

Original article

Serum vitamin E concentration in Iranian population with angiography defined coronary artery disease

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Background: Oxidative modification of low-density lipoprotein (LDL) seems to play a role in the development of atherosclerosis and coronary artery disease (CAD). Although in many experiments a role for vitamin E in prevention of LDL oxidation and therefore CAD has been suggested, some clinical studies have failed to confirm these findings.

Objective: A case- control study was conducted to find out the association between serum vitamin E levels and coronary artery disease susceptibility in Iranian patients with CAD.

Methods: Ninety-one patients with angiographically confirmed CAD (defined as coronary obstruction >50%) and 39 age and sex matched controls that their atherosclerosis was not proven according to angiography were included. Serum vitamin E concentration was measured in plasma by high performance liquid chromatography (HPLC) for all patients.

Results: No significant difference in serum levels of vitamin E and standardized vitamin E [vitamin E/total cholesterol, and vitamin E/ (total cholesterol+triglycerides)] and lipid profile parameters was observed between patients and control groups. The association between vitamin E and CAD remained unchanged independent of age, sex, smoking habit, hypertension, hyperlipidemia and diabetes. Serum vitamin E levels were positively associated with waist/hip ratio, high-density lipoprotein cholesterol (HDL-C) and total cholesterol in the control group and inversely associated with HDL-C in the CAD group. Vitamin E/total cholesterol levels were inversely associated with HDL-C and low-density lipoprotein cholesterol (LDL-C).

Conclusion: Our data does not support the results of other studies which have shown an association between lower amounts of vitamin E and enhanced risk of coronary heart disease.

Keywords: Coronary artery disease, HPLC, LDL-cholesterol, LDL oxidation, vitamin E

Coronary artery disease (CAD) is the most common cause of death in developed countries. Multiple factors such as hypertension, diabetes mellitus, smoking, obesity and dyslipidemia may play a role in the pathogenesis of CAD. After demonstration of a probable role for low-density lipoprotein (LDL) oxidation in the development of atherosclerosis and presentation of the theory termed oxidative modification hypothesis, there was a surge of interest considering the effects of antioxidants in the prevention and treatment of CAD. Indeed, this hypothesis states that accumulation of circulating

oxidized LDL particles in macrophages inside the arterial walls leads to atherosclerotic plaque formation and consequently coronary heart disease [1, 2] and thus it seems that antioxidant agents including antioxidant vitamins and particularly vitamin E may be effective in prevention of these processes [3].

Since vitamin E is the major lipid-soluble lipoprotein antioxidant and α -tocopherol is the predominant antioxidant in LDL, vitamin E may be effective in prevention of CAD [4]. Several human and animal studies have been conducted to determine the mechanisms by which vitamin E may prevent the LDL oxidation process [5]. In spite of many studies performed for demonstration of the association between plasma vitamin E levels and risk of coronary artery disease, the effectiveness of this antioxidant in prevention or treatment of CAD is still equivocal.

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According to some different epidemiological studies, vitamin E supplementation seems to be beneficial in atherosclerosis treatment, since an inverse relationship between coronary artery disease and plasma vitamin E levels has been observed [6, 7]. There are also evidences showing an inverse relationship between LDL vitamin E contents and susceptibility to CAD [8, 9]. In contrast, no consistent relationship was found between vitamin E and myocardial infarction or CAD death risks in other epidemiological prospective studies [10-13].

In the present study we examined the association of serum vitamin E status and presence of coronary artery disease in an Iranian population.

Methods

Study population

Ninety-one patients with angiographically confirmed CAD (defined as coronary obstruction >50%), aged 32-83 years and 39 sex and age matched control individuals in whom CAD was not proven through angiography were recruited from the patients of cardiology clinic of Ghaem Hospital, Mashhad, Iran. Written informed consents were obtained from patients.

CAD patients were allocated into three groups (svd, 2vd, and 3vd) according to the number of their obstructed coronary arteries. The svd group included patients who had only one obstructed coronary artery and 2vd and 3vd groups included patients with two and three obstructed coronary arteries, respectively. Information about the smoking habits, drug consumption, and major risk factors of CAD including hypertension, diabetes, hyperlipidemia and etc were collected from case and control group individuals via a designed questionnaire. The waist/hip ratio and body mass index (BMI) of both case and control group subjects were also calculated.

Blood sampling

Fasting blood samples were collected into tubes and immediately centrifuged at 1500g for 10 minutes. Plasma was kept at -80°C, in dark conditions, until vitamin E analysis. Besides, an aliquot of all serum samples were stored separately in 4°C for lipid assays.

Analytical methods

Serum vitamin E was determined via HPLC as

described by Ghayour Mobarhan et al [14]. In short, 200 µL internal standard (10 µg/ml δ-tocopherol in isopropyl alcohol) was added to 200 µL serum and vortex mixed. 200 µL aqueous ammonium sulphate 3.9 M was added to the solution and vortex mixed. After centrifugation for five minutes (1000g), 25 µL of supernatant was utilized for evaluating the amounts of vitamin E by HPLC (Shimatzu, Japan), using a 25 µm ODS2 (50 × 4.6 mm) column with methanol as mobile phase, and a Ultra Violent detector (294 nm). The flow rate was 1.0 ml per minute.

Serum total cholesterol (TC) and TGs values were analyzed by enzymatic methods. According to the strong correlation between serum vitamin E levels and lipids content, vitamin E concentrations were standardized for TC and TC+TGs (representing as VE/TC and VE/TC+TGs respectively).

Statistical analysis

For analyzing the complete set of data, we used SPSS version 11.5. All values in this report are presented as the mean±SD and percentages. One-way ANOVA was used for data with normal distribution and Mann Whitney test for non-parametric data.

Results

Demographic and anthropometric characteristics and lipid values of both patient and control groups are given in **Table 1**. As shown, there were no significant differences in age, sex, smoking habit, BMI, hypertension, hyperlipidemia, diabetes, and C-reactive protein status (hs-CRP) between control and case groups.

Serum levels of vitamin E and lipid-standardized vitamin E were also compared between CAD patients and control group individuals. No significant difference was found in the mean concentrations of vitamin E, standardized vitamin E for TC and standardized vitamin E for TC+TG between the two groups as well as between control group and different subgroups of patients based on the number of narrowed vessels ($P>0.05$) as shown in **Tables 2** and **3**.

Furthermore, comparing vitamin E, standardized vitamin E for TC and standardized vitamin E for TC+TGs concentrations between three distinct patient subgroups (svd, 2vd, and 3vd, $P<0.05$) did not revealed any statistically significant difference (**Table 4**).

Table 1. General characteristic and Lipid profiles of study groups.

Groups	Controls	CAD Patients	p-value
N	39	91	NS ¹
Female (%)	69.2	41.8	NS
Smoker (%)	41	37.4	NS
Diabetics (%)	20.5	24.7	NS
Hyperlipidemics (%)	33.3	43.8	NS
Hypertensive (%)	33.3	51.7	NS
Age (year)	59.59±11.81	60.25±10.03	P=0.103
BMI (Kg/m ²)	27.18±6.37	27.62±6.54	P=0.705
FBS (mg/dl)	107.94±44.65	120.32±61.91	P=0.312
Total Cholesterol (mmol/l)	4.22±1.15	4.24±1.55	P=0.220
Triglycerides (mmol/l)	1.24 (0.87-2.12)	1.35 (1.02-2.07)	P=0.455
HDL-C (mmol/l)	1.07±0.30	1.11±0.46	P=0.148
LDL-C (mmol/l)	2.36±0.96	2.32±1.07	P=0.386
Hs-CRP(mg/dl)	1.58 (0.92-6.90)	2.07 (1.21-5.08)	P=0.394

BMI: body mass index, FBS: fasting blood sugar, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol. Values are expressed as mean±SEM, or medial and interquartile range. Between-group comparison were assessed using Independent-Samples T Test for normally distributed data and Mann-Whitney for non-parametric data (*P<0.05, **P<0.01, ***P<0.001). NS¹ means non-significant

Table 2. Comparison of VE and VE/TC, VE/TC+TGs ratios between patients and controls

	Patients (n=91)	Controls (n=39)	Sig
VE, μmol/L	0.91±1.39	1.01±1.41	0.971
VE/TC, mmol/mol	0.21±0.31	0.25±0.35	0.647
VE/TC+TG, mmol/mol	0.15±0.22	0.17±0.24	0.798

Table 3. Comparison of VE and VE/TC, VE/TC+TGs ratios within patient sub-groups

	Patients (n=91)			Sig
	svd (n=26)	2vd (n=26)	3vd (n=39)	
VE, μmol/L	0.99±1.67	0.86±1.20	0.87±1.32	0.960
VE/TC, mmol/mol	0.20±0.30	0.21±0.29	0.22±0.32	0.925
VE/TC+TG, mmol/mol	0.15±0.22	0.16±0.24	0.15±0.22	0.944

Table 4. Comparison of VE and VE/TC, VE/TC+TGs ratios between CAD patient sub-groups and control individuals.

	Trial groups		Sig
	control	svd	
VE, μ mol/L	control	svd	1.000
		2vd	0.976
		3vd	0.972
VE/TC, mmol/mol	control	svd	0.917
		2vd	0.968
		3vd	0.975
VE/TC+TG, mmol/mol	control	svd	0.947
		2vd	0.995
		3vd	0.961

Correlation between CAD risk factors and serum vitamin E status in CAD patients

Bivariate correlations between vitamin E and vitamin E / total cholesterol levels with CAD risk factors showed that vitamin E is positively correlated with waist / hip ratio ($P < 0.05$), LDL ($P < 0.01$) and total cholesterol ($P < 0.05$). No significant correlation was observed for vitamin E/ total cholesterol ($P > 0.05$) as shown in **Table 5**.

Correlation between CAD risk factors and serum vitamin E status in control group

In control group subjects, a significant negative correlation between vitamin E concentrations and HDL was found ($P < 0.05$). With respect to vitamin E/ total cholesterol, significant negative correlations were observed with HDL and LDL ($P < 0.05$).

Lipid profile of control and patient groups

Comparing the information about lipid profile of patients and control group individuals including TC, TGs, LDL-C and HDL-C did not revealed any significant difference between these two groups.

Discussion

Coronary heart disease is known as one of the most important causes of mortality in developed countries and several studies have been done yet to prescribe the different risk factors and treating agents of this harmful disease. Despite the lack of data showing the prevalence of CAD in the whole Iranian population, there are some studies demonstrating the

incidence of CAD and its known risk factors in different parts of Iran [15, 16, 38].

After clarifying the effects of Low-density lipoprotein oxidation as an obvious pathomechanism for CAD and subsequently the role of antioxidants in suppressing this process, a hypothesis about the efficacy of vitamin E as an antioxidant involved in CAD prevention emerged.

Although in various studies an association between lower amounts of vitamin E levels and enhanced risk of coronary artery disease has been suggested [6-9, 17, 18], our data failed to confirm these results. In other word, we did not find any association between normalized vitamin E concentrations and susceptibility to CAD. These results confirm the result of our previous study which evaluated whether there is a relationship between vitamin E status and coronary risk factors in dyslipidaemic patients [14]. Our results also are consistent with the results of some other studies that have not found any benefit for vitamin E in the primary and secondary prevention of cardiovascular disease [19, 20-24].

Vitamin E is the major lipid soluble antioxidant vitamin and several animal and human studies have suggested an antioxidant role for this micronutrient in preventing LDL from oxidation [25]. In spite of proposed antioxidant effects of vitamin E, some large-scale trials have not shown any benefit of supplementary vitamin E in prevention or treatment of CAD [26-28]. In some other studies an increased risk of cardiovascular disease mortality with high dose of vitamin E supplementation has been observed [29].

Table 5. Correlation between CAD risk factors and serum vitamin E status in both case and control groups.

	Patients (n=91)		Controls (n=39)	
	Vitamin E $\mu\text{mol/L}$	Vitamin E/T mmol/mol	Vitamin E $\mu\text{mol/L}$	Vitamin E/TC mmol/mol
Age	0.066	0.117	0.025	0.014
Fasting blood glucose	0.066	0.026	0.144	0.110
BMI	0.025	-0.050	-0.156	-0.181
w/h ratio	0.248*	0.196	0.000	-0.030
HDL	0.017	-0.088	-0.335*	-0.324*
LDL	0.331**	0.083	-0.250	-0.320*
Triglyceride	0.138	0.028	0.264	0.195
Total cholesterol	0.230*	-0.003	-0.046	-0.142
Systolic BP	0.100	0.110	0.008	-0.042
Diastolic BP	0.184	0.057	0.140	0.051
hs-CRP	-0.020	-0.076	-0.238	-0.237

Besides, high dose oral consumption of vitamin E, vitamin C and a combination of these two, have not indicated any improvement in coronary and brachial endothelial function and failed to reduce circulating oxidized LDL or auto-antibodies to oxidized LDL [30]. Similarly, vitamin E supplementation in an outpatient population with advanced heart failure did not revealed any significant effect on any marker of in vivo oxidative stress and additionally any significant improvements in prognostic or functional indexes of heart failure [31].

The efficiency of antioxidant vitamin E in prevention of atherogenesis process or treatment of cardiovascular disease or furthermore, any disease related to different extents of vascular obstruction has been studied for many years. According to multiple-step variable pathway hypothesis, atherogenesis is a progressive multi-factorial process which requires a sequence of events to occur and the progress along the disease is caused by risk factor bundles that change with the stage of disease and vary between different persons. In other word, transition along one stage to another might occur through different pathways that can be dependent on individual differences. Besides, some of the risk factors of this disease show non-uniform importance during the entire disease pathway [32]. Regarding to this hypothesis, the possibility of non-uniform effects of vitamin E and lesser importance of this antioxidant vitamin through different stages of atherogenesis may be considered as a factor for unpredictable results of CAD antioxidant studies, such as our study. Moreover, despite the undeniable effects of oxidative modification of LDL in pathogenesis of CAD, the role of inflammatory process in atherogenesis is something that should also be concerned. According to "oxidative response to inflammation hypothesis" oxidative modifications in the vessel wall may occur as a process secondary to inflammation. In other words oxidative events may be a consequence, rather than a cause, of the atherosclerotic process [33]. As a consequence, evaluation of antioxidant vitamin E status, regardless to the stages of atherosclerogenesis may lead to unpredictable results of antioxidant studies, such as the result we have obtained in this study.

Although a proper diet including foods rich of antioxidants is still one of the safest ways known for prevention of CAD and for lowering plasma levels of LDL, these benefits may occur as a result of other components found in these food rather than vitamin

E. High- antioxidant containing foods may also have other micronutrients such as carotenoids, flavonoids, minerals and also lower cholesterol and saturated fat [34].

The amount of serum vitamin E levels might be affected by many factors such as age, obesity, cigarette smoking and dietary intake of antioxidant vitamins [35-37]. Therefore, the non-significant difference in serum levels of vitamin E between CAD patients and control group subjects in our study might be resulted from the similarity of anthropometric factors, smoking habit and lipid values of case and control groups.

Furthermore, in the present study the concentration of vitamin E in both case and control groups was relatively lower than that reported with other studies in Iran [9, 38]. It is postulated that the dietary intake of vitamin E is the most important way for providing daily need to this antioxidant. Inappropriate diet and malnutrition have undesirable impressions on plasma antioxidant vitamin E values. In one of our recent studies performed on eating habits of elderly people and possibility of malnutrition regarding to nutritional status and socioeconomic conditions in a part of Khorasan province, north Iran, we found 12% malnutrition prevalence with an adverse association between socioeconomic parameters and risk of malnutrition [39]. Pall et al [40] also indicated that socioeconomic factors, life-style and specific nutrient intake are related to plasma levels of antioxidant. In other word, insufficient intake of antioxidant containing foods, particularly foods rich of vitamin E because of any of the reasons mentioned above can explain this low values. Most subjects included in our study were from lower-income classes of society. This might be a possible explanation for the lower values of vitamin E in this study.

Conclusion

In this study, evaluation of vitamin E status and its relation to CAD did not reveal any association between lower amounts of vitamin E and enhanced risk of coronary heart disease. Current investigation was limited by the relatively few number of control subjects. This lower size of control group compared to case group might negatively affect the statistical power of the study. Therefore, this issue must be considered upon interpretation of the present findings. In addition, more systematic and detailed analysis of the disease process with an accurate control of diet

and consumption of LDL lowering drugs is needed to realize the effects of vitamin E as a protective antioxidant. Evaluation of any association between vitamin E antioxidant action and other non-enzymatic and enzymatic protective agents involving in atherogenesis is also suggested. Furthermore, determination of vitamin E status and its association with life style and dietary habits in a larger population of Khorasan province, Iran, is recommended.

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