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Diminished Plasma Levels of Vitamin E in Patients with Severe Viral Hepatitis

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RRR-alpha-Tocopherol (Vitamin E) was assayed in plasma of 48 patients with viral hepatitis and of 32 healthy controls. In patients with highly elevated serum transaminases (ALT >100 U/L) vitamin E plasma levels were significantly lower (17.5 ± 4.8 μmol/L) than in controls (22.7 ± 4.2 μmol/L, $p < 0.01$). The vitamin E/ lipid ratios (3.12 ± 0.63 μmol/g) in these patients were 33% lower than those of the controls (4.68 ± 0.54 μmol/g). The lowered vitamin E levels in patients with acute or chronic viral hepatitis with high activity of disease may be due to free radical-mediated liver injury.

Keywords: Alpha-tocopherol, vitamin E, liver, viral hepatitis, free radicals, antioxidants, oxidative stress

INTRODUCTION

Vitamin E is a lipophilic antioxidant located in cell membranes and in lipoproteins[1,2]. In vitro experiments demonstrate that vitamin E protects lipophilic cellular compartments against lipid peroxidation, thus contributing to sustain biological functions under the conditions of oxidative stress[3,4]. Oxidative stress has been associated with the development of various diseases, and the role of vitamin E deficiency as a risk factor is under investigation[5,6]. Diminished vitamin E plasma levels have been reported in hemolytic disorders[7], neurological afflictions[8] and in arteriosclerosis[9]. Recently, we found decreased alpha-tocopherol levels in plasma of patients suffering from alcoholic liver disease, hemochromatosis and Wilson's disease[10].

In this study, we address the question whether viral hepatitis is associated with diminished tocopherol plasma levels.

MATERIALS AND METHODS

Forty-eight patients with viral hepatitis (age 45 ± 13 years, 30 male and 18 female) were studied. Fifteen patients suffered from hepatitis B (13

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chronic hepatitis B, 2 acute hepatitis B), thirty-two patients suffered from chronic hepatitis C and one patient from acute hepatitis A. Five of the patients with hepatitis C were studied while being treated with alpha-interferon, all others prior to therapy with alpha-interferon. Thirty-two healthy adults served as controls (age 34 ± 6 years, 21 male and 11 female).

Evaluation of Vitamin E Status

Blood samples: Subjects fasted overnight for 12 h. Whole blood (10 ml) was collected in plastic tubes containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. Plasma was separated by centrifugation (3000 rpm, 10 min, room temperature) and was then pipetted into another vessel, and one part butyl-hydroxy-toluene (BHT, 1% in ethanol) was then added to 100 parts of plasma (0.45 mM final conc.). Plasma samples were stored at –24° for less than 2 months before vitamin E contents were determined. Data were expressed as μmol alpha-tocopherol/L plasma or as the ratio of vitamin E to lipids. Serum lipids were calculated as the sum of serum cholesterol, triglycerides and phospholipids[15]. Vitamin E analysis was performed essentially as described in[16] with modifications as described previously[10].

Statistics: Data are expressed as means ± SD. Comparison among multiple groups was made by analysis of variance with Student's t-test. Differences were designated as significant when p was < 0.01.

RESULTS

Vitamin E in Plasma of 48 Patients with Viral Hepatitis

Forty-eight patients (age 45 ± 13 years) with acute or chronic viral hepatitis A, B or C were examined. Eight patients with chronic hepatitis B or C had normal serum transaminases (ALT < 25 U/l, AST < 22 U/L; see Table 1). The other 40 patients exhibited elevated ALT > 25 U/L. In 14 of these patients ALT levels were higher than 100 U/L. The increase of ALT in plasma of patients with viral hepatitis is taken as an indicator of liver cell damage.

The vitamin E level in plasma of the total group of patients with viral hepatitis (n = 48) was not significantly different from controls (Table 1). However, low alpha-tocopherol plasma levels were clearly associated with increased liver transaminases. Patients with highly elevated transaminases (ALT > 100 U/L, n = 14) exhibited vitamin E levels 23% lower than controls.

Vitamin E/Lipid Ratios

When vitamin E levels were corrected for plasma lipids, significantly lower vitamin E/lipid ratios were found in the group designated "all patients" with viral hepatitis as compared to controls (Table 1). The lowest vitamin E/lipid ratio was determined in patients with ALT > 100 U/l, 33% lower than controls (p < 0.01).

TABLE I Plasma Vitamin E and Vitamin E/Lipid Ratios in 48 Patients with Viral Hepatitis

Group	n	Vitamin E (μmol/l)	Vitamin E/lipids (μmol/g)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Bilirubin (mg/dl)
ALT < 25 U/l	8	28.5 ± 6.7	4.69 ± 0.49	219 ± 41	133 ± 28	256 ± 36	0.7 ± 0.4
ALT > 25 < 100 U/l	26	20.7 ± 5.4	3.78 ± 0.70**	200 ± 37	115 ± 33	233 ± 40	0.8 ± 0.4
ALT > 100 U/l	14	17.5 ± 4.8**	3.12 ± 0.63**	204 ± 30	122 ± 42	235 ± 57	2.2 ± 2.4
All Patients	48	21.1 ± 6.7	3.74 ± 0.83**	204 ± 37	122 ± 39	238 ± 46	1.2 ± 1.5
Controls	32	22.7 ± 4.2	4.68 ± 0.54	178 ± 26	84 ± 31	221 ± 38	0.5 ± 0.2

Results are expressed as means ± SD
**Significantly different from controls (p < 0.01)

As with vitamin E plasma levels, the vitamin E/lipid ratios correlate with the activity of disease indicated by transaminase levels. The correlation coefficient between vitamin E/lipid ratios and transaminases (ALT) was r = −0.50 (see Fig. 1). No significant difference was found between vitamin E/lipid ratios in chronic hepatitis B (vitamin E/lipids = 3.82 ± 1.04 μmol/g; 13 patients) and chronic hepatitis C (vitamin E/lipids = 3.76 ± 0.73 μmol/g; 32 patients), indicating that low vitamin E plasma concentrations are independent of the type of viral hepatitis.

Time Course of Vitamin E Level During Acute Hepatitis A

In one patient with acute hepatitis A (male, 29 years) the time course of vitamin E levels in plasma was monitored for 17 days after admission to the hospital (Fig. 2). The lowest alpha-tocopherol level was found at the outset, when the transaminases were highly elevated (ALT 768 U/L, AST 357 U/L). On day 1, the alpha-tocopherol level was 19.2 μmol/L and the vitamin E/lipid ratio was 2.50 μmol/g. On day 17, when the transaminases were lower (ALT 41 U/L, AST 13 U/L), a 25% higher vitamin E level was observed (23.9 μmol/L). The vitamin E/lipid ratio was 3.55 μmol/g, which was 42% higher as compared to day 1 of hospitalisation. The vitamin E levels and the transaminases changed inversely during the course of the first two weeks, the correlation coefficient being r = −0.92.

In addition to decreasing transaminase levels, a lowering of bilirubin was detected in serum, further indicating a recovery from acute liver damage.

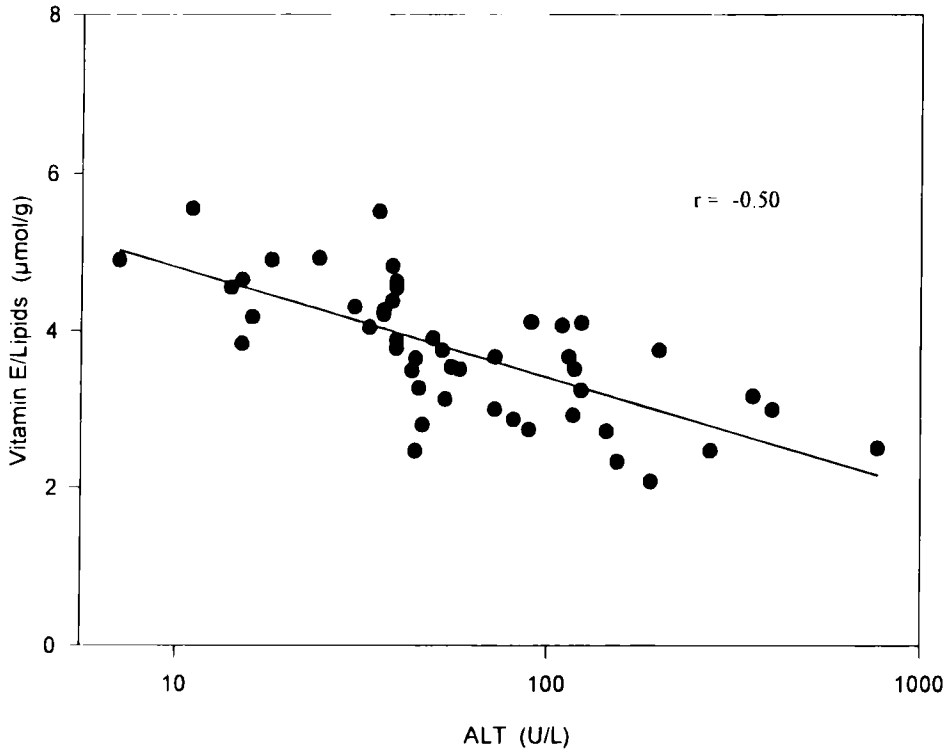

FIGURE 1 Correlation between vitamin E/lipid ratios and the serum transaminases (ALT) in 48 patients with viral hepatitis. The data are given in a log scale.

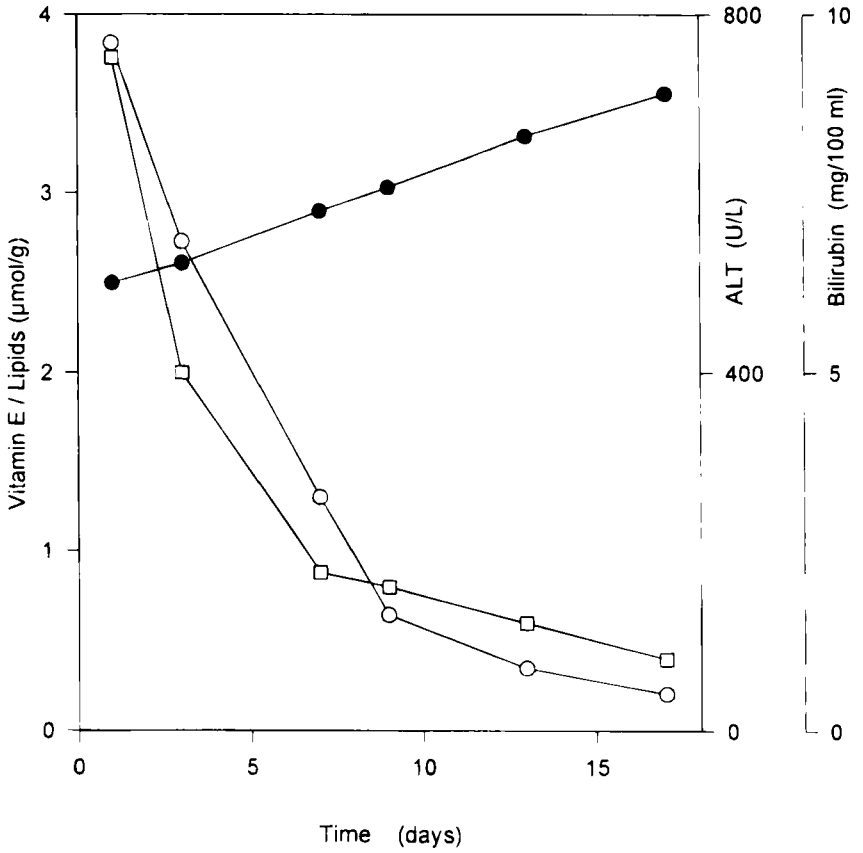

FIGURE 2 Vitamin E/lipid ratios (●) in plasma of a 29-year-old male patient during the course of acute hepatitis A. ALT (○) and bilirubin (❑) levels are also shown. Admission to the hospital was on day 1.

DISCUSSION

Decreased vitamin E serum levels and vitamin E/lipid ratios correlate with acute liver cell injury in patients with acute hepatitis as indicated by increasing transaminase levels (Fig. 1, Table 1).

The low vitamin E levels in plasma might reflect the consumption of this antioxidant by reactive oxygen species generated in progressive inflammatory processes. Upon recovery from the disease, vitamin E levels in plasma were restored, likely due to a diminished consumption while intake was constant (Fig. 2).

An alternative reason could be related to cholestasis. An inverse but not significant relationship between vitamin E uptake and parameters of cholestasis, such as bilirubin levels has been described[20]. In the present study, low vitamin E/lipid ratios and elevated bilirubin levels (>5 mg/dl) were only found in patients with ALT > 100 U/l. However, decreased vitamin E/lipid ratios were also detected in patients without clinical signs of cholestasis (normal serum bilirubin, see Table 1). Thus, diminished vitamin E associated with cholestasis appears not to be the major reason for low vitamin E levels.

Severe liver disease is often accompanied by elevation of serum lipids. In this study only small increases in serum lipids were found in viral hepatitis as compared to controls and the serum lipids did not correlate with the activity of the disease.

Vitamin E deficiency might be a factor increasing the susceptibility of the liver for viral infec-

TABLE II Vitamin E and vitamin E/lipid ratios in patients with various liver disease: Overview from the literature.

Disease	Vitamin E Related to Controls	Vitamin E/Lipids Related to Controls	Reference
viral hepatitis normal ALT and AST	1.25	1.00	this paper
viral hepatitis ALT > 100 U/l	0.77	0.67	this paper
alcoholic liver cirrhosis elevated ALT and AST	0.59	0.72	Bell et al. 1992
alcoholic liver cirrhosis normal ALT and AST	0.93	0.95	von Herbay et al. 1994 (10)
alcoholic liver cirrhosis	0.66	0.86	Munoz et al. 1989 (20)
hemochromatosis untreated	0.68	0.66	von Herbay et al. 1994 (10)
Wilson's disease	0.57	0.63	von Herbay et al. 1994 (10)
primary biliary cirrhosis	0.94	0.62	Sokol et al. 1985 (14)
	0.90	0.62	Munoz et al. 1989 (20)
beta-thalassemia major	0.41		Rachmilewitz et al. 1976 (21)
Gaucher's disease	0.40		Rachmilewitz et al. 1982 (22)
sickle-cell anemia	0.69		Chiu et al. 1982 (23)

Vitamin E and vitamin E/lipids are given as the ratio of the mean patient level to the mean level of healthy controls.

tions. Previous studies showed that liver injury was especially severe in cases of vitamin E deficiency (10,17–19; see Table 2). In vivo experiments demonstrated that toxic hepatitis occurred in LEC rats fed a vitamin E-depleted diet but not in vitamin E-supplemented animals[18].

Since no hepatotoxic side effects are known for vitamin E[24], vitamin E is considered a safe antioxidant for treatment studies in patients with acute viral hepatitis. Vitamin E might also be used in the protection even when low transaminase levels indicate that no acute inflammatory processes are ongoing in patients with chronic viral hepatitis.

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References

[1] G. W. Burton and M. G. Traber (1990) Vitamin E: Antioxidant activity, biokinetics and bioavailability, *Annual Reviews of Nutrition*, **10**, 357–382.
[2] M. G. Traber and H. Sies (1996) Vitamin E in Humans: Demand and Delivery, *Annual Reviews of Nutrition*, **16**, 321–347.
[3] E. Cadenas, M. Ginsberg, U. Rabe and H. Sies (1984) Evaluation of alpha-tocopherol antioxidant activity in microsomal lipid peroxidation as detected by low-level chemiluminescence, *Biochemical Journal*, **223**, 755–759.
[4] P. Palozza, S. Monalla and N. I. Krinsky (1992) Effects of beta-carotene and alpha-tocopherol on radical-initiated peroxidation of microsomes, *Free Radical Biology and Medicine*, **13**, 127–136.
[5] M. J. Stampfer, C. H. Hennekens, J. E. Manson, G. A. Colditz, B. Rosner and W. C. Willett (1993) Vitamin E consumption and the risk of coronary heart disease in women, *New England Journal of Medicine*, **328**, 1444–1449.
[6] E. B. Rimm, M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz and W. C. Willett (1993) Vitamin E consumption and the risk of coronary heart disease in men, *New England Journal of Medicine*, **328**, 1450–1456.
[7] L. M. Corash, M. Sheetz, J. G. Bieri, C. Bartsocas, S. Moses, N. Bashan and J. D. Shulman (1982) Chronic hemolytic anemia due to glucose-6-phosphate dehydrogenase deficiency or glutathione synthetase deficiency: The role of vitamin E in its treatment, *Annals New York Academy of Sciences*, **393**, 348–360.
[8] D. P. R. Muller and M. A. Goss-Sampson (1990) Neurochemical, neurophysiological and neuropathological studies in vitamin E deficiency, *Critical Reviews in Neurobiology*, **5**, 239–264.

[9] D. R. Janero (1991) Therapeutic potential of Vitamin E in the pathogenesis of spontaneous atherosclerosis, *Free Radical Biology and Medicine*, **11**, 129–144.
[10] A. von Herbay, H. de Groot, U. Hegi, W. Stremmel, G. Strohmeyer and H. Sies (1994) Low vitamin E content in plasma of patients with alcoholic liver disease, hemochromatosis and Wilson's disease, *Journal of Hepatology*, **20**, 41–46.
[11] S. G. Pak and E. V. Nikitin (1991) Status of the process of free-radical oxidation and the antioxidation system in patients with a severe course of hepatitis B, *Klin Med Mosk*, **69**, 54–57.
[12] T. Suzuki, T. Kawase and T. Harada (1991) Clinical study of vitamin E status in patients with chronic liver diseases, *Nippon Shokakibyo Gakkai Zasshi*, **88**, 1066–1074.
[13] T. Yoshikawa, S. Takemura and M. Kondo (1982) Alpha-tocopherol level in liver diseases, *Acta Vitaminologica Enzymologica*, **4**, 311–318.
[14] R. J. Sokol, W. F. Balistreri, J. H. Hoofnagle and E. A. Jones (1985) Vitamin E deficiency in adults with chronic liver disease, *American Journal of Clinical Nutrition*, **41**, 66–72.
[15] M. K. Horwitt, C. C. Harvey, C. H. Daum *et al.* (1972) Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy, *Annals New York Academy of Sciences*, **203**, 223–236.
[16] M. E. Murphy and J. B. Kehrer (1987) Simultaneous measurement of tocopherols and tocopheryl quinones in tissue fractions using high performance liquid chromatography with redox cycling electrochemical detection, *Journal of Chromatography*, **421**, 71–82.
[17] B. R. Bacon and R. S. Britten (1990) The pathology of hepatic iron overload: a free radical-mediated process? *Hepatology*, **11**, 127–137.
[18] K. Yamazaki, H. Ohyama, K. Kurata and T. Wakabayashi (1993) Effects of dietary vitamin E on clinical course and plasma glutamic oxalacetic transaminase and glutamic pyruvic transaminase activities in hereditary hepatitis of LEC rats, *Laboratory Animal Sciences*, **43**, 61–67.
[19] H. Bell, A. Bjorneboe, B. Eidsvoll *et al.* (1985) Increased free-radical activity in alcoholics, *Lancet*, **ii**, 291–194.
[20] S. J. Munoz, J. E. Heubi, W. F. Balistreri and W. C. Maddrey (1989) Vitamin E deficiency in primary biliary cirrhosis: gastrointestinal malabsorption, frequency and relationship to other lipid-soluble vitamins, *Hepatology*, **9**, 525–531.
[21] E. A. Rachmilewitz, B. H. Lubin and S. B. Shohet (1976) Lipid membrane peroxidation in beta-thalassemia major, *Blood*, **47**, 495–505.
[22] E. A. Rachmilewitz, A. Kornberg and M. Acker (1982) Vitamin E deficiency due to increased consumption in beta-thalassemia and in Gaucher's disease, *Annals New York Academy of Sciences*, **393**, 336–360.
[23] D. Chiu, E. Vichinsky, M. Yee, K. Kleman and B. Lubin (1982) Peroxidation, vitamin E and sickle-cell anemia, *Annals New York Academy of Sciences*, **393**, 323–335.
[24] H. Kappus and A. Diplock (1992) Tolerance and safety of vitamin E: a toxicological position report, *Free Radical Biology and Medicine*, **13**, 55–74.