Serum vitamin-E levels and its relation to clinical features in nonalcoholic fatty liver disease with elevated ALT levels

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Abstract

Background : **Oxidative stress and free oxygen radicals play an important role in the progression from simple fatty liver to steatohepatitis. Deficiency of antioxidants like vitamin-E has been reported to trigger this progression. The main aims of our study were to measure plasma vitamin-E levels in nonalcoholic fatty liver disease (NAFLD), to explain its relationship with biochemical parameters and to examine the possible therapeutic and prophylactic role of vitamin-E.**

Methods : **52 patients with NAFLD and elevated liver function tests were enrolled. After 6 months of follow up with a standard low-fat, low-calorie diet, changes in liver enzymes were evaluated.**

Results : **Deficiency of vitamin-E was detected in 16 patients with NAFLD. Homogenous echo pattern of the liver and attenuation was found to be significantly higher in the low vitamin-E group (p = 0.03). The low vitamin-E group had significantly higher levels of triglyceride (p = 0.02). After 6 months, patients in the low vitamin-E group did not respond to the diet and no decrease in ALT levels was detected (p = 0.04).**

Conclusion : **This is the first study measuring the serum vitamin-E levels in nonalcoholic fatty liver disease. A correlation was found between low vitamin-E levels, high triglyceride levels, as well as sonographic findings, both of which are negative prognostic factors causing progression of fatty liver to steatohepatitis. Patients with low vitamin-E levels did not respond to a classical diet for fatty liver disease. Based on the data, we suggest that diet alone is not adequate for patients with fatty liver, and vitamin-E supplementation should be added.** (Acta gastroenterol. belg., **2006**, 69, **5-11**).

Key words : nonalcoholic fatty liver disease, oxidative stress, vitamin-E.

Introduction

Non-alcoholic steatohepatitis (NASH) is one of the causes of hepatic injury with elevated transaminase levels. NASH, along with other forms of nonalcoholic fatty liver disease (NAFLD), is a chronic liver disease that is gaining increasing importance. In the general population the prevalence of NAFLD and NASH has been reported as 20% and 3%, respectively in recent studies (1). A two-hit hypothesis has been postulated to explain the pathogenetic mechanisms in NASH ; the first is the development of steatosis and the second is the triggering of necroinflamation which includes endotoxin induced cytokin release, oxidative stress and an increased supply of potentially toxic free fatty acids (2-4). Oxidative stress and free oxygen radicals, key components of the so called second hit hypothesis, play a major role in the progression from simple fatty liver to steatohepatitis. Deficiency of antioxidants like vitamin E and vitamin C has been reported to trigger this progression (5). However, large and long term placebo controlled studies are lacking.

Vitamin E is known to be an antioxidant substance, chemically scavenging free radicals, which is fat soluble, readily available, and relatively safe with less adverse effects than other antioxidants (6-10). In recent years, the role of vitamin-E in the prophylaxis and treatment of NASH has been studied (3). It has been professed to slow the progression of fatty liver to steatohepatitis, which occurs due to oxidative damage attributed to the reactive oxygen species mentioned on the second hit hypothesis. Recent studies have shown that increased liver enzymes are lowered by vitamin E supplementation regardless of the baseline serum levels (11-13). It is suggested that vitamin E treatment combined with vitamin C, improves fibrosis in patients with nonalcoholic steatohepatitis (14, 15). Vitamin E treatment itself resulted in decreased plasma TGF-ß levels and transaminase levels in a recent study (16). In this study, improvement of inflammation and fibrosis on liver biopsies were detected as well.

Aim

The main aims of our study was to measure plasma vitamin-E levels in nonalcoholic fatty liver disease patients with elevated liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT)), and to try to explain the relationship between vitamin-E and clinical features and biochemical parameters of nonalcoholic fatty liver patients with elevated enzymes. Based on the data of this study, the secondary aim was to examine the possible therapeutic and prophylactic role of vitamin-E in nonalcoholic fatty liver patients with elevated liver enzymes after 6 months of follow up with a standard low-fat, low-calorie diet by measuring the plasma ALT levels.

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Table 1. — **USG findings of the patients according to groups**

Ultrasonography		*Vitamin-E deficient group (n = 16)* (number, %)	*Normal Vitamin-E group (n = 36)* (number, %)
Echopattern of liver	Homogenous	15 (93.8)	29 (80.6)
	Non-homogenous	1 (6.3)	7 (19.4)
Liver size	Normal	11 (68.8)	29 (80.6)
	Minimal hepatomegaly	4 (25)	4 (11.1)
	Evident hepatomegaly	1 (6.3)	3 (8.3)
Attenuation*	Absent	4 (25)	12 (33.3)
	Present	12 (75)	24 (66.7)
Hepatic venous flow	Monophasic	4 (25)	12 (33.3)
	Biphasic	1 (6.3)	2 (5.6)
	Triphasic	11 (68.8)	22 (61.1)

* Attenuation frequency was significantly higher in vitamin E deficient group. Other ultrasonographic findings were not significantly different between groups.

Material and methods

Subjects

A total number of 214 patients who were referred to Gastroenterology, Cardiology, and Internal Medicine outpatient clinics with elevated liver function tests was enrolled in the study. Patients whose ALT and/or AST were higher than 40 U/L, and aged between 18 and 65 were enrolled. After abdominal ultrasound was carried out, and markers for viral and autoimmune liver disease such as Hepatitis B, C, D, anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (ASMA), anti-liver kidney muscle (anti-LKM) anticores were found negative, diagnosis of non-alcoholic fatty liver disease was made. Enhanced echogenicity had to be detected for non-alcoholic fatty liver disease diagnosis. In ultrasound findings, diffuse enhanced echogenicity in the entire liver was named as homogenous echo pattern, whereas enhanced echogenicity detected together with the spared areas was named as non-homogenous echopattern. While detecting the size of the liver passing the lower border of the kidney was accepted as hepatomegaly. Hepatic venous flow of the patients, which is normally triphasic, was evaluated as monophasic, biphasic and triphasic. The ultrosounograpic findings of the patients according to Vitamin E deficient and normal groups are depicted in Table 1 (Table 1).

Exclusion criteria were diabetes mellitus, exposure to hepatotoxic drugs, alcohol consumption, and positive HBs and anti-HCV serology. Hepatic fat may account for up to 5% of the weight of normal liver and is often increased in other liver diseases including Wilson's disease, autoimmune liver disease, hemochromatosis, galactosemia, hepatitis C virus (HCV) infection, and alcoholic liver disease. Therefore, these diseases as well as the secondary causes of NAFLD should be excluded before a reliable diagnosis of primary NAFLD could be made. The exclusion of HCV and alcoholic liver disease is particularly important because of their high prevalence. The reliable diagnosis of NAFLD can be made after excluding alcohol consumption. The limit of the amount of alcohol consumed is controversial in different studies. The amount suggested as hepatotoxic is 20-30 g daily in female and 40 g daily in male (17-19). Hepatosteatosis develops even with daily consumption of 20 g alcohol (20). Therefore, the limit for alcohol consumption is usually chosen as 20 g daily in most studies. As a result we excluded either male and female consuming 20 g or more alcohol daily. Fatty liver is a common problem in diabetic patients. To examine the exact relationship between vitamin E and NAFLD, and to minimize the effect of diabetes mellitus on fatty liver disease progression, diabetic patients were not included in study. Seventy two diabetic patients, 32 patients using hepatotoxic drugs (i.e. phenytoin, amiodorone, L-thyroxin, tamoxifen, statins and lithium), 41 patients who had a history of alcohol consumption were excluded from the study. HBs antigen was positive in 4 patients, and anti-HCV antibodies were detected in 3 patients. These patients were excluded as well (Table 2). After exclusion, 52 nonalcoholic fatty liver disease patients with elevated liver function tests were enrolled in the study. Inclusion criteria for the study are listed in Table 3 (Table 3). Verbal informed consent was obtained from all of the patients.

After the listed conditions interacting with NAFLD were excluded, 52 patients with nonalcoholic fatty liver disease and elevated liver function tests underwent further assessment including a detailed lipid profile, measurement of serum ferritin, iron, transferrin, immunglobulins, fasting glucose, C-peptide and insulin levels. Oral glucose tolerance test was also performed. Antiinsulin, antigliadin, antiendomicial antibodies, serum vitamin-E levels were evaluated. Physical measurements included body mass index and umbilical gluteal circumference. After 6 months of follow up with a standard low-fat, low-calorie diet, changes in liver enzymes, particularly ALT levels, were compared in both low and normal vitamin-E groups.

Table 2. — **Subjects enrolled in the study**

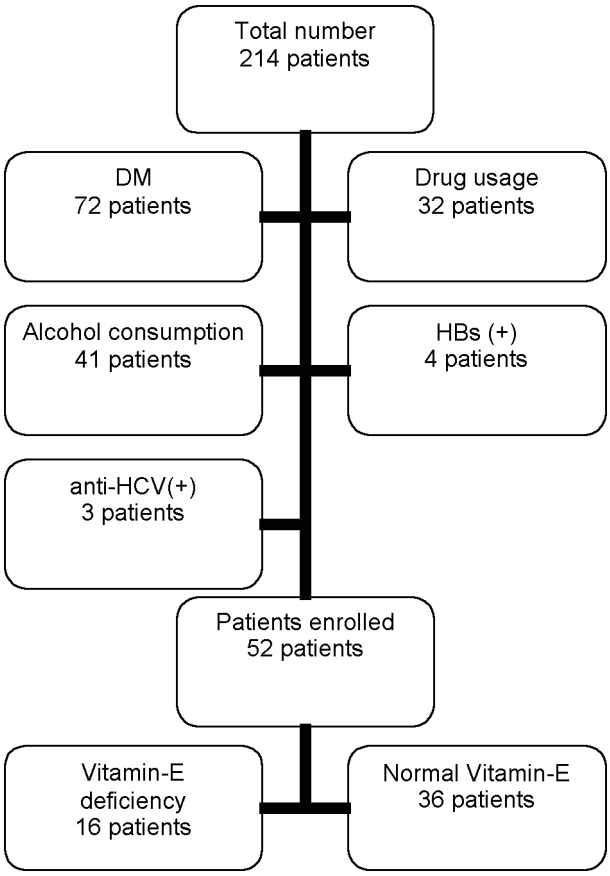

Table 3. — **Inclusion criteria for the study**

Patients without diabetes mellitus
Patients not using hepatotoxic drugs
Patients not consuming > 20 g alcohol/day
Patients with negative serology for the hepatitis viruses and autoimmune hepatitis
Patients with normal serum serruloplasmin level
Ages between 18 and 65
ALT levels > 40 U/L and/or AST levels > 40U/L
Fatty liver detected on abdominal ultrasound
Patients without cognitive impairment
Patients admitting the verbal inform consent for the study

Biochemical analysis

Blood samples were obtained after a 12 hour overnight fast. Serum vitamin E levels were measured in Hacettepe University's Pediatric Metabolism Division Laboratories by the same biologist, and the samples were taken simultaneously with liver function tests and other laboratory parameters. Vitamin E was measured by using the Kimble test tube, Beckmann spectrometer and terpyridyl, and the normal range was 0.8-1.5 mg/dl.

Statistical analysis

Categorical variables are demonstrated as frequencies, and continuous variables are demonstrated as means and standard deviations. Descriptive and frequency analysis of the results were first done and then the chi-square test was used to explain the relationship between vitamin-E levels and other parameters. Data of vitamin E levels are reported as mean ± standard deviation (SD). The Pearson chi-square test was used to compare categorical variables. Age distribution between groups was tested by using one-way analyses of variance method. Sex distribution between groups was tested by using Pearson chi-square test. Means were compared by ANOVA. Comparison of the data before and after 6 months of diet was examined by paired samples T-test. Statistical Package for Social Sciences (SPSS) for Windows version 10.0 statistical package was used.

Results

Demographic features of the total sample, vitamin E deficient group and the normal vitamin E group are depicted in Table 4, laboratory findings such as liver enzymes and lipid-lipoprotein levels are demonstrated in Table 5, and results of the metabolic status of the patients are depicted in Table 6 (Table 4, Table 5 and Table 6). When the serum vitamin E levels were examined, the mean value was found to be 0.96 mg/dl ± 0.26SD and deficiency of vitamin-E was detected in 16 patients (30.8%). After statistical analysis of the two groups of patients with normal and low vitamin E levels, it was found that the sex and age distribution of both groups were similar.

When sonographic findings of the liver were examined, a homogenous echo pattern was observed in 93.8% and attenuation was present in 75.0% of the patients with low serum E-vitamin levels. The relationship between sonographic findings and low vitamin E levels was not statistically significant (Table 1).

The association between lipid levels, lipoprotein levels and low vitamin E were examined. Deficiency of vitamin E was found to be correlated with high triglyceride levels. When the cut-off value for triglyceride levels was set at 150 mg/dl in accordance with to NCEP ATP-III criteria (21), 40% of the low vitamin E group and 11% of the normal vitamin-E group had high triglyceride levels ($p = 0.02$). No relationship was found between low vitamin E levels and other lipoprotein levels such as lipoprotein (a), apolipoprotein A1, apolipoprotein B, low density lipoprotein, high density lipoprotein or total cholesterol.

The difference between the low and normal vitamin E groups with regards of hepatocyte enzymes levels, fasting plasma glucose, oral glucose tolerance test results, fasting insulin levels, fasting C-peptide levels, anthropometric measures, antinsulin antibodies and serum iron parameters was not significant. Fifty percent of the

Table 4. — **Demographic features of the patients**

	Total Sample (n = 52) (number, %)	*Normal Vitamin E group (n = 36)* (number, %)	*Vitamin-E deficient group (n = 16)* (number, %)
Sex Male Female	32, 61.5 20, 38.5	22, 61.1 14,38.9	10, 62.5 6, 37.5
Age ≤ 45 > 45	30, 57.7 22, 22.3	23, 63.9 13, 36.1	7, 43.9 9, 56.3
Family history of DM	19, 36.5	13, 36.1	6, 37.5
Hypertension history	12, 23.1	9, 25.0	3, 18.8
Smoking > 10 pack/year	19, 36.5	17, 47.2	2, 12.5

Demographic features were similar between groups.

Table 5. — **Serum lipid-lipoprotein levels and liver function tests of the patients**

Laboratory findings		*Total Sample (n = 52)* (number, %)	*Normal Vitamin E group (n = 36)* (number, %)	*Vitamin-E deficient group (n = 16)* (number, %)
AST levels	< 40U/L 40-80U/L > 80U/L	33, 63.5 12, 23.0 7, 13.5	23, 63.9 8, 22.2 5, 13.9	10, 62.5 4, 25.0 212.5
ALT level	50-80U/L ≥ 80U/L	33, 63.5 19, 36.5	21, 58.3 15, 41.7	12, 75.0 4, 25.0
GGT level	< 40U/L ≥ 40U/L	28, 53.8 24, 46.2	19, 52.8 17, 47.2	9, 56.2 7, 43.8
ALP level	< 280U/L ≥ 280U/L	36, 69.2 16, 30.8	24, 66.7 12, 33.3	12, 75.0 4, 25.0
Albumin level	< 3.5 mg/dl ≥ 3.5 mg/dl	5, 9.6 47, 90.4	3, 8.3 33, 91.7	2, 12.5 14, 87.5
HDL level	< 45 mg/dl ≥ 45 mg/dl	24, 46.2 28, 53.8	18, 50.0 18, 50.0	6, 37.5 10, 62.5
LDL level	≤ 100 mg/dl 100-160 mg/dl ≥ 160 mg/dl	11, 21.2 28, 53.8 13, 25	7, 19.4 19, 52.8 10, 27.8	4, 25.0 9, 56.2 3, 18.8
TG level*	< 200 mg/dl ≥ 200 mg/dl	35, 67.3 17, 32.7	21, 58.3 15, 41.7	14, 87.5 2, 12.5
TC	< 200 mg/dl ≥ 200 mg/dl	39, 75 13, 25	26, 72.2 10, 27.8	13, 81.2 3, 18.8
Apo-A**	≤ 100 mg/dl 100-199 mg/dl	6, 11.5 46, 88.5	6, 16.7 30, 83.3	0, 0.0 16, 100.0
Apo-B	50-110 mg/dl ≥ 110 mg/dl	22, 42.3 30, 57.7	14, 38.9 22, 61.1	8, 50.0 8, 50.0
Lipoprotein-a	< 30 mg/dl ≥ 30 mg/dl	31, 59.6 21, 40.4	20, 55.6 16, 44.4	11, 68.8 5, 31.3

* TG levels were significantly higher in the normal vitamin E group ($p = 0.002$). ** APO A levels were significantly higher in vitamin E deficient group ($p = 0.002$). The relationship between the other parameters and Vitamin E were non-significant.

patients with antigliadine antibody seropositivity and 23% of the patients with antigliadin antibody seronegativity had low vitamin E levels, but this difference was not statistically significant. It has been stated that antigliadin and tissue transglutaminase antibodies can be seropositive in NASH (22,33). Besides it is known that liver enzymes may be elevated in asymptomatic Celiac disease. Therefore, antigliadin antibodies were studied to exclude asymptomatic Celiac disease and to find out the relationship between the antibodies and ALT, AST and Vitamin E levels, but no significant relation was found.

A standard diet was given to all patients provided by the same nutritionist for a period of 6 months. All of the patients enrolled were evaluated at follow-up visits every 3 months. Compliance to the diet was asked and reinforced at each visit. Anthropometric measurements were measured again after 6 months of diet therapy.

Table 6. — Metabolic properties of the patients

Clinical parameters		*Total Sample (n = 52)* (number, %)	*Normal Vitamin-E group (n = 36)* (number, %)	*Vitamin-E deficient group (n = 16)* (number, %)
Diastolic pressure	< 85 mmHg	28, 53.8	18, 50.0	10, 62.5
	≥ 85 mmHg	24, 46.2	18, 50.0	6, 37.5
Systolic pressure	< 135 mmHg	31, 59.6	19, 52.8	12, 75.0
	≥ 135 mmHg	21, 40.4	17, 47.2	4, 25.0
Fasting blood glucose	≤ 110 mg/dl	40, 76.9	28, 77.8	12, 75.0
	110-126 mg/dl	12, 23.1	8, 22.2	4, 25.0
2nd hr plasma glucose	≤ 140 mg/dl	38, 73.1	25, 69.4	13, 81.2
	140-200 mg/dl	14, 26.9	11, 30.6	3, 18.8
Fasting c-peptide levels	0.6-2.8 ng/ml	20, 38.5	14, 38.9	6, 37.5
	≥ 2.8 ng/ml	32, 61.5	22, 61.1	10, 62.5
Fasting insulin levels	2.1-22 yIU/ml	46, 88.5	33, 91.7	13, 81.2
	22 yIU/ml	6, 11.5	3, 8.3	3, 18.8
Antiinsulin anticore	0-7%	46, 88.5	31, 86.1	15, 93.8
	≥ 7%	6, 11.5	5, 13.9	1, 6.2
Waist circumference basal	< 90 cm	7, 13.5	4, 11.1	3, 18.8
	90-110 cm	30, 57.7	21, 58.3	9, 56.3
	≥ 110cm	15, 28.8	11, 30.6	4, 25.0
Hip circumference	< 100cm	10, 19.2	6, 16.7	4, 25.0
	≥ 100cm	42, 80.8	30, 83.3	12, 75.0
Body Mass index	< 30	29, 55.8	20, 55.6	9, 56.3
	≥ 30	23, 44.2	16, 44.4	7, 43.8
Waist-hip ratio	< 0.9	11, 21.2	7, 19.4	4, 25.0
	≥ 0.9	41, 78.8	29, 80.6	12, 75.0

No significant relationship was found between clinical parameters and vitamin E.

Levels of liver enzymes, lipid and lipoprotein levels were measured at control visits as well. After 6 months of follow up, liver function tests of 45 patients (87%) returned to normal levels with the standard diet. Despite the diet, 7 patients (13%) continued to have high liver enzyme levels and liver biopsy had to be performed. The baseline vitamin E levels were low in 6 of the 7 patients who failed to achieve liver enzyme of normal levels ($p = 0.04$). In the low vitamin E group, 6 patients did not respond to the diet and a decrease in ALT levels was not observed at the end of the sixth month ($p = 0.04$). The results of the liver biopsy showed focal micro and macro vesicular steatosis in 2, diffuse micro and macro vesicular steatosis in 2 and necroinflamation in 3 patients.

Evaluation of the anthropometric parameters and lipid levels were made at each control visit. Waist circumference, hip circumference, BMI, body weight, LDL and total cholesterol significantly decreased after 6 months of diet therapy ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p = 0.001$, $p < 0.001$ and $p < 0.001$ respectively). Changes in the serum HDL cholesterol and TG levels were not statistically significant. In the vitamin E deficient group, decrease in only waist circumference, hip circumference, BMI, LDL and total cholesterol were significant ($p = 0.003$, $p < 0.001$, $p < 0.001$, $p = 0.024$ and $p = 0.014$ respectively) whereas, body weight, HDL cholesterol and TG were not.

Discussion

Oxidative stress plays one of the major roles in progression from fatty liver to steatohepatitis. Reactive oxygen metabolites cause steatohepatitis by means of lipid peroxidation, cytokine induction and fas ligand induction (23). Free oxygen radicals primarily affect unsaturated fats by triggering the lipid peroxidation, which is the pathogenesis of NASH. Oxidative stress also causes the release of inflammatory cytokines such as tumor necrosis factor alpha (TNF-ß) which triggers necroinflamation of the hepatocytes (24). The main reason causing liver damage is the decrease in the amount of glutathione sulphate (GS) which is the main antioxidant in the liver. In animal models, it has been shown that a decrease in GS levels causes steatohepatitis (25).

The antioxidant property of vitamin E is well known. Vitamin E increases the production of transforming growth factor-b which plays a major role in fibrosis and also increases mRNA-procollagen-I expression in stellate cell (9). Vitamin E suppresses TNF-a and decreases interleukin 1, 6 and 8 expressions (9). Because of its antioxidant effects, its possible role in NAFLD is being studied in recent years.

Vitamin E is a well known molecule with antioxidant properties and is widely being used in several clinical settings such as cardiovascular diseases (26,27), stroke

prevention (28-30), prevention of dementia (28,31), and prevention and treatment of steatohepatitis (32). Recently, studies regarding relationship between liver pathologies and vitamin-E have gradually increased (4). Strauss et al reported that obese children have low vitamin-E levels and carotene levels compared to children with normal weight. It has been claimed that vitamin E treatment decreases necroinflamation but not the steatosis (6). Lavine *et al.* had studied 16 obese children with normal vitamin E levels who had steatohepatitis, and they had shown that 400-1200 IU/day vitamin E supplementation normalizes the hepatocyte enzyme levels within 3 months, but the stage of steatosis remained unaffected. An increase was detected in hepatocyte enzyme levels after discontinuation of vitamin E, in this study (11). This was the pilot study regarding the therapeutic role of vitamin E in NASH. In a second study, 12 patients with NASH proven by liver biopsy were given 300 mg/day vitamin E for 1 year, and improvement in liver enzymes were observed (13). Based on the data of these two studies, vitamin E supplementation in addition to weight loss is recommended to patients with NASH (2,23,33). Harrison *et al.* treated 45 NASH patients with a combination of vitamin E and C for 6 months (15) and have found significant improvement in fibrosis, but no changes in inflammation and hepatocyte necrosis. Kugelmas et al found that TNF, interleukin 8, and interleukin 6 concentrations were significantly elevated and vitamin E therapy did not influence plasma cytokine levels in patients with NASH (10). In the above-mentioned studies, vitamin E was given to the patients regardless of their baseline serum level and circulating levels of vitamin E were not measured.

The specific aim of our study was to examine the relationship between serum vitamin E levels and the clinical and anthropometric features of NAFLD and the possible effect of serum vitamin E levels after 6 months of diet therapy. Our study is the first study measuring the serum vitamin E levels in nonalcoholic fatty liver disease ; however, further studies involving a greater number of patients are needed to fully establish such an association. In our study, low vitamin E levels were in correlation with high triglyceride levels, and ultrasonographic findings such as homogenous echo pattern and attenuation. This is an important finding which suggests vitamin E deficiency can trigger this progression since hyperlipidemia, homogenous echopattern and attenuation of the liver in liver sonography are among the well-known negative prognostic factors leading to the progression of fatty liver to steatohepatitis. It is for this reason that vitamin E deficient patients should be treated with vitamin E supplements for the prevention of steatohepatitis.

All of our patients had elevated liver function tests and the fatty liver disease diagnosis was demonstrated with sonography and computerized tomography of the liver. Although the number of patients enrolled in the study is small, the difference in ALT levels observed between the low and normal vitamin E level groups gives some clues about the role of vitamin E or antioxidants in the progression of fatty liver. A limitation of this study is the absence of liver biopsy, but some authors propose to use the term possible steatohepatitis in nonalcoholic fatty liver disease for patients with elevated liver function tests. In our study, patients with low vitamin E levels did not respond to a classical diet for fatty liver disease when compared to the patients with normal vitamin E levels. However, improvement in anthropometric measures and serum lipid levels, except in HDL and total cholesterol levels, were detected in both vitamin E deficient and normal vitamin E groups. In vitamin E deficient group, only body weight did not decrease significantly unlike the normal vitamin E group. Based on the data of this study, we suggest that diet alone is not adequate for patients with fatty liver, and vitamin E supplementation should be added to the therapy. Further studies which would be carried out with liver biopsies and measurement of the local vitamin E levels or its activity in liver tissue will be necessary to understand the relationship between vitamin E and fatty liver disease progression. This is the first study which examines the relationship between fatty liver disease and levels of vitamin E, together with other parameters such as lipids, lipoprotein levels, ultrasonographic findings of the liver and antigliadin antibodies.

Vitamin E has both systemic and local effects. If the local effects of the vitamin on the liver necroinflamation stage can be studied in future studies, clinicians would attain more knowledge about the vitamin's role in the pathogenesis, prevention and treatment of fatty liver and steatohepatitis. Moreover, because of its few side effects and its antioxidant properties, vitamin E should be given to fatty liver patients for the prevention of steatohepatitis.

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