ORIGINAL ARTICLE

Effect of long-term Vitamin C intake on vascular endothelial function in diabetic children and adolescents: A pilot study

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Background: This study attempted to determine the effects of long-term use of Vitamin C on vascular endothelial function. **Materials and Methods:** During a pilot clinical trial study conducted at Imam Hussein Hospital (Isfahan) in 2014–2015, a total of forty diabetic patients were selected and then assigned randomly into two twenty-subject groups receiving Vitamin C and placebo tablets. The patients were treated with Vitamin C or placebo for 6 months. All patients were examined through echocardiography in terms of cardiac function before and after treatment. To evaluate the endothelial function (flow-mediated dilatation [FMD], intima-media thickness), they underwent arterial Doppler. Moreover, the chemical indices of vascular function were tested through intercellular adhesion molecule and vascular cell adhesion molecule (VCAM). Finally, the results were compared between the two groups. **Results:** Based on the results, the mean left ventricular mass significantly reduced after the intervention in the group treated with Vitamin C (from 76.35 ± 25.6–68.62 ± 22.66; $P = 0.015$) while there was no significant difference observed in the control group (from 67.58 ± 25.38–71.63 ± 26.84; $P = 0.19$) but no statistically difference between the two groups-based repeated measures ANOVA test ($P = 0.6$). In addition, the mean of VCAM changes was significantly difference between the two groups ($P < 0.001$). **Conclusion:** Long-term use of Vitamin C in diabetic patients can improve certain echocardiographic parameters such as ejection fraction, fractional shortening, and FMD, which in turn enhances vascular endothelial function.

Key words: Adolescents, children, diabetes, endothelial function, Vitamin C

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INTRODUCTION

Over the last two decades, it has been proven that endothelium plays an essential role in homeostasis.[1] In addition to atherosclerosis, endothelial dysfunction has been observed in various pathological conditions such as hypercholesterolemia, diabetes, hypertension, heart failure, smoking, and aging.[1] Under basic conditions, endothelial functions are supposed to sustain a relatively dilated vascular system. Although endothelial capacity responds to physical stimuli such as shear stress, blood vessels are dilated in response to stress.

Endothelial dysfunction induced by dyslipidemia is the initial step in atherosclerosis.[1,2] Among patients with normal coronary arteries or minimal coronary artery disease, positive family history of coronary artery disease can be a significant predictor of the risk of a coronary artery low blood flow, reflecting any potential coronary endothelial dysfunction in the microcirculation.[3]

Brachial artery ultrasound imaging during reactive hyperemia provides a tool for vascular tone measurement associated with endothelium and may verify vascular endothelial dysfunction.[4] Measurement of vascular tone impairment (endothelial dysfunction) plays an important role as a screening tool. Vasodilatation through blood flow is technically called flow-mediated dilatation (FMD). FMD measurement is a clinical method to assess endothelial function. This

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endothelium-dependent response is essentially regulated by the release of nitric oxide from the endothelium.[1]

In patients with hypercholesterolemia, Vitamin C, folate, 5-methyl tetra-dihydrofolate (active form of folic acid) can improve endothelial function without changes in plasma lipid levels.[4] Vitamin C can also reverse the microcirculatory coronary response, thus delaying coronary flow damage in smokers with normal coronary arteries.[5] Vitamin C and folate may reduce oxidative stress and prevent the destruction of nitric oxide.

Vitamin C is a water-soluble vitamin and is a nutritional supplement essential for collagen formation and tissue repair. Moreover, it engages in a number of other metabolic reactions such as reductase reactions.[6]

Vitamin C is a potent endogenous antioxidant, regulating the intracellular reductase through glutathione, capable of destroying oxygen-derived free radicals.[7] Vitamin C can reverse damages caused by smoking, including oxidative stress and increased monocyte adhesion to endothelium.[8] Vitamin C may affect coronary artery disease through curtailing lesion activity.[9] Observational studies of Vitamin C have yielded mixed results in the prevention of coronary heart disease (CHD). A few have demonstrated the beneficial effects of Vitamin C.[10] Others identified small benefits.[11-13] However, there has been no consensus about the effects of Vitamin C on improving endothelial function, since the previous studies have been limited. Considering the high prevalence of cardiovascular diseases in diabetes patients and the role of endothelial dysfunction in this process, this study intended to determine the long-term administration of Vitamin C on vascular endothelial function in children and adolescents with diabetes.

MATERIALS AND METHODS

The current study was a pilot randomized, double-blind study carried out at Imam Hussein Hospital in Isfahan during 2014–2015.

Inclusion criteria were age range 5–18 years, diagnosed with diabetes, at least 5 years of diabetes, absence of any other systemic diseases, no intake of Vitamin C in the 3 months before the start of the study, no smoking, no pregnancy, normal diet regimen (no special diet such vegetarian habit), and no suffering to dietary diseases.[6]

The exclusion criteria were nonintake of Vitamin C or placebo during the study for any reason, failure to attend upcoming sessions, and development of any new disease during the study.

The smallest sample size was estimated to be twenty patients using the sample size formula taking into account the 95% confidence level, 80% test power, and 10% standard deviation of FMD estimated to be 0.9. The minimum significant difference between the two treatment procedures was 0.8. Moreover, the samples were selected through a convenient procedure.

After approval of the proposal and obtaining permission from the University Ethics Committee, forty patients fulfilling the inclusion criteria were visited in Imam Hussein Hospital where they were diagnosed with diabetes. Having spoken with patients and their parents and having obtained their written consent to participate in the study, the patients were distributed into two twenty-member groups through randomized block design. In fact, the first patient was randomly by lot placed in one of the groups supplemented with Vitamin C or placebo while the patients were in order of inclusion distributed consecutively into the two groups until the sample size was realized.

The patients were blinded since they were unaware of the type of drug or placebo administered. Moreover, the drug or placebo was similarly supplied by an identical pharmaceutical company and then coded by a third person unaware of the study details while the project researcher was unaware of the drug specifications.

At baseline, demographic data and patient records were gathered together with heights and weights recorded in the data collection form. Before intervention, all patients were examined through echocardiography in terms of cardiac function. The endothelial function was evaluated through arterial Doppler. FMD, intima-media thickness (IMT), and chemical indices of vascular function were tested through intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAM). Before the intervention, the level of hemoglobin A1C was measured to determine glycemic control, where 7 and lower values were considered desirable blood glucose control and 7 and above values were considered undesirable blood glucose control.

Patients in intervention group were treated with 250 mg of single daily dose of oral Vitamin C. Patients in the control group received a placebo with the same shape and size of Vitamin C tablets and with the same duration.[11] At the end of the 6th-month period, the tests were repeated for all patients.

Given that, many factors affect the FMD and IMT (such as medicines, food, sympathetic stimulation, etc.) patients were fast for at least 6 h before doing FMD. Moreover, the test took place in a quiet environment at a constant temperature of 25°C.[3] In addition, all cardiovascular-effective drugs such

as nitrates were cutoff for at least four half-life. Moreover, 24 h before the FMD test, prohibition was imposed on any exercise and consumption of foods containing caffeine and similar substances and high-fat foods and Vitamin K (either natural or supplementary) and folic acid. For women in the first 7 days of menstrual cycle, FMD test was not performed to reduce the impact of hormones on the test.[3]

Patients underwent full echocardiogram to evaluate cardiac chambers, systolic and diastolic functions, valvular dysfunction, and hypertrophy.

The thickness of the carotid artery intima and media was measured through two-dimensional high-resolution ultrasound images by a 7–12 MHz transducer of EKO 7 machine by Samsung Medison Company. The patients rested in the supine position for 10 min at room temperature 24–26°C. The right carotid artery was scanned while the patient looked to the left. An artery length where intima can be clearly seen was used to measure the thickness of intima. That was repeated three times until the average thickness value was achieved for thickness of carotid intima and media.

The right-hand brachial artery located in the extension position was scanned in longitudinal cross-section of approximately 2–5 cm above right elbow hole. Transducer was placed at an angle of 70–80° to the body. Settings were made for optimum lumen artery wall. Electrocardiogram (ECG) monitor was attached to the patient along with ultrasound machine. The size of the lumen was recorded at the end of diastole coincident with R wave in ECG. All images were stored in the hard drive of the echo machine. A pneumatic tourniquet was inflated on the forearm distal to the scanning area, 50 mmHg over the systolic blood pressure of the patient for 5 min. An increase in blood flow was observed by sudden emptying of cuff while the measurements from the anterior to posterior in the midline at the end of diastole were calculated again. The lumen diameter was scanned 60 s after cuff was deflated. The average vascular diameter and dilatation percentage were obtained for each patient. FMD was calculated during hyperemia as percentage increase in diameter compared with the resting period.[3] Moreover, after 6 months of intervention, FMD, IMT, and echocardiographic indices of heart as well as biochemical markers of endothelial function (ICAM, VCAM) were measured for all subjects.

After completion of the study, the drug codes were recognized, and the data were analyzed with SPSS 23 (IBM corporation, USA). Statistical tests used for data analysis included Chi-square (comparison of the two groups in terms of qualitative and nominal data), *t*-test (for comparison of continuous data between the two groups), T-paired (to compare continuous data in each group), and repeated measures ANOVA (for examining how variables changed in the two groups).

RESULTS

In this study, forty patients with diabetes were examined in two groups of twenty patients receiving Vitamin C and placebo. During the study period, no patient was excluded from the study, and forty patients participated to the end. Table 1 displays the distribution and demographic characteristics of the two groups. Based on Chi-square and *t*-test, there were no differences between the two groups in terms of age, gender, blood pressure, height, weight, body mass index, family history of the disease, and duration of diabetes.

Hypoglycemia occurred in the case and control groups in two and four cases, respectively (10% vs. 20%). However, it was not statistically significant. One patient had coma in the control group.

Table 2 displays the biochemical findings in patients of both groups. Based on *t*-test, the mean 2-h fasting blood glucose level, HbA1C, insulin, high-density lipoprotein and low-density lipoprotein cholesterol, and triglyceride levels had no significant statistical differences between the groups.

The mean hemoglobin A1C at baseline and after intervention in the group supplemented with Vitamin C were 8.5 ± 1.9 and 8.4 ± 2.15 ($P = 0.82$). Moreover, hemoglobin A1C levels at baseline and after intervention in the placebo group were 9.61 ± 1.75 and 8.21 ± 1.4 ($P = 0.18$). In addition, according to repeated measures ANOVA test, changes of echocardiography parameters and biochemical findings

Table 1: Distribution of the demographic and general variables in the two groups

Variables	Groups		*P*
	Vitamin C	Placebo	
Mean age (year)	13.18±3.63	13±3.4	0.88
Sex, *n* (%)*			
Male	12 (60)	10 (50)	0.53
Female	8 (40)	10 (50)	
Systolic blood pressure (mmHg)	112±10.6	111.8±11.6	0.94
Diastolic blood pressure (mmHg)	66.8±5.7	68.5±8.8	0.46
Height (cm)	146.7±21.9	150.1±16	0.58
Weight (kg)	45±14.7	45.9±15.4	0.85
BMI (kg/m^2)	19.14±3.5	19.83±3.64	0.54
Family history, *n* (%)			
Coronary diseases	2 (10)	7 (35)	0.13
CVA[a]	2 (10)	4 (20)	0.66
Hyperlipidemia	7 (36.8)	8 (40)	0.99
Duration of diabetes mellitus (year)	7.98±2.37	7.71±2.86	0.76

*n (%), [a]CVA=Cardiovascular disease; BMI=Body mass index

Table 2: The mean and standard deviation of glucose and lipid profiles in the two groups before intervention

Variables	Groups		*P*
	Vitamin C	Placebo	
Fasting blood sugar (mg/dl)	130.9±61.9	112.8±53.6	0.36
Random blood sugar	203.3±82.5	205.4±100.1	0.95
HbA1C	8.46±1.79	8.67±1.67	0.71
Insulin	38.88±73.9	27.5±16.6	0.51
HDL	58.3±25.7	56.7±19.6	0.83
LDL	97.1±29	86.6±20.7	0.21
Total cholesterol	166.1±26.1	164.1±29.6	0.83
Triglyceride	87.8±36.7	89.9±48.1	0.89

HDL=High-density lipoprotein; LDL=Low-density lipoprotein; HbA1C=Hemoglobin A1C

aren't significant between the two groups except VCAM value that was ststisticaly decreased in the intervention groups.

Table 3 shows the mean and standard deviation of echocardiographic parameters in both groups before and after the intervention. Based on the results of paired *t*-test, the mean left ventricular (LV) mass significantly reduced after the intervention in the group treated with Vitamin C while there was no significant difference observed in the control group. The mean ejection fraction (EF), systolic function (SF), FMD, and Myocardial Performance Index (MPI) significantly altered in the intervention group while there was no significant difference in the control group.

The results indicated that 13 patients (32.5%) had adequate control of blood glucose levels, and 27 patients (67.5%) had inadequate control of blood glucose levels during the 6-months period based on the level of hemoglobin A1C. On the other hand, 13 patients in the intervention group and 14 patients in the control group had improper control blood glucose levels (65% vs. 70%) ($P = 0.73$).

Table 4 displays the mean and standard deviation of echocardiographic findings and indicators related to vascular endothelial function in two groups of intervention and control as well as blood glucose control over the 6 months of intervention. Based on the group of patients with well-controlled diabetes, there was a significant difference between the two groups receiving Vitamin C and placebo in terms of EF and SF, whereas the group of patients whose diabetes was not controlled, showed a significant difference in EF and SF as well as LV mass changes.

DISCUSSION

There have been numerous studies demonstrating that the incidence of atherosclerosis and cardiovascular diseases in diabetic patients is by far more than that in

Table 3: The mean and standard deviation of echocardiographic parameters and vascular endothelial function indicators before and after intervention in both groups

Variables	Groups		*P** (between)
	Vitamin C	Placebo	
Left ventricle mass			
Before	76.35±25.6	67.58±25.38	0.28
After	68.62±22.66	71.63±26.84	0.71
*P*** (within)	0.015	0.19	0.6***
EF			
Before	59.73±2.48	61.35±3.4	0.09
After	63.8±5.39	59.9±3.15	0.01
*P*** (within)	0.002	0.12	0.28***
FS			
Before	30.43±2.83	32.4±2.85	0.034
After	34.39±3.82	31.63±2.36	0.011
*P*** (within)	<0.001	0.27	0.67***
IMT			
Before	0.194±0.043	0.181±0.002	0.16
After	0.179±0.009	0.183±0.004	0.15
*P*** (within)	0.1	0.1	0.37***
FMD			
Before	8.35±4.2	10.12±5.00	0.23
After	16.3±9.1	11.97±5.68	0.09
*P*** (within)	0.002	0.36	0.42
MPI			
Before	0.37±0.06	0.34±0.04	0.13
After	0.32±0.07	0.35±0.08	0.2
P** (within)	0.023	0.7	0.73***
E wave to A wave			
Before	1.75±0.41	1.68±0.25	0.56
After	1.8±0.4	1.72±0.22	0.41
*P*** (within)	0.57	0.42	0.4***
Velocity time integral			
Before	188.3±56.5	175.2±34.9	0.38
After	204.7±46.9	187.2±39.4	0.22
*P*** (within)	0.19	0.032	0.24***
ICAM			
Before	80.36±38.15	93.1±44.37	0.33
After	75.87±31.18	95.59±44.09	0.14
*P*** (within)	0.001	0.77	0.21***
VCAM			
Before	111.2±51.41	189.38±68.01	<0.001
After	122.88±56.93	193.16±57.7	0.001
*P*** (within)	0.46	0.82	<0.001
HbA1C			
Before	8.46±1.8	8.67±1.67	0.71
After	8.43±2.09	8.21±1.4	0.71
*P*** (within)	0.82	0.18	0.99***

*Difference between the intervention and control groups according to *t*-test; **Difference before and after intervention in each group according to paired *t*-test; ***Trend of changes of echocardiography, biochemical changes in the two groups based on repeated measure ANOVA. HbA1C=Hemoglobin A1C; VCAM=Vascular cell adhesion molecule; ICAM=Intercellular adhesion molecule; MPI=Myocardial Performance Index; IMT=Intima-media thickness; FS=Fractional shortening; EF=Ejection fraction; FMD=Flow-mediated dilatation

Table 4: The mean and standard deviation of echocardiographic parameters and endothelial function indicators in the two groups receiving Vitamin C and placebo separately in terms of controlled and uncontrolled diabetes

Variables	Diabetes well controlled			Diabetes was not controlled very well		
	Treatment	Control	*P**	Treatment	Control	*P**
Left ventricle mass						
Before	68.2±21.6	71.3±18.7	0.79	80.7±27.3	66±28.3	0.18
After	68.9±25.2	75.2±20.3	0.64	68.4±22.2	70±30	0.88
*P*** (within)	0.79	0.64	0.69	0.006	0.23	
EF						
Before	59.7±3.1	63.5±2.8	0.04	59.7±2.2	60.4±3.3	0.53
After	64.3±5.9	59.7±1.5	0.09	63.5±5.3	60.1±3.7	0.07
*P*** (within)	0.038	0.06	0.005	0.031	0.65	
FS						
Before	30.9±2	34.3±2.3	0.014	30.2±3.2	31.6±2.7	0.24
After	34.6±4.3	31.3±1.5	0.11	34.3±3.7	31.8±2.7	0.06
*P*** (within)	0.028	0.09	0.005	0.006	0.93	
IMT						
Before	0.176±0.02	0.18±0.001	0.59	0.204±0.05	0.181±0.003	0.09
After	0.174±0.02	0.172±0.004	0.27	0.182±0.004	0.183±0.004	0.37
*P*** (within)	0.85	0.36	0.71	0.1	0.19	
FMD						
Before	8.52±1.53	8.28	0.73	8.15±3.08	10.68±5.73	0.25
After	17.56±8.28	15.53±6.45	0.62	14.77±9.18	9.89±4.16	0.12
*P*** (within)	0.013	0.1	0.48	0.08	0.69	
MPI						
Before	0.36±0.06	0.36±0.04	0.99	0.38±0.06	0.33±0.04	0.043
After	0.32±0.08	0.35±0.05	0.42	0.32±0.06	0.35±0.1	0.37
*P*** (within)	0.21	0.039	0.58	0.03	0.44	
E wave to A wave						
Before	1.59±0.26	1.53±0.21	0.028	1.69±0.46	1.75±0.24	0.65
After	1.92±0.36	1.65±0.15	0.12	1.74±0.42	1.75±0.24	0.95
*P*** (within)	0.63	0.17	0.69	0.71	0.97	
Velocity time integral						
Before	190.8±87.3	168.7±29.9	0.57	187±35.1	178±37.5	0.53
After	241.3±49.3	174.7±37.9	0.021	185±32.5	192.9±40.1	0.59
*P*** (within)	0.12	0.28	0.17	0.81	0.06	
ICAM						
Before	85.4±35	118.1±28.1	0.09	77.63±40.87	82.4±46.5	0.78
After	65±21	98.7±40.8	0.037	83.49±35.7	93.87±47.6	0.58
*P*** (within)	0.15	0.023	0.94	0.52	0.14	
VCAM						
Before	118.7±71.8	202.7±52.2	0.14	107.17±39.5	183.66±74.8	0.003
After	143.7±59.4	192.7±58.6	0.14	108.29±53.2	193.42±63.5	0.004
*P*** (within)	0.19	0.69	0.25	0.8	0.48	

*Difference between the intervention and control groups according to *t*-test; **Difference before and after intervention in each group according to paired *t*-test. VCAM=Vascular cell adhesion molecule; ICAM=Inter cellular adhesion molecule; MPI=Myocardial Performance Index; IMT=Intima media thickness; FS=Fractional shortening; EF=Ejection fraction; FMD=Flow-mediated dilatation

the general population[1] and such a phenomenon mostly arises from vascular endothelial dysfunction. On the other hand, the studies have shown that antioxidants can improve the vascular endothelial function. In this regard, Vitamin C is a powerful antioxidant applied in some studies. Hence, this study aimed to determine the effect of long-term Vitamin C intake on vascular endothelial function in pediatric and adolescent patients with diabetes.

Our results showed that taking Vitamin C for 6 months can affect and improve a number of echocardiographic and sonographic parameters such as EF, SF, LV mass, MPI, and FMD and the significantly higher in case rather than the control group. However, chemical parameters of vascular function including ICAM and VCAM showed no significant difference between the two groups, although changes in the level of ICAM and VCAM was higher in the intervention group than the control group.

In another study by Sabri *et al*. at Isfahan University of Medical Sciences on 18 patients with type I diabetes and 19 healthy children, IMT was reduced in both groups of patients and healthy subjects after taking Vitamin C for 1 month.[14] Furthermore, FMD increased in both groups, but it was significant only in the control group. The results of this study and the previous one could indicate the fact that the use of Vitamin C may have a protective effect on endothelial dysfunction. At the same time, in another study conducted by Erbs *et al*., administration of Vitamin C on patients with coronary artery disease and congestive heart failure was associated with improved vascular endothelial function.[15] In a review study by Ashor *et al*., evaluation of 14 randomized clinical trials demonstrated a positive effect of Vitamin C on EF, which was evident in three groups of patients with atherosclerotic disease, diabetes, and heart failure.[16] Moreover, the results of this study had a significant correlation with an increase in EF with the prescribed dose of Vitamin C. In another review in 2015, Ashor *et al*. examined the effect of Vitamins C and E to improve cardiac EF, concluding that the effect of Vitamin C; this parameter was significant and more dependent on the patient's age.[17] Raitakari *et al*. (1999) administered supplementation of Vitamin K improved vascular endothelial function in smoking patients.[18] Ceriello *et al*. showed that administration of Vitamin C in diabetic patients improved the endothelial dysfunction and oxidative stress in diabetic patients.[19] In a study conducted by Ling *et al*., the postprandial serum triglyceride concentration increased significantly at 2–5 h after the high-fat meal in all groups. The fasting FMD ($P < 0.02$) and nitroglycerin-induced dilatation (NID) ($P < 0.05$) of patients with CHD were impaired compared with those of non-CHD subjects. Postprandial FMD was significantly aggravated in the non-CHD/control group ($P < 0.01$) and the CHD/control group ($P < 0.001$), but the postprandial FMD in patients and subjects taking Vitamin C showed no significant change; however, the CHD/Vitamin C group had a mild tendency toward improvement ($P = 0.064$) and non-CHD/Vitamin C group had a mild tendency toward aggravation ($P = 0.852$). The change of NID after a high-fat meal did not reach statistical significance in the four groups. The decrement of postprandial FMD correlated positively with the increment of 2-h serum triglyceride concentration in the patients without Vitamin C ($n = 62$, $r = 0.545$, $P < 0.001$).[20]

CONCLUSIONS

The results of the current study showed that long-term use of Vitamin C in diabetic patients can improve certain echocardiographic and sonographic parameters such as EF, fractional shortening, MPI, and FMD, which in turn may show improvement in vascular endothelial function. However, because this study was done in a pilot and due to the lack of significant difference in the biochemical markers, such as ICAM and VCAM as well as the limitations of this study, including a small sample size and low dose of Vitamin C, it is recommended that further studies could be carried out with larger sample size and higher Vitamin C dosage in diabetic patients.

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Conflicts of interest

The authors have no conflicts of interest.

AUTHORS' CONTRIBUTION

All of the authors contributed in all stages of this work.

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