Original Article

Effect of vitamin C on endothelial function of children with chronic renal failure: An experimental study

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Abstract

Background: It is well established that improvement of endothelial dysfunction (ED) could prevent or delay the occurrence of cardiovascular disease (CVD) and its related morbidity and mortality in patients with chronic kidney disease (CKD). In this study we investigated whether administration of vitamin C could be effective by improving brachial artery flow-mediated dilation (FMD) and intima media thickness (IMT), two surrogate markers of ED, in children with CKD or chronic renal failure (CRF).

Materials and Methods: In this analytic-experimental study children aged 3-18 years with a diagnosis of CRF and a group of healthy children were enrolled. Vitamin C (250 mg/day) administrated for the two studied groups for 1 month. Endothelial function was evaluated by FMD and IMT measurement using vascular Doppler ultrasonography, before and after trial.

Results: In this study 18 patients with CRF and 19 normal children as the control group 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 the two studied groups ($P < 0.05$). FMD increased in the two studied groups but the difference was significant in the control group ($P < 0.05$).

Conclusion: The findings of this interventional trial have demonstrated that vitamin C could have protective effect on ED of patients with CRF possibly in those with severe form of the disease but for obtaining more conclusive results larger sample size is needed.

Key Words: Chronic renal failure, endothelium, flow-mediated dilation, vitamin C

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INTRODUCTION

Chronic kidney disease (CKD) in children is an important medical problem which is associated with high rate of complications including impaired growth and development and quality of life as well as chronic renal failure (CRF) and is associated with increased risk of cardiovascular disease (CVD).[1]

Prevalence of CKD in children has been reported to be approximately 18.5-58.3 per million children. Though the rate is much lower than an adult population but it is suggested that due to under reporting, the rate is higher in a pediatrics population.[2,3]

Increased risk of CVD is the major health consequences of CRF or CKD in children.[4,5] Evidences indicated that

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CVD-related mortality rates are significantly higher in younger adults with history of childhood CKD. Moreover, previous studies reported that the presence of markers of subclinical CVD including concentric left ventricular hypertrophy and increased intima media thickness in children with CRF are independent predictors of CVD morbidity and mortality.[6-9]

The pathogenesis of CVD in CRF has a multi-factorial basis but evidences indicated that endothelial dysfunction (ED) have a crucial role in this field. Potential mechanisms responsible for ED in CKD are oxidative stress and L-Arginine deficiency.[10-12]

Considering the underlying mechanisms of ED in CKD and CRF some trials especially with antioxidants supplements such as vitamin C have been conducted.[13] Accordingly, vitamin C could improve ED in this group of patients but the results were not conclusive enough.[14,15]

However, it is well established that improvement of endothelial dysfunction could prevent or delay the occurrence of CVD and its related morbidity and mortality in patients with CRF. In this study we investigated whether administration of vitamin C could be effective at improving brachial artery flow-mediated dilation (FMD) and intima media thickness (IMT), two surrogate markers of ED, in children with CKD or CRF.

MATERIALS AND METHODS

In this analytic-experimental study a group of children aged 3-18 years with a diagnosis of CRF, and a group of matched healthy children who were referred to Emam Hossein children hospital, the only pediatric referral center in Isfahan, affiliated to Isfahan University of Medical Sciences were enrolled.

Patients with CRF were selected by the non-randomized convenience method. Children in the control group were selected from outpatients without appreciable cardiovascular risk factors who were referred for routine annual checkup or from healthy brothers and sisters of selected patients.

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

This work complies with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the 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 them 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 for all studied groups with a dose of 250 mg, daily for 1 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 the studied children was measured before procedure. The subjects were recommended to do not exercise or use caffeine, acid folic, nitrate, high fat diet, for at least 24 hours before the procedure and do not take vitamin C (as supplement).

The procedure was performed in the morning after 8 hours of 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.

FMD 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 make 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-minute interval of ischemia. After cuff deflation, the image of the radial artery and the Doppler signal recorded alternatively for 5 minutes with 20-second 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 was analyzed by a pediatric cardiologist.[16]

IMT measurement

Carotid arteries were 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.[17]

Statistical analysis

Obtained data analyzed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) software and paired t-test and ANOVA tests.

RESULTS

In this study 18 patients with CRF and 19 normal children as the control group were studied. Demographics characteristics and mean ± SD of endothelial function parameters of studied population are presented in Table 1.

Table 1: Demographic characteristics and mean ± SD of endothelial function parameters of studied patients with chronic renal failure and the control group

Variables	Patients with CRF No = 18	Control group No = 19	*P* value
Gender (female/male)	12/6	9/10	0.19
Age (years)	13.00±5.25	12.63±4.5	0.82
BMI (kg/m^2)	18.02±4.62	20.55±4.95	0.12
IMT	31.6±6.43	27.66 ±5.66	0.06
FMD	5.51±2.69	6.53±2.36	0.30
LV mass	127.59±117.21	48.50±6.66	0.21
Systolic blood pressure (mmHg)	118.70±22.67	97.05±18.46	0.01
Diastolic blood pressure (mmHg)	73.52±18.35	55.58±11.02	0.002

The patients with CRF were on hemodialaysis, peritoneal dialysis, and pharmacological treatment and had renal transplantation in 9, 4, 3 and 2 of patients. The mean duration of CRF was 7.0 ± 4.6 years. Arterioventricular (AV) fistula was implanted in 10/17 (58.8%) of patients with CRF.

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

Echocardiographic findings and endothelial function markers, blood pressure and mean differences (after intervention-before intervention) of IMT2-IMT1, FMD2-FMD1 and left ventricular (LV) mass 2-LV mass 1 were not significantly different among male and female in two studied groups ($P > 0.05$).

Comparing patients with and without AV fistula, IMT1 (34.6 ± 6.4 vs. 26.6 ± 3.30, $P = 0.009$), diastolic blood pressure at baseline (82.0 ± 19.9 vs. 61.4 ± 3.4, $P = 0.01$) and IMT2-IMT1 (9.2 ± 5.6 vs. 2.2 ± 2.6, $P = 0.013$) were significantly higher in CRF with AV fistula than those without fistula. FMD1 was lower in CRF with AV fistula than those without fistula (4.7 ± 2.4 vs. 7.3 ± 3.1, $P = 0.07$).

IMT1 was significantly higher in hemodialaysis patients ($P = 0.003$) and FMD was significantly higher in patients with renal transplantation ($P = 0.001$) than those who were on peritoneal dialysis and pharmacological treatment. Mean differences of IMT2-IMT1 and FMD2-FMD1 was significantly higher in patients with hemodialaysis ($P = 0.006$ for IMT and $P = 0.017$for FMD).

Duration of CRF had no significant effect on echocardiographic findings and endothelial function parameters, blood pressure and mean differences (after intervention-before intervention) of IMT2-IMT1, FMD2-FMD1 and LVmass2-LVmass1.

Mean differences of IMT2-IMT1, FMD2-FMD1, LV mass 2-LV mass 1 and blood pressure were not

Table 2: Echocardiographic findings of patients with CRF before and after intervention

Echocardiographic variables	CRF group			Control group		
	Before intervention	After intervention	*P* value	Before intervention	After intervention	*P* value
EF	63.6±9.47	60.21±9.59	0.187	64.44±6.37	64.50± 5.68	0.980
SF	34.14±6.29	31.5±6.30	0.152	34.33±4.85	33.83±4.70	0.768
IMT	31.6±6.43	25.29±3.29	0.000	27.66±5.66	23.33±3.66	0.001
FMD	5.51±2.69	5.85±2.07	0.460	6.53±2.36	7.82±2.14	0.021
LV mass	127.59±117.21	100.44±66.85	0.316	48.50±16.66	43.92±7.50	0.658
Systolic blood pressure (mmHg)	118.70±22.67	114.41±15.60	0.302	97.05±18.46	99.70±13.63	0.198
Diastolic blood pressure (mmHg)	73.52±18.35	69.11±16.32	0.144	55.58±11.02	60.0±9.84	0.074

EF: Ejection fraction; SF: Shortening fraction

significantly different in patients with CRF with Hb < 10 and Hb ≥ 10 ($P > 0.05$).

DISCUSSION

In this study we evaluated the effect of vitamin C administration on markers of endothelial function of children with CRF. Our findings indicated that vitamin C have significantly proper effect on reducing IMT in both patients and control group. FMD increased in two studied groups but it was only significantly different in the control group.

Several studies reported ED among patients with CRF. Hussein *et al*. in Egypt and Civilibal *et al*. in Turkey indicated that pediatric patients with CRF have impaired endothelial function and are at higher risk of CVD.[18,19]

Ece *et al*. in Turkey investigated markers of oxidative stress, inflammation and early cardiovascular damage in 29 children with CRF. Their results showed that studied population had increased LVM and IMT values. They concluded that children with CRF have increased oxidative stress and inflammation and early cardiovascular abnormalities.[20]

In this study patients with CRF had lower FMD and higher IMT than control group. Though it was not statistically significant, it may be due to small sample size but the differences especially for IMT had trend to be significant.

On the other hand, there are evidences which indicate that renal dysfunction is associated with a decreased level of plasma vitamin C. Takahashi and colleagues in Japan have reported that children with CKD have lower level of vitamin C which could lead to ED by increasing oxidative stress.[21] Deicher *et al*. demonstrated that decreased vitamin C level in hemodialaysis patients is associated with CVD.[22]

There are evidences which support the effectiveness of vitamin C administration on improving ED and decrease the formation of atherosclerotic lesions. Though the pathophysiological role of vitamin C in this field and in this group of patients was not completely determined, but it is suggested that vitamin C could improve endothelial function through two possible mechanisms. First as an antioxidant agent, it could increase nitrous oxide (NO) bioavailability and reduce oxidative stress. It could restore cellular uptake of L-arginine and consequently improve endothelial-dependent relaxation.[23,24]

In this study we evaluated the effect of vitamin C on ED of children with CRF using IMT and FMD measurement. IMT and FMD measurements are reliable, reproducible and noninvasive technique to assess endothelial function as well as detecting and monitoring progression of atherosclerosis.[25]

There were few studies on the effectiveness of vitamin C in patients with CRF and there was not similar study in pediatrics population.

Cross *et al*. in the UK have reported that acute intra-arterial use of vitamin C increased endothelial-dependent dilation in the resistance vasculature of predialysis and hemodialaysis renal failure patients.[26] Dupont *et al*. in the USA showed that cutaneous vasodilation impairment, which is found in stage 3-4 of CKD, normalized by local infusion of ascorbic acid.[27]

In our study, IMT decreased significantly in children with CRF after vitamin C use and FMD increased but it was not statistically significant. It may be due to small sample size or shorter duration of vitamin C administration.

ED was more significant in CRF patients with AV fistula. In addition, IMT changes were more significant among CRF patients with AV fistula. The findings could be justified that the effect of vitamin C administration would be more appropriate in CRF patients with higher grade of CRF. Significant changes of IMT and FMD among hemodialysis patients than others also could be a confirmatory explanation for the above-mentioned justification.

The limitation of current study was small sample size specially for evaluating different subgroups of CRF patients i.e. hypertensive, with and without fistula, anemic and non-anemic and on different treatment strategies. Another limitation is that we did not measured baseline vitamin C in the studied population.

The findings of this interventional trial have demonstrated that vitamin C could have protective effect on ED of patients with CRF possibly in those with severe form of the disease but for obtaining more conclusive results larger sample size is needed. It is recommended to study the effectiveness of vitamin C on different markers of renal failure and their relationship with endothelial markers.

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