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Serum vitamin E as a significant prognostic factor in patients with dyslipidemia disorders

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Abstract

Objectives: Obesity and overweight are among the main causes of cardiovascular disease (CVD) mortality. Dyslipidemia, fatty liver index, is strongly related to CVD. Vitamin E as an antioxidant protects the hepatic cells against oxidative stress and prevents fatty liver disease. The aim of the current study is to evaluate the relationship between anthropometric parameters and fasted lipid profile with serum vitamin E levels.

Study Design: A randomized trial was designed based on data from the Mashhad stroke and heart atherosclerotic disorders (MASHAD: 2010-2020).

Methods: 363 CVD subjects (173 males and 190 females) was selected at random, among 9704 subjects in three regions of Mashhad, northeast of Iran to investigate the specific correlations among their serum vitamin E, lipid profile (TG, HDL-C, LDL-C and TC), and anthropometric features (height, weight, BMI, hip and waist circumferences.

Result: The results indicated the significant relationships between vitamin E, and fasting serum lipid profile in subjects. Serum vitamin E was negatively correlated to TC, TG, and LDL-C and positively related to HDL-C. Also, statistically negative correlations were found between vitamin E and anthropometric parameters (weight, waist and hip circumference, middle Arm, and Systolic Blood Pressure). Moreover, vitamin E ratios such as vitamin E/(TC + TG) and vitamin E/TC values as standardized vitamin E, had significant negative correlation with BMI, the whole of anthropometric parameters, and dyslipidemia risk factors including TC, TG and LDL-C.

Conclusion: We found that vitamin E profile was significantly lower in the dyslipidemia subjects. It is generally suggested that vitamin E monitoring might be used as a useful prognostic and therapeutic agent in dyslipidemia disorder.

Keywords: Serum vitamin E; dyslipidemia disorders; prognostic and therapeutic factor; lipid profile; anthropometric parameters.

1 Introduction

Cardiovascular disease (CVD) is the major cause of death worldwide [1]. Dyslipidemia is considered as a prominent risk factor for cardiovascular disease [2, 3]. Moreover dyslipidemia is the main cause of atherosclerosis, which is associated with the combination of fatty acids and cholesterol levels, fiber and vitamins in an inappropriate diet [4-6]. The main features of dyslipidemia refer to low levels of decreased high-density lipoprotein cholesterol (HDL-C) (< 1.04 mmol/L or < 40 mg/dL), high levels of low density lipoprotein cholesterol (LDL-C) (3.37-4.13mmol/L or 130-159 mg/dL) and triglyceride (TG) (1.70-2.25 mmol/L or 150-199 mg/dL), which increase the risk of cardiovascular disease [7-9].

According to the World Health Organization (WHO) reports, obesity is one of the main public health problems [10]. In addition to the Body Mass Index (BMI), central obesity is used to measure overweight by measuring waist circumference (WC) or waist to hip ratio (WHR) [11]. Obesity is the main risk factor for cardiovascular disease (CVD), which is leading to death in adults. In general, symptoms of CVD reveal in the fourth decade of life, but the progression of atherosclerotic begins at earlier decades and is closely related to dyslipidemia [12]. Components of the metabolic syndrome such as high blood pressure and insulin resistance (IR), as well as obesity and dyslipidemia, are considered as progression risk factors for cardiovascular disease [13]. For the prevention of primary and secondary cardiovascular disease, dyslipidemia controlling strategies have always been helpful [14].

Many factors affect plasma lipid concentrations, including non-modifiable agents such as age and genetic, and modifiable factors such as dietary regimen and physical activity [15]. It has been shown that a good diet and weight loss strategies are associated with lowering serum cholesterol levels. One of the most important dietary strategies for decreasing cholesterol levels is to reduce cholesterol consumption up to 200 mg/day and total fat intake up to 20% of total calories. Also, the presence of phytosterol esters, soluble fibers, nuts and soy isoflavines in the dietary regimens reduce 5-10 mg/dl of LDL-C [16]. Altogether, by reducing weight, cholesterol and fat intake, LDL-C can be decreased by about 10 to 15 percent [17]. Moreover, Studies in people without coronary heart disease suggest that dietary intake of vitamin E may prevent cardiovascular disorders [18].

The most abundant form of vitamin E is α-tocopherol which exists in common edible oils such as peanuts, corn, and soybean [19]. Dietary tocopherols are absorbed along with dietary fat in intestinal lumen and secreted in chylomicron particles. Chylomicron remnants containing vitamin E are absorbed by the liver. In order to transfer vitamin E to peripheral tissues, such as bone, muscle, skin, brain, lung and adipose tissues, the liver returns it to the bloodstream as part of VLDL and HDL [20, 21]. Vitamin E has an important role in protecting cells from reactive

oxygen species [22].Tocopherol molecules, in addition to their antioxidant properties, also play as an anti-atherogenic agents [23].

Researches on the varius aspects of its physiological roles have suggested this vitamin as an essential nutrient to achieve and maintain optimal health. In other words low levels of the plasma circulating vitamin E are detrimental to immune function and oxidative damage control [24].

The aim of the present study is to show the impact of vitamin E as an important lipid soluble antioxidant, which is considered as an effective inhibitor in dyslipidemia formation.

2 Methods

2.1 Study design and population

A population of 363 subjects (173 males and 90 females) aged 35-65 years was selected from Mashhad stroke and heart atherosclerotic disorders (MASHAD: 2010-2020) at random. Pregnant and breast feeding women, patients who had systemic disease, and patients taking any drug (including lipid lowering drugs) were excluded from the study. Demographic data including age and gender were collected using a questionnaire.

2.2 Anthropometric measurements and Data collection

Anthropometric parameters including body weight, height, waist and hip circumference were measured using a standard protocol. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m2); and height and weight were measured with a standard scale to an accuracy of +0.1 cm and +0.1 kg, respectively. The systolic and diastolic blood pressure was measured using a standard mercury sphygmomanometer three times with an interval of 30 minutes in participants and the average of the 3 measurements was taken as the blood pressure. High Blood Pressure is defined as BP $\geq$ 140/90. All statistical data were obtained through face-to-face research. The questionnaire was provided by trained interviewers with information about demographic characteristics (gender, age), lifestyle (alcohol consumption, cigarette smoking, physical activity), History of individual disease (cardiovascular disease, atherosclerosis, etc.), blood pressure, heart rate and psychological tests (anxiety and depression tests). Serum samples (10 ml) were collected by an expert nurse with at least 14 hours of fasting between 8:00 and 10:00 am. Blood samples were collected by a vacuum blood tubes and centrifuged at a speed of 2500 rpm for 15 minutes.Then, serum samples were used for measuring lipid profile.

2.3 Lipid profile measurements

After 12 hours fast, the values of triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentrations were assayed as described previously [25, 26].

2.4 Phenotypic definition of dyslipidemia

Dyslipidemia was identified based on the NCEP ATPIII criteria [27], if one or more of the following criteria were met: 1) Hypercholesterolemia: serum cholesterol levels ≥200 mg/ dl (≥5.2 mmol/l); 2) Low HDL cholesterol: HDL cholesterol levels <40 mg/dl (<1.04 mmol/l) for men and <50 mg/dl (<1.3 mmol/l) for women, 3) High LDL cholesterol: LDL cholesterol levels ≥130 mg/dl ≥3.4 mmol/l) calculated using the Friedewald equation, and 4) Hypertriglyceridemia: serum triglycerides ≥150 mg/dl.

2.5 Serum vitamin E measurements and normalization

Serum concentration of vitamin E was measured by HPLC using a modification of the procedure of Papas et al. (2003). Briefly, 750 μL ethanol was added to 250-μL serum and centrifuged at 5000–7000 rpm for 10 min. In the following, to prepare the injection sample into HPLC column, 1 mL n-hexane was added to supernatant, and recentrifuged. The supernatant vaporization under nitrogen gas and the deposits merging by 250 μL methanol was performed subsequently. 25 μL of the solution was then injected to the HPLC column. α-Tocopherol extraction was done and measured by the spectroscope at 295 nm.

According to the nature of vitamin E fat-solubility, the vitamin E can be found in triglycerides of chylomicrons and liver-derived lipoproteins, vitamin E normalization has done to correct vitamin-free lipoproteins and trigycerids. In order to normalize the serum vitamin E levels, measured vitamin E values has divided into TotalChol and TotalChol plus TG. Therefore, two functional criterions were created to correlate with anthropometric and serum lipid profile variables. So the term vitamin E refers to its both normalized values in this study.

2.6 Statistical analysis

Data analyses were undertaken using the Statistical Package for Social Sciences (SPSS version 16). The normality of distribution was assessed using the Kolmogorov-Smirnov test. Quantitative data were expressed as the mean ± SD for normally distributed variables or as the Median and IQR for not normally distributed variables. Mann-Whitney U and Kruskal-Wallis tests were used for non-normally distributed variables. Qualitative variables compared using Chi-square test. A multivariate and univariate linear regression was used to calculate the effect of dyslipidemia risk factors and BMI on the vitamin E profile. The Spearman test was used to investigate the relationship between vitamin E and normal quantitative variables. Control group was considered as a reference. A P-value of less than 0.05 was considered as statistically significant.

2.7 Ethics statement

The protocol was approved by the Medical Ethics Committee of Mashhad Medical Sciences University. All participants gave written consent.

3 Result

3.1 General features of participants in the study

Anthropometric anthropometric and blood lipid profile of individuals according to their gender are described in Table 1. Of the total 363 participants, 312 individuals (males(144) and females(168)) had dyslipidemia. The mean values of age, height, weight, Hypercholesterolemia, LDL, HDL and triglyceride are mentioned in males and females in details. Also the correlation of each parameter was analyzed with males and females separately.

3.2 Vitamin E correlation with anthropometric and lipid profile variables

The correlation of vitamin E with anthropometric variables has been shown clearly in Table 2. vitamin E ratios such as vitamin E/(TC + TG) and vitamin E/TC values as standardized vitamin E, had significant negative correlation with BMI and the whole of anthropometric parameters including weight, waist and hip circumference, middle Arm, and Systolic Blood Pressure. Table 3 displays the relationship between fasted lipid profile with serum vitamin E levels. The results proved the strong negative correlation among vitamin E with LDL-C (p-value: 0.048), TC (p-value: 0.016), and TG (p-value: <0.001). Also vitamin E/(TC + TG) values had significant negative correlation with plasma triglyceride concentration (p-value: < 0.001).

3.3 Association of vitamin E with Dyslipidemia characteristics

The association of vitamin E with dyslipidemia characteristics has been explained in Table 4. Vitamin E had negative correlation with cholesterol (p-value: 0.044) in both normal and abnormal patients cholesterol, triglyceride (p-value: 0.003) generally in subjects, and positive correlation with HDL (p-value: 0.044) just in both normal and abnormal men HDL profile. In the other word, high levels of vitamin E were accrued along with low values of serum cholesterol and triglyceride, and high values of HDL. Therefore, high values of vitamin E reduce the dyslipidemia development by affecting its corresponding characteristics.

3.4 Effect of BMI and risk factors Dyslipidemia on vitamin E in Multivariate and univariate linear regression model

Table 5 refers to an important association of vitamin E with LDL, TG, and TC. The results show the vitamin E negative correlation with BMI (p-value: 0.01), LDL (p-value: 0.004), triglyceride (p-value: 0.003), and total Cholesterol (p-value: 0.006). In addition, there is a positive association with HDL concentration in men and women.

4 Discussion

To the best of our knowledge, this is the first study evaluating the association between serum vitamin E profile with anthropometric characteristics and dyslipidemia risk factors in Iranian population. The results showed that low serum vitamin E was significantly related to increased waist and hip circumference, weight, cholesterol and triglyceride levels. There also was a significant association between Vitamin E/TC and BMI, waist, hip circumferences, LDL and TC.

In particular vitamin E/(TC + TG) values had significant correlation with TG, TC, HDL(in men), weight, weight/ height ratio , BMI , waist circumference, hip circumference, middle Arm, systolic Blood Pressure, heart rate and dyslipidemia.

Cardiovascular disease is the main cause of death worldwide. Interestingly dyslipidemia is an important leading cause of cardiovascular disease [14], Which is characterized by high TG, LDL-C and low HDL-C levels in serum [7] and closely linked to obesity [28],fatty liver disorders, CVD, and nonalcoholic fatty liver disease (NAFLD) [29].

Defining the risk of cardiovascular disease in people who seem to be healthy and the appropriate treatment in order to primary prevention have critical implications for public health.

The liver, as an important metabolic organ, regulates the blood lipid compounds and manages lipoproteins concentration [30, 31]. Triglycerides are the main form accumulation of fat in the liver, which belongs to dietary chylomicron (15%), nonesterified fatty acids (60%) derived from lipolysis of adipose tissue or hydrolyzed lipoproteins, and freshly synthesized fatty acids (25%) [32]. Fatty liver is a common disease and is characterized by dysglycemia and dyslipidemia independent of visceral adipose tissue (VAT). Fatty liver disease (FLD) occurs in the majority of alcoholic and obese people worldwide [33, 34]. It is worth noting that many metabolic and genetic conditions that affect the metabolism of fatty acids also cause FLD [33, 35, 36].

Defective oxidation of fatty acids leads to excessive lipid storage in the liver. Oxidation of fatty acids in hepatic cells is approximately proportional to the plasma FFA released from adipose tissue. Three cellular organelles are specialized in fatty acids oxidation, mitochondria, peroxisomes, and endoplasmic reticulum [35, 37]. Since catabolism of FFAs in the liver is mainly due to the mitochondrial β-oxidation, if the FFA delivery to the liver has increased, this process can lead to the ROS productions, such as superoxide, hydroxyl radicals and hydrogen peroxide. ROS, through the formation of reactive aldehydes, including 4-hydroxyenonal (4-HNE) and malondialdehyde (MDA), causes peroxidation of liver triglycerides and ultimately damage to mitochondrial components [38].

Mitochondrial dysfunction due to a disabled activity of the mitochondrial electron transport chain may cause some structural mitochondrial disorders, including megamitocandria, lose cristae, and paracrystalline inclusions [39, 40], Which are lead to lower levels of ATP, ROS permeation and excessive accumulation of fat in the cell [41]. In other words the production of toxic acetaldehyde achieved through the alcohol dehydrogenase and cytochrome P450 2E1 (CYP2E1) pathways, and leads to fatty acid accumulation in liver by alcohol consumption [42]. The imbalance between ROS production and antioxidant defense is called oxidative stress, leading to DNA damage and disorders in cell biology [43].

Oxidative stress can lead to direct damage to the biomolecules, causing the activation of inflammatory signalling pathways and fibrogenesis. It may also have different effects on antioxidant mechanisms. ROS, in addition, to inhibit the activity of antioxidant enzymes such as

superoxide dismutase (SOD), directly damage antioxidant molecules such as glutathione (GSH) [44]. Setting the status of reducing and oxidizing (redox) is critical for survival, activation, reproduction, and organ function [43]. Antioxidants such as vitamin C and E prevent atherosclerosis by inhibition of LDL oxidative alteration.

Vitamin E supplementation provides benefit health status in CVD and inhibition of lipoproteins oxidation through its antioxidant activity [45].The biological activity of vitamin E is not only limited to its antioxidant properties but also play an important role in the regulation of inflammatory response, gene expression, membrane-attached enzymes, cell signaling and proliferation [46, 47]. Since the vitamin E ability to scavenge fat radicals and terminates ROS generation [48], It has been proposed that vitamin E biological activities are due to its antioxidant activity [49].

Several case-control studies have reported the association of vitamin E with serum lipid profiles and its related diseases. Zahra and et al demonstrated that three months consuming vitamin E in patients with type 2 diabetes (T2DM) leads to lower blood pressure, lipid profile levels and improved insulin function [50]. Also Duangkamol and et al shown the same results for the antioxidant vitamins A, E, and c correlation with lipid profiles in their case-control study [51].

According to the concepts mentioned, in addition to genetic factors, dietary regimen and physical activity define the plasma lipoprotein compounds. It is worth noting that liver as the central manager of plasma lipoproteins alternatively may be exposed to high levels of oxidative stress. Also as it mentioned, Fat accumulation in patients' liver generally leads to dyslipidemia due to not-compensated oxidative stress. Moreover excessive fatty acids storage in adipose tissues refers to obesity [52] and increased BMI index and anthropometric parameters. Interestingly, vitamin E has been suggested as a therapeutic approach for primary and secondary protection of cardiovascular events [53]. It has been shown that vitamin E as a potent antioxidant improves CVD symptoms by recruiting mechanisms such as inhibiting the lipoprotein LDL cholesterol oxidation in plasma [54] and reducing oxidative stress in NAFLD [23]. Moreover epidemiological studies have shown that consuming a diet rich in vitamin E has led to a reduction in CVD rates [45, 55].

In the current study, we have been shown that participants with low levels of serum vitamin E had increased waist circumference, weight, cholesterol, and triglyceride levels. Moreover the BMI index and waist circumferences parameters had ascending values in the majority of subjects, and people with high levels of α-tocopherol, exhibited descending values of blood lipids (Tables: 2, 3, 4, and 5).

5 Conclusion

According to the vitamin E antioxidant activity and its essential role in regulating oxidative stress, it can be argued that it regulates the lipoprotein rates and prevents dyslipidemia by preventing fatty liver production and reducing the oxidative stress of hepatic cells. Moreover, it

was shown that vitamin E indexes have a significance association with lipid profile and anthropometric parameters. Therefore, supporting studies are needed to serve vitamin E as a preventing and therapeutic factor in dyslipidemia.

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Table1: baseline anthropometric characteristics of subjects according to sex

Variable	Male (n=173)	Female (n=190)	P-value
Height (m)	1.69±0.059	1.56±0.061	<0.001**
Weight (kg)	73.89±12.36	70.07±12.33	<0.003*
age	48.42±8.19	47.63±7.75	0.345
Hypercholesterolemia (TC≥200)	60(34.7%)	83(44.4%)	0.060
High LDL (LDL≥130)	55(31.8%)	71(38.2%)0.206	0.206
Low HDL (M<40 and F<50)	97(56.1%)	133(71.1%)	0.003*
High triglycerides (TG≥150)	53(30.6%)	52(27.8%)	0.555
Dyslipidemia	144(82.8%)	168(89.8%)	0.05
Serum α-Tocopherol (µmol/l)	0.150(0.029-0.727)	0.150(0.029-0.523)	0.983

Table2: Vitamin E correlation with anthropometric factors

Variable	Vit E		Vit E/TC		Vit E/(TC+TG)	
	r	p	r	p	r	p
Age (year)	-0.016	0.754	-0.074	0.164	-0.075	0.0156
Height (m)	0.029	0.587	-0.040	0.446	0.006	0.906
Weight (kg)	-0.070	0.184	-0.083	0.115	0.130	0.013*
Weight/ Height (kg/m)	-0.083	0.115	-0.098	0.062	-0.138	0.009*
BMI (kg/m2)	-0.087	0.100	-0.104	0.048*	-0.131	*0.013
Waist circumference (cm)	-0.102	*0.052	-0.122	*0.021	-0.166	*0.002
Hip circumference (cm)	-0.110	*0.037	-0.118	*0.025	-0.142	*0.007
Middle Arm (cm)	-0.075	0.155	-0.098	0.062	-0.124	*0.011
Systolic Blood Pressure (mmhg)	-0.068	0.199	-0.091	0.086	-0.112	*0.034
Diastolic Blood Pressure (mmhg)	-0.058	0.269	-0.074	0.161	-0.095	0.074
Heart rate (number/min)	-0.092	0.083	-0.098	0.066	-0.101	*0.058

BMI: Body Mass Index. * P value<0.05 and ** P value<0.001

Table 3: Vitamin E Correlation with lipid profile

Variable	Vit E		Vit E/TC		Vit E/(TC+TG)	
	r	p	r	p	r	p
LDL (mg/dl)	0.045	0.398	-0.104	*0.048	-0.041	0.437
Total Cholesterol (mg/dl)	0.053	0.313	-0.127	*0.016	-0.097	0.067
Triglycerides (mg/dl)	0.018	0.734	-0.040	0.448	-0.168	**<0.001
HDL (mg/dl)	0.036	0.492	-0.048	0.367	0.030	0.570

HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol. * P value<0.05 and ** P value<0.001

Table 4: Association between Vitamin E, Vit E/TC, Vit E/(TC+TG) with Dyslipidemia characteristics

Variable		Vit E	Vit E/TC	Vit E/(TC+TG)
Cholesterol(mg/dl)	Normal	0.15(0.50)	0.093(0.32)	0.058(0.17)
	Hypercholesterolemia (TC≥200)	0.15(0.98)	0.065(0.38)	0.040(0.24)
	p.value	.760	.016	.044
LDL (mg/dl)	Normal	0.15(0.49)	0.093(0.25)	0.058(0.17)
	High LDL (LDL≥130)	0.15(0.99)	0.062(0.42)	0.0402(0.28)
	p.value	.550	.072	.345
Triglycerides (mg/dl)	Normal	0.15(0.608)	0.086(0.35)	0.056(0.24)
	High triglycerides (TG≥150)	0.15(0.502)	0.071(0.30)	0.036(0.12)
	p.value	.855	.394	.003
HDL (mg/dl)	Normal	0.19(0.53)	0.11(0.28)	0.059(0.21)
	Low HDL (M<50), M	0.06(0.75)	0.048(0.40)	0.029(0.21)
	p.value	.050	.230	.044
	Normal	0.056(0.89)	0.027(0.45)	0.017(0.28)
	Low HDL (F<40), F	0.15(0.46)	0.095(0.29)	0.058(0.16)
	p.value	.591	.221	.504
Dyslipidemia	Without Dyslipidemia	0.16(0.49)	0.11(0.31)	0.08(0.24)
	With Dyslipidemia	0.15(0.61)	0.07(0.35)	0.05(0.20)
	p.value	0.410	0.205	0.033

Value are expressed as interquartile range for non-normally distributed variable. HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol. * P value<0.05 and ** P value<0.001

Table5: Effect of BMI and risk factors Dyslipidemia on vitamin E in Univariate and Multivariate linear regression model

		Univariate linear regression model					
Variable		Vit E		Vit E/TC		Vit E/(TC+TG)	
		B	P	B	P	B	P
BMI (kg/m^2)		-0.010	0.399	-0.005	0.324	-0.005	0.101
LDL (mg/dl)		0.004	0.010*	0.000	0.495	0.000	0.250
Triglycerides (mg/dl)		0.003	<0.001**	0.001	0.007*	-1.37	0.920
Total Cholesterol (mg/dl)		0.006	<0.001**	0.001	0.074	0.000	0.165
HDL (mg/dl)	Female	0.009	0.234	0.002	0.489	0.003	0.196
	Male	0.009	0.264	-1.57	0.996	0.002	0.258
		Multivariate linear regression model					
Variable		Vit E		Vit E/TC		Vit E/(TC+TG)	
		B	p	B	p	B	p
BMI (kg/m^2)		-0.021	0.070	-0.008	0.156	-0.005	0.099
LDL (mg/dl)		0.000	0.834	0.000	0.806	-5.569	0.992
Triglycerides (mg/dl)		0.002	0.007*	0.001	0.040*	-8.362	0.971
Total Cholesterol (mg/dl)		0.004	0.187	0.000	0.700	0.000	0.670
HDL (mg/dl)		0.006	0.358	0.003	0.399	0.002	0.249

HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; BMI: Body Mass Index.
* P value<0.05 and ** P value<0.001