ORIGINAL ARTICLE

Does Vitamin C improve endothelial function in patients with Kawasaki disease?

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Background: Improvement of endothelial dysfunction could prevent or delay the occurrence of the atherosclerosis process in patients with Kawasaki disease (KD). It is suggested that Vitamin C could improve endothelial dysfunction. In this study, we investigated whether administration of Vitamin C as a water-soluble antioxidant could be effective in this regard among patients with KD. **Materials and Methods:** In this case control analytic-experimental study, children aged 3-18 years with KD, and a group of healthy children evaluated. Vitamin C (250 mg/daily) administrated for the two studied groups for 1 month. Endothelial function evaluated by flow-mediated dilatation (FMD) and intima-media thickness (IMT) measurement using vascular Doppler ultrasonography, before and after trial. **Results:** In this study, 16 patients with KD and 19 normal children were studied. At baseline mean of IMT and FMD was not different in the two studied groups ($P > 0.05$). After Vitamin C administration IMT decreased significantly in two studied groups (from 27.06 ± 6.27 to 21.56 ± 3.77 in KD group and from 27.66 ± 5.66 to 23.33 ± 3.66 in control group [$P < 0.05$]). FMD increased in two studied groups, but the difference was significant in the control group (from 6.84 ± 2.51 to 7.03 ± 2.87 in KD group and from 6.53 ± 2.36 to 7.82 ± 2.14 in the control group). **Conclusion:** Vitamin C might improve the endothelial function of patients with KD.

Key words: Endothelium, Kawasaki disease, Vitamin C

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INTRODUCTION

Kawasaki disease (KD) is a systemic acute self-limited vasculitis with predominant involvement of coronary arteries.[1,2] Coronary artery involvement in acute and long term follow-up is represented by coronary artery aneurysms and stenosis.[3,4]

Studies revealed that KD patients with or without coronary complications may be at increased risk for premature atherosclerosis.[5-7] Though the underlying mechanisms of accelerated atherosclerosis was not clearly determined, but it is suggested that arterial dysfunction, the consequence of endothelial dysfunction and stiffening of both the coronary and systemic arteries, a state of chronic inflammation and alterations in the lipid profile are the most important risk factors.[8-10]

Evidences indicated that the endothelial dysfunction, the key event in the pathogenesis of premature atherosclerosis, developed in post-KD patients regardless of the degree of coronary artery involvement.[11,12] In addition, increased carotid intima-media thickness (IMT) and diminished flow-mediated dilatation (FMD) are surrogate parameters of atherosclerosis and endothelial dysfunction which have been shown to correlate with late cardiovascular events of patients with KD.[13,14]

Endothelial dysfunction is characterized by vasodilatation impairment. Experimental studies showed that the impairment depends on the ability of the endothelium to release nitric oxide (NO) in response to shear stress. NO release reduced both in endothelial dysfunction and atherosclerosis.[15,16] It is suggested that reactive oxygen species has a crucial role in this process by reducing the bioavailability of NO.[17]

Some studies reported that regarding the described mechanism, Vitamin C administration could improve endothelial dysfunction. Its effectiveness has been reported in patients with coronary artery disease, diabetes, hypertension as well as KD.[18-21]

So improvement of endothelial dysfunction could prevent or delay the occurrence of the atherosclerosis process in patients with KD. In this study, we investigated whether administration of oral Vitamin C a water-soluble antioxidant could be effective in this field among patients with KD.

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MATERIALS AND METHODS

In this case control analytic-experimental study children aged 3-18 years with a diagnosis of KD, and a group of healthy children referred to Emam Hossein children hospital, the only pediatric referral center in Isfahan, affiliated to Isfahan University of Medical Sciences were evaluated. Patients with KD were selected by non-randomized convenience method from patients with mentioned disease who were referred to the cardiology clinic of the hospital for follow up. KD diagnosis was based on standard criteria. Children in the control group were selected from outpatients without appreciable cardiovascular risk factors who were referred for routine annual check-up or from healthy brothers and sisters of selected patients.

All subjects were nonsmokers, non-pregnant and without any history of systematic disease.

The protocol of the study was approved by Regional Bioethics Committee of Isfahan University of Medical Sciences. Written informed consent was obtained from all selected patients or their parents.

Basal characteristics of the studied patients were recorded from their medical files. Selected patients recalled; a pediatric cardiologist examined their clinically. Endothelial function of studied subjects during the first visit was evaluated by FMD and IMT measurement using vascular Doppler ultrasonography (Eko 7 machine by Samsung Medison Company and by a 7 MHz vascular transducer).

Vitamin C (Osvah Pharmaceutical Company, Tehran, Iran) was administrated orally to all studied groups with a dose of 250 mg, daily[22] for one month. After that period studied subjects underwent vascular Doppler ultrasonography for the second FMD and IMT measurement. Mean of studied endothelial parameters before and after Vitamin C administration was compared in each studied groups.

Endothelial function measurement

Weight, height and blood pressure of studied children was measured before the procedure. The subjects were recommended to do not exercise or use caffeine, acid folic, nitrate, high fat diet, for at least 24 h before the procedure and do not take Vitamin C (as a supplement).

The procedure was performed in the morning after 8 h fasting, in a temperature-controlled room at 25°C in a supine position.

All subjects were examined by the same physician, who was blinded to their clinical conditions at the time of examination.

Flow-mediated dilatation measurement

The measurement was assessed based on the guidelines of the International Brachial Artery Reactivity Task Force. Studied subjects were examined in the supine position with their forearm placed in a semi open splint. The high-frequency (7 MHz) vascular transducer (EKO 7 by Samsung Medison Company) was fixed with a stereotactic probe-holding device. In order to made a flow stimulus by reactive hyperemia a pediatric BP cuff was fixed on the wrist of the subjects and radial artery was imaged in a longitudinal plane 5 cm distal from the antecubital fossa. After performing a baseline rest image, the blood flow velocity was estimated by time averaging the Doppler signal from a mid-artery sample volume. Cuff deflation was followed by a brief high-flow state after a 5 min interval of ischemia. After cuff deflation, the image of the radial artery and the Doppler signal recorded alternatively for 5 min with 20 s intervals. After the procedure, obtained images saved on the EKO 7 hard disk and analyzed. Distance measurements of radial artery were made at maximum systolic extension. FMD analyzed by a pediatric cardiologist.[22]

Intima-media thickness measurement

Carotid arteries imaged using a high-frequency (7 MHz) vascular linear transducer. Subjects were in the supine position with the head turned 45° away from the scanner. Two segments including the distal 1 cm of the common carotid artery and its bifurcation were evaluated on each side. Measurements of the two segments were performed at 2-mm intervals at near and far wall, and maximum and mean of IMT were calculated for them. Sonography and reading were assessed by a pediatric cardiologist.[23]

Statistical analysis

Obtained data analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, U.S.A.) software. Normality of studied variables was evaluated using Kolmogorov–Smirnov test. Differences in demographic characteristics and mean ± standard deviation (SD) of endothelial function parameters between and within groups was determined using independent samples *t*-test and paired *t*-test respectively. $P < 0.05$ considered significant.

RESULTS

In this study, 16 patients with KD and 19 normal children (control group) were studied. Demographic characteristics and mean ± SD of endothelial function parameters of studied population in KD and control groups are presented in Table 1. Mean duration of KD was 5.8 ± 3.7 years.

Echocardiographic findings and endothelial function markers and blood pressure of patients in the two studied groups before and after intervention are presented in Table 2.

Mean differences (after intervention-before intervention) of IMT2-IMT1, FMD2-FMD1 and LVmass2-LV mass 1 were not significantly different in the two studied groups and among male and female patients ($P > 0.05$).

Coronary artery involvement was detected in 5/16 (31.2%) of patients with KD. Mean differences of IMT2-IMT1, FMD2-FMD1, LVmass2-LVmass1 and blood pressure were not significantly different in patients with and without coronary involvement ($P > 0.05$).

In 8 (50%) of patients duration of KD was <5 years and in reminders (50%) duration of the disease was more than 5 years. Mean differences of IMT2-IMT1, FMD2-FMD1, LVmass2-LVmass1 and blood pressure, were not significantly different in patients with duration of KD of less or more than 5 years and control group ($P > 0.05$).

DISCUSSION

In this study, the effectiveness of Vitamin C administration on improvement of endothelial function was evaluated by noninvasive method of FMD and IMT measurement in patients with KD. The findings of the current study indicated that Vitamin C had a significant effect on improving IMT of both control group and patients with KD. FMD increased in two studied groups, but the difference was significant in the control group.

Kawasaki disease has an important role in developing systemic inflammation and endothelial dysfunction and consequently the risks of cardiovascular events are higher in this group of patients.[24,25] Accordingly KD could lead to functional sequelae in coronary arteries in long term period even in those patients without obvious coronary artery involvement during the acute phase of the disease.[26]

Though several studies indicated the presence of endothelial dysfunction in patients with KD regardless of coronary artery involvement but there were studies which reported no significant difference in endothelial function of KD patients and control group.[21,27-30]

In this study, there was not significant difference in this field. Our results were in line with Borzutzky *et al*. study in Chile, which indicated that endothelial function was not significantly different between KD patients and control group. They suggested that the main cause could be the duration of KD.[31] Accordingly during the first years of KD, the patients have low grade inflammation. In our study, the duration of KD in studied population was 5.8 ± 3.7 years, and there was no statistical differences in the studied parameters in those patients with less or more than 5 years duration of KD.

There was only one similar study in the literature review in this field. Deng *et al*. have evaluated weather acute administration of Vitamin C could improve endothelial dysfunction using the percent change in diameter of the brachial artery induced by reactive hyperemia. They showed that a systemic endothelial dysfunction develops after KD even after early treatment with high-dose gamma globulin but it could be restored after acute use of Vitamin C. They also indicated that Vitamin C administration could improve FMD in this group of patients. They suggested that the

Table 1: Demographic characteristics and endothelial function parameters of studied patients with KD and control group at baseline

Variables	KD group	Control group	*P*
Gender			
Female/male	9/7	9/10	0.6
Age (years)	12.12±4.99	12.63±4.5	0.51
BMI (kg/m^2)	21.12±5.43	20.55±4.95	0.08
IMT	27.06±6.27	27.66±5.66	0.80
FMD	6.84±2.51	6.53±2.36	0.83
LV mass	60.48±20.87	48.50±16.66	0.53
EF	63.06±3.99	64.44±6.37	0.58
SF	33.68±3.73	34.33±4.85	0.72
Systolic blood pressure (mmHg)	99.06±18.36	97.05±18.46	0.95
Diastolic blood pressure (mmHg)	55.00±9.48	55.58±11.02	0.81

BMI = Body mass index; KD = kawasaki disease; IMT = intima-media thickness; FMD = Flow-mediated dilatation; LV = Left ventricle; EF = ejection fraction; SF = shortening fraction

Table 2: Echocardiographic and BP findings of patients with KD and control group before and after intervention

Echocardiographic and BP variables	KD group*		*P*	Control group		*P*
	Before intervention	After intervention		Before intervention	After intervention	
EF	63.06±3.99	62.06±6.76	0.630	64.44±6.37	64.50±5.68	0.980
SF	33.68±3.73	32.93±4.44	0.647	34.33±4.85	33.83±4.70	0.768
IMT	27.06±6.27	21.56±3.77	0.004	27.66±5.66	23.33±3.66	0.001
FMD	6.84±2.51	7.03±2.87	0.788	6.53±2.36	7.82±2.14	0.021
LV mass	60.48±20.87	61.41±20.97	0.751	48.50±16.66	43.92±7.50	0.658
Systolic blood pressure (mmHg)	99.06±18.36	99.37±14.24	0.915	97.05±18.46	99.70±13.63	0.198
Diastolic blood pressure (mmHg)	55.00±9.48	55.31±7.40	0.892	55.58±11.02	60.00±9.84	0.074

**P* value after intervention between KD and control groups were >0.05 for all variables; EF = ejection fraction; SF = shortening fraction; IMT = intima-media thickness; FMD = flow-mediated dilatation; LV = left ventricle; BP = Blood pressure; KD = kawasaki disease

underlying mechanism was endothelin-1 which could increase superoxide anion production. Vitamin C is a potent water soluble antioxidant and effective scavenger of free radicals, such as the superoxide anion which could improve endothelial dysfunction by increasing availability of NO.[32]

In this study effectiveness of Vitamin C on endothelial function of patients with or without coronary involvement was not different significantly. As reported by McCrindle *et al*. in Canada and others, the degree of coronary artery involvement have no association with systemic endothelial function.[29,30]

The limitations of the current study were small sample size of patients, short duration of disease in studied patients with KD and we did not measure the metabolic and inflammatory factors such as lipid profile or CRP.

In summary, Vitamin C might improve the endothelial function in patients with KD, but further studies with larger sample size and consideration of mentioned limitation is recommended.

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AUTHOR'S CONTRIBUTIONS

All authors have contributed in designing and conducting the study. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

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