuff on the forearm inflated to a

pressure of 250 mmHg for 5 min, while isosorbide dinitrite (ISDN) (1.25 mg) is used as an endothelium-independent vasodilator.

For reactive hyperaemia scanning, the diameter measurements were performed 45–70 s after cuff deflation. The percentage change from the baseline diameter to the value during reactive hyperaemia was calculated to determine flow-mediated dilatation (%FMD). Further, the percentage change from the baseline diameter to the maximum diameter after ISDN administration was calculated to determine endothelium-independent dilatation (%EID). The FMD/EID ratio was calculated by dividing %FMD by %EID.

Measurement of arterial stiffness

We measured the baPWV by a previously described non-invasive volume plethysmographic technique (formPWV/ABI, Colin Co., Komaki, Japan) [17, 24]. Briefly, the occlusion and monitoring cuffs were wrapped around the brachia and ankles, electrocardiogram electrodes were placed on both wrists, and a microphone was placed on the left edge of the sternum. Pulse wave contours in the four extremities were then simultaneously recorded, and brachial-ankle PWV was determined from the pulse transit time and the distance between the brachial and ankle regions. Ankle brachial index (ABI), which is calculated by dividing ankle systolic blood pressure (SBP) by brachial SBP, was evaluated simultaneously.

Laboratory measurements

Urine and venous blood samples were collected in bottles; these samples were immediately aliquoted and stored at -80°C.

Table 1 Clinical characteristics of the study groups

	Control group (n=36)	KD group (n=35)	<i>p</i>
Age (years)	25.5±3.9	27.0±4.2	0.097
Male/Female	19/17	16/19	0.552
Age at onset (years)	–	2.7±0.3	–
Interval (years)	–	24.1±4.5	–
Height (cm)	165.9±8.1	164.7±8.1	0.525
Weight (kg)	57.5±7.9	57.0±10.3	0.553
BMI	20.8±1.8	20.7±2.3	0.564
SBP (mmHg)	112.9±9.1	109.2±12.3	0.112
DBP (mmHg)	63.4±7.7	61.6±7.1	0.222
MBP (mmHg)	79.9±7.7	77.7±8.2	0.220
Medication	0 (0%)	8 (22.9%)	<0.01

Data are mean ± SD or *n* value

Table 2 Blood sugar and cholesterol levels in the control and KD groups

	Control group (n=36)	KD group (n=35)	<i>p</i>
BS (mg/dl)	95.6±15.2	95.6±12.7	0.593
TC (mg/dl)	161.3±24.5	168.3±27.9	0.242
LDL-C(mg/dl)	93.2±19.4	97.3±25.3	0.454
HDL-C (mg/dl)	55.4±8.9	56.5±12.8	0.690
HDL-C (mg/dl)	64.5±32.7	64.2±24.4	0.490
TC/HDL-C ratio	3.0±0.5	3.1±0.8	0.391

Data are mean ± SD

Capillary electrophoresis was performed using a Quanta-4000 system (Waters Corp., Milford, MA, USA) to determine the urinary NOx [16]. The urinary NOx values obtained were standardized with those of the urinary creatinine (Cre) levels (urinary NOx/Cre, μmol/mg Cre).

Both sICAM-1 and sVCAM-1 were measured using ELISA (sICAM-1, Bender MedSystem; sVCAM-1, R&D Systems). TAT and tPA PAI-1 were estimated by EIA. Hs-CRP and vWV were determined by the latex agglutination reaction.

Statistical analysis

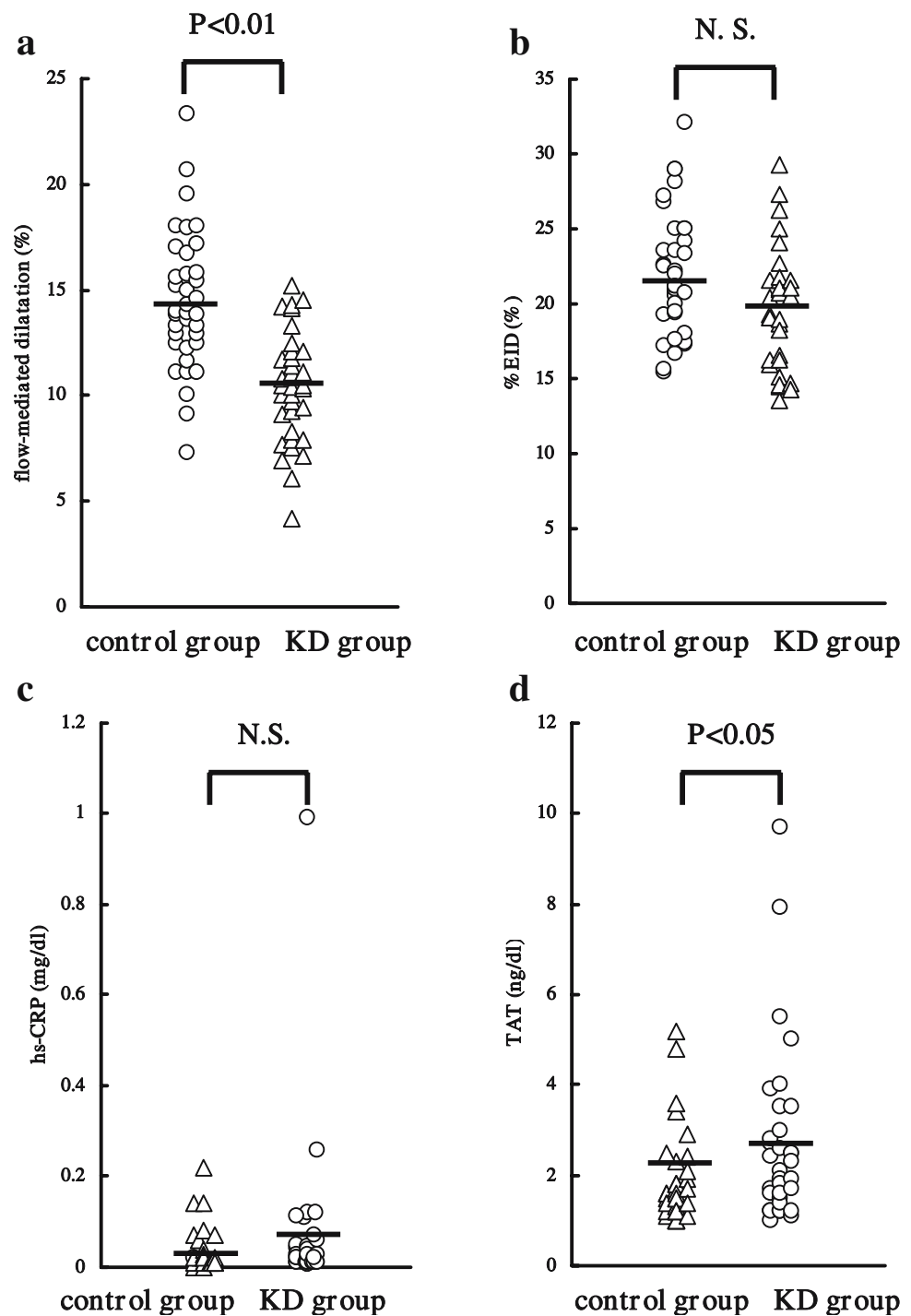
All values are expressed as mean ± SD unless otherwise specified. All data analyses were performed using the SPSS 13.0J software (SPSS, Chicago, USA). For comparison of the two groups, a student’s t-test for parametric variables or the Mann-Whitney U test for non-parametric variables was performed. In the analysis of clinical characteristics, the differences in proportions were evaluated using χ² analysis. To compare the differences among the three KD groups, a one-way ANOVA followed by the Bonferroni test for parametric variables or the Kruskal-Wallis test for non-

Table 3 Results of the endothelial function tests

	Control group (n=36)	KD group (n=35)	<i>p</i>
TAT (ng/dl)	2.4±2.9	2.7±1.9	<0.05
vWV (%)	80.1±21.0	88.0±33.6	0.504
urinary NOx/Cre (μmol/mg Cre)	1.22±0.92	1.11±0.55	0.662
sICAM-1 (ng/ml)	217.0±58.2	222.3±65.3	1.000
sVCAM-1 (ng/ml)	1169.9±243.5	1192.9±317.7	0.951
%FMD (%)	14.4±3.2	10.4±2.6	<0.01
%EID (%)	21.9±4.1	19.9±4.7	0.352
FMD/EID ratio	0.67±0.15	0.53±0.15	<0.01
hs-CRP (mg/dl)	0.035±0.05	0.066±0.166	0.179

Data are mean ± SD

Fig. 1 %FMD, %EID, hs-CRP, and TAT in both the KD and control groups. Horizontal lines represent the mean of the groups



parametric variables was performed. Since 25 out of 71 subjects had tPA PAI-1 levels below the lowest detectable level, the proportions of subjects having tPA PAI-1 above and below the lowest detectable level in each group were compared by χ^2 analysis. The correlation was analysed with Pearson's and Spearman's correlation analyses to assess a possible relation. A p value of less than 0.05 was considered to be statistically significant.

Results

Clinical characteristics

The clinical data are shown in Table 1. No difference was observed between the KD group and the control group in terms of age, weight, body mass index (BMI), and blood pressure. The levels of glucose, TC, HDL-C, TG, and the

Table 4 Comparison among the KD subgroups

	Group A (n=9)	Group B (n=6)	Group C (n=20)
TAT (ng/dl)	3.1±3.0	3.0±2.4	2.5±1.4
%FMD (%)	8.8±2.2 ^{a,b}	9.6±2.1 ^a	11.5±2.8 ^a
FMD/EID ratio	0.52±0.18 ^a	0.47±0.13 ^a	0.56±0.15 ^a
hs-CRP (mg/dl)	0.153±0.317 ^a	0.016±0.006	0.041±0.047

Data are mean ± SD

^a*p*<0.05 vs control group

^b*p*<0.05 vs group C

TC/HDL-C ratio in the KD group were all within normal limits and were not significantly different from the data in the control group (Table 2). The ECG findings in all the KD patients and the control subjects were normal. Also, the echocardiogram findings in the control subjects were entirely normal.

Markers for endothelial function

With regard to endothelial function, the TAT values in the KD group were significantly higher than those in the control group (Table 3, Fig. 1), while the vWV levels were

not higher in the KD group than in the control group (Table 3). The proportion of tPA PAI-1 under the detectable levels was not higher in the KD group than in the control group as revealed by χ^2 analysis. No significant difference was observed in the urinary NOx/Cre values between the KD and control groups (Table 3). Moreover, there were no differences between the two groups with regard to the values of ICAM-1 and VCAM-1 values (Table 3).

Endothelium-dependent and endothelium-independent dilatation

There was no difference between the KD group and the control group with regard to the values for the baseline vessel diameter of the brachial artery (KD group, 3.7±0.7 mm; control group, 3.5±0.6 mm); however, both %FMD and the FMD/EID ratio were markedly reduced in the KD group (Table 3, Fig. 1). In contrast, the %EID value did not differ between the KD and the control groups (Table 3, Fig. 1). Both %FMD and FMD/EID values were significantly reduced in each of the three KD subgroups when compared with the control group (Table 4).

Next, we compared the %FMD and FMD/EID ratio among the three KD subgroups. Flow-mediated dilatation was significantly reduced in group A as compared to group

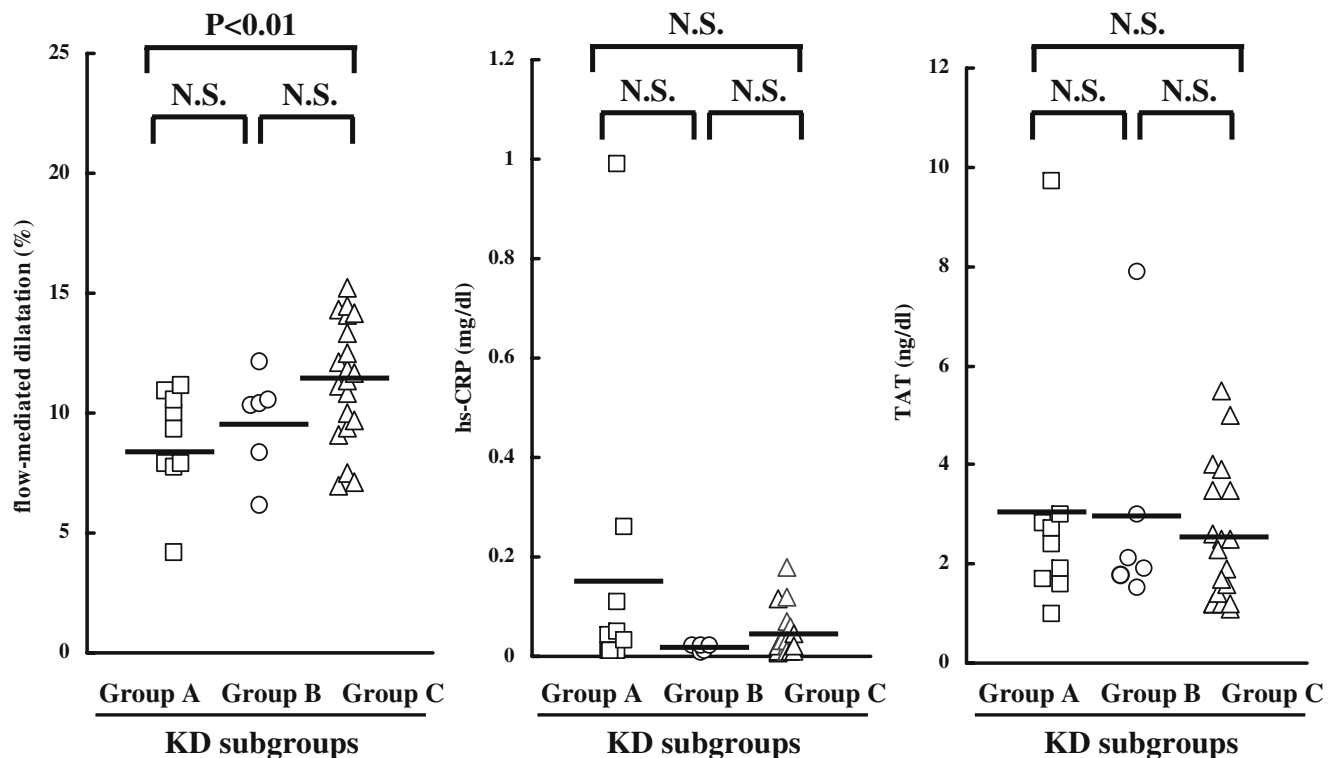


Fig. 2 Endothelium-dependent dilatation among three KD subgroups. A comparison among the three KD subgroups revealed that the % FMD values in group A were significantly lower than those in group

C. There were no differences in the hs-CRP and TAT values among the three KD subgroups. Horizontal lines represent the mean of the groups

C (Table 4, Fig. 2), while there was no difference in the FMD/EID values among the three KD subgroups. These results suggested that endothelium-dependent dilatation reduced in KD patients, particularly in those with CALs.

A marker for chronic inflammation

We compared the hs-CRP values between the KD and control groups and the results showed no difference (Table 3, Fig. 1). However, we noted that some of the patients with CAL had high hs-CRP values; therefore, we compared the hs-CRP values of each of the three KD subgroups with those of the control group. The results revealed a significant increase in group A as compared to the control group (Table 4, Fig. 2).

Arterial stiffness

We compared the baPWV values of both the sexes between the KD and in the control groups. There were no differences in terms of ages in both sexes (male KD patients 26.9±4.2 years; male control subjects 25.7±4.3 years; female KD patients 27.1±4.4 years; male control subjects 25.2±3.6 years). The baPWV values were significantly increased in the male KD patients (KD group 1248±136 cm/sec; control group 1161±114 cm/sec, $p<0.05$) (Fig. 3). No significant difference in baPWV was observed in the female KD patients when compared with the female control subjects (KD group 1061±92 cm/sec; control group 1040±140 cm/sec). As for ABI, we did not find any differences between the two groups.

Discussion

This is the first report suggesting that endothelial dysfunction probably exists in adults with KD since the results that were obtained revealed increased TAT and reduced %FMD in adult KD patients. This residual endothelial dysfunction in the chronic stage of KD is supposed to be induced by the strong systemic inflammation during the acute stage of KD [6, 18]. Endothelium-dependent dilatation in the brachial artery was reduced in the KD patients both with and without CALs, but it was more evident in the patients with CALs in our study. This result might be consistent with the histological findings that revealed widespread vascular inflammation in both coronary and other medium-sized muscular arteries in acute KD, even in children, without echocardiographically detectable aneurysms [18, 22, 23].

We evaluated several markers for endothelial function, but endothelial damage was not evident in all the markers. Our results showed that in KD patients who were in their twenties and thirties, endothelium-dependent dilatation was

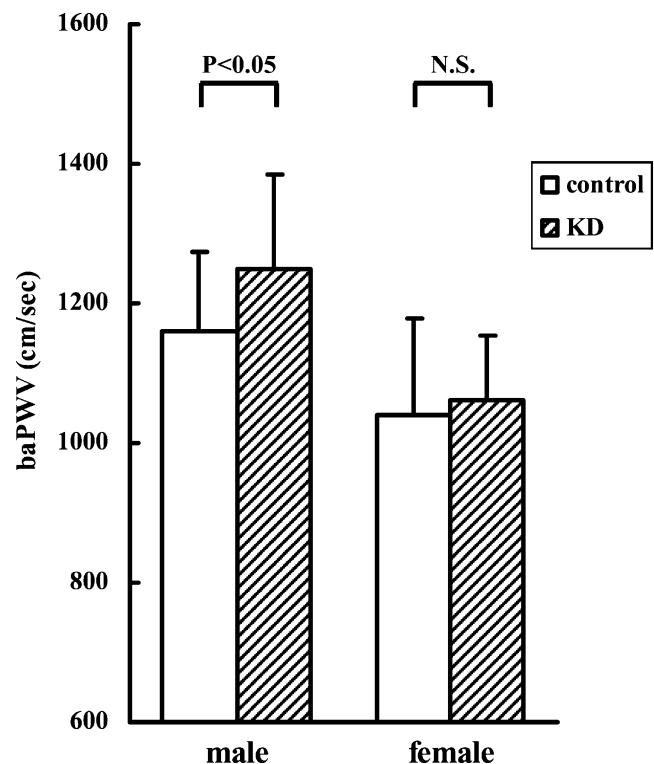


Fig. 3 Brachial-ankle pulse wave velocity in the KD and control groups for both sexes. While there were no differences in terms of ages in both sexes, the baPWV values were significantly higher in the male KD patients than those in the male control subjects

apparently reduced, but the levels of endothelial cell-specific molecules in the blood (adhesion molecules, vWV, etc.) were not found to be increased. We assumed that this was because endothelial dysfunction in patients in the chronic stage of KD might not be severe, and it may be revealed only when endothelial cells are under some load, for example, increased blood flow. Molecules such as adhesion molecules might be produced when endothelial cells are more activated and injured. Moreover, we could not find a decrease in the urinary NOx/Cre values in the KD group. This might be due to a large variation in urinary NOx/Cre values, which are strongly influenced by diet and exercise [13, 20]. Our results that endothelium-dependent dilatation is reduced even in patients late after the onset of KD suggest that further follow-up study is required to reveal whether this systemic endothelial dysfunction could lead to an early onset of atherosclerosis in the chronic stage of KD because impaired endothelial function might be the initial stage of atherosclerosis [21].

It is well known that inflammatory processes play an important role in the pathogenesis of atherosclerosis. In this study, the hs-CRP values in group A, that is, in the patients with CALs, were significantly higher than those in the control group, although there was no difference in the hs-CRP values between all the KD and the control groups.

Mitani et al. showed that hs-CRP levels were elevated in KD patients, particularly in those with CAL [15]. Similarly, Cheung et al. reported that MCP1, CCR2 and iNOS gene expression in THP-1 macrophages was induced by serum of children late after KD [3]. These interesting observations demonstrated that chronic low-grade inflammation could persist late after the onset of KD. We believe that inflammatory processes would be related to endothelial dysfunction not only in the vascular wall of coronary arteries but also in the vascular wall of systemic arteries in the chronic stage of KD.

We also measured baPWV to evaluate systemic atherosclerosis. These values were significantly increased in the male KD patients. It might suggest that systemic arteritis caused during the acute stage of KD could induce a change in the arterial wall structure, and render the age-related increase in arterial stiffness to be more evident in KD patients in the chronic stage.

These problems regarding why endothelial dysfunction exists even in the chronic stage of KD remain unsolved. With respect to this, we have previously shown that in weanling rabbits suffering from KD-like allergic coronary arteritis, smooth muscle cells that migrate into the intima in the acute stage of arteritis remained in the intima even in the chronic stage [14]. We also confirmed that by administering a hypercholesterol diet in the chronic stage of arteritis, atherosclerotic changes in coronary arteries were more evident in these allergic arteritis rabbit models than in the control rabbits [14]. Therefore, we assume that in KD patients, residual smooth muscle cells might exist in the subendothelium. Further, an interaction between smooth muscle cells and endothelial cells might be associated with endothelial dysfunction, which might be the cause of development of the atherosclerotic change.

Based on our results that endothelial dysfunction could exist in some of the adults in the chronic stage of KD, we should establish a better, effective treatment strategy for atherosclerosis late after the onset of KD. Based on the result that systemic vascular endothelial function was restored on vitamin C administration in KD patients in the chronic stage, Deng et al. reported that oxidative stress could be one of the major causes of endothelial dysfunction, and an antioxidant would be effective for endothelial dysfunction late after the onset of KD [4]. Additionally, 3-hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, or statins, are widely recognized as the anti-atherosclerotic agents, which act through both cholesterol-dependent and cholesterol-independent mechanisms [26]. One cholesterol-independent mechanism involves inhibiting reactive oxygen species to inactivate nitric oxide [19]. Therefore, we hope that statins will be effective for improving endothelial function in patients late after the onset of KD as well.

Study limitations

This study has several limitations. First, we were unable to resolve an important question regarding whether endothelial function is influenced by treatment in the acute stage of KD, particularly by intravenous gamma-globulin, because of the small number of patients. In this study, two patients received intravenous gamma-globulin, and no patients received steroids. All of the patients were treated with aspirin. For further study, we would like to clarify the effects of the treatment during the acute stage of KD on endothelial function in the chronic stage. Second, we did not eliminate the influence of estrogen, although there were no female subjects who were in menstrual phase. Further prospective trials are required to elucidate these problems.

In conclusion, this is the first study demonstrating that endothelial dysfunction exists in adult KD patients both with and without CALs. In further studies, we aim to clarify the causes or the mechanisms of endothelial dysfunction to investigate an important strategy for a long-term follow-up or treatment for the early onset of atherosclerosis in the chronic stage of KD.

References

1. Blake GJ, Ridker PM (2001) Novel clinical markers of vascular wall inflammation. *Circ Res* 89:763–771
2. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 340:1111–1115
3. Cheng YF, Karmin O, Tam SC, Siow YL (2005) Induction of MCP1, CCR2, and iNOS expression in THP-1 macrophages by serum of children late after Kawasaki disease. *Pediatr Res* 58:1306–1310
4. Deng YB, Li TL, Xiang HJ, Chang Q, Li CL (2003) Impaired endothelial function in the brachial artery after Kawasaki disease and the effects of intravenous administration of vitamin C. *Pediatr Infect Dis J* 22:34–39
5. Dhillon R, Clarkson P, Donald AE, Powe AJ, Nash M, Novelli V, Dillon MJ, Deanfield JE (1996) Endothelial dysfunction late after Kawasaki disease. *Circulation* 94:2103–2106
6. Fujiwara H, Hamashima Y (1978) Pathology of the heart in Kawasaki disease. *Pediatrics* 61:100–107
7. Hamaoka K, Onouchi Z, Kamiya Y, Sakata K (1998) Evaluation of coronary flow velocity dynamics and flow reserve in patients with Kawasaki disease by means of a Doppler guide wire. *J Am Coll Cardiol* 31:833–840
8. Iemura M, Ishii M, Sugimura T, Akagi T, Kato H (2000) Long term consequence of regressed coronary aneurysms after Kawasaki disease: vascular wall morphology and function. *Heart* 83:307–311
9. Ikemoto Y, Ogino H, Teraguchi M, Kobayashi Y (2005) Evaluation of preclinical atherosclerosis by flow-mediated dilatation of the brachial artery and carotid artery analysis in patients with a history of Kawasaki disease. *Pediatr Cardiol* 26:782–786
10. Ishii M, Ueno T, Ikeda H, Iemura M, Sugimura T, Furui J, Sugahara Y, Muta H, Akagi T, Nomura Y, Homma T, Yokoi H,

- Nobuyoshi M, Matsuishi T, Kato H (2002) Sequential follow-up results of catheter intervention for coronary artery lesions after Kawasaki disease: quantitative coronary artery angiography and intravascular ultrasound imaging study. *Circulation* 105:3004–3010
11. Kadono T, Sugiyama H, Hoshiai M, Osada M, Tan T, Naitoh A, Watanabe M, Koizumi K, Nakazawa S (2005) Endothelial function evaluated by flow-mediated dilatation in pediatric vascular disease. *Pediatr Cardiol* 26:385–390
 12. Kato H, Ichinose E, Kawasaki T (1986) Myocardial infarction in Kawasaki disease: clinical analyses in 195 cases. *J Pediatr* 108:923–927
 13. Kim SS, Kim H, Lee SJ, Chang HK, Shin ML, Jang MH, Shin MS, Kim CJ (2003) Treadmill exercise suppresses food-deprivation-induced increase of nitric oxide synthase expression in rat paraventricular nucleus. *Neurosci Lett* 353:41–44
 14. Liu Y, Onouchi Z, Sakata K, Ikuta K (1996) An experimental study on the role of smooth muscle cells in the pathogenesis of atherosclerosis of the coronary arteritis. *J Japan Pediatr Soc* 100:1453–1458
 15. Mitani Y, Sawada H, Hayakawa H, Aoki K, Ohashi H, Matsumura M, Kuroe K, Shimpo H, Nakano M, Komada Y (2005) Elevated levels of high-sensitivity C-reactive protein and serum amyloid-A late after Kawasaki disease: association between inflammation and late coronary sequelae in Kawasaki disease. *Circulation* 111:38–43
 16. Morcos E, Wiklund NP (2004) Nitrite and nitrate measurements in human urine by capillary electrophoresis. *Methods Mol Biol* 479:21–34
 17. Munakata M, Ito N, Nunokawa T, Yoshinaga K (2003) Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. *AJH* 16:653–657
 18. Naoe S, Shibuya K, Takahashi K, Wakayama M, Masuda H, Tanaka N (1991) Pathological observations concerning the cardiovascular lesions in Kawasaki disease. *Cardiol Young* 1:212–220
 19. O'Driscoll G, Green D, Taylor RR (1997) Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. *Circulation* 95:1126–1131
 20. Roberts CK, Chen AK, Barnard RJ (2007) Effect of a short-term diet and exercise intervention in youth on atherosclerotic risk factors. *Atherosclerosis* 191(1):98–106
 21. Ross R (1999) Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126
 22. Suzuki A, Miyagawa-Tomita S, Komatsu K, Nishikawa T, Sakomura Y, Horie T, Nakazawa M (2000) Active remodeling of the coronary arterial lesions in the late phase of Kawasaki disease. *Circulation* 101:2935–2941
 23. Takahashi K, Oharaseki T, Naoe S (2001) Pathological study of postcoronary arteritis in adolescents and young adults: with reference to the relationship between sequelae of Kawasaki disease and atherosclerosis. *Pediatr Cardiol* 22:138–142
 24. Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N, Hinohara S (2003) Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement—a survey of 12517 subjects. *Atherosclerosis* 30:303–309
 25. Vallance P, Chan N (2001) Endothelial function and nitric oxide: clinical relevance. *Heart* 85:342–350
 26. Yoshida M (2003) Potential role of statins in inflammation and atherosclerosis. *J Atheroscler Thromb* 10:140–144