

Vitamin E-based therapy is effective in ameliorating transaminasemia in nonalcoholic fatty liver disease

Kaushal Madan, Yogesh Batra, S Datta Gupta,* Bal Chander,*
K D Anand Rajan,* Rajvir Singh,** S K Panda,* S K Acharya

Departments of Gastroenterology, *Pathology, and **Biostatistics,
All India Institute of Medical Sciences, New Delhi 110 029

Background: Comparative trials of ursodeoxycholic acid (UDCA), vitamin E and weight management programs among patients with nonalcoholic fatty liver disease (NAFLD) are lacking. **Aim:** To find an effective single agent or combination of agents for management of NAFLD. **Methods:** In this retrospective study, consecutive patient with histologically confirmed NAFLD with raised ALT were included. The patients received either weight management (exercise and therapeutic lifestyle changes [TLC] diet with a target to reduce body weight 10% in 6 months) (group I) ; weight management + UDCA (300 mg BID) (group II); or weight management + UDCA + vitamin E (400 mg OD) (group III). Outcome measure was normalization of ALT. **Results:** 42 patients (18, 12 and 12 in groups I, II and III, respectively) were included between 1996 and 2004. All patients in group III normalized their ALT levels, which was significantly higher than numbers in group I (8/18) and group II (5/12); ($p=0.003$). Post treatment ALT was significantly lower in group III (28.6 [9.3]) as compared to group I (59.3 [32.2]) and group II (49.0[31.8]); ($p=0.01$). Type of therapy received was the only factor predictive of ALT normalization. **Conclusion:** Combination regimen containing vitamin E appears to be effective in normalizing ALT among NAFLD patients. [*Indian J Gastroenterol* 2005;24:251-255]

Nonalcoholic fatty liver disease (NAFLD) is being recognized as an important cause of chronic liver disease, especially in affluent societies where there is a high prevalence of the metabolic syndrome. The advanced stages of NAFLD have been shown to progress to cirrhosis^{1,2} and there have been reports of hepatocellular carcinoma arising in patients with this disease.³ Insulin resistance has been shown to be the basic pathophysiological mechanism responsible for both the fatty transformation of liver (first hit) as well as the second hit which leads to hepatocyte injury.^{4,5}

Multiple therapeutic agents such as ursodeoxycholic acid (UDCA),⁶ vitamin E,⁷

metformin,⁸ PPAR-gamma agonists⁹ and the lipase inhibitor orlistat¹⁰ have been demonstrated to be useful in NAFLD in small case series. Dietary restriction and exercise have also been shown to improve liver function in patients with NAFLD.¹¹ Conflicting data on the therapeutic efficacy of the above mentioned drugs have been reported in the literature. A recent randomized trial documented lack of benefit of UDCA in comparison to placebo among patients with NAFLD.¹² Vitamin E, by virtue of being an antioxidant, has been shown to be effective in reducing raised transaminases in a significant proportion of patients with NAFLD. Other antioxidants such as betaine have also been shown to be effective in improving transaminase levels.¹³

At our center, we counsel all our patients with NAFLD about dietary restriction and exercise. In the early years of this study, we also prescribed UDCA alone or in combination with vitamin E to those who were willing to try these medications. The present retrospective review was undertaken to assess the therapeutic efficacy of the above three therapeutic regimens among patients with NAFLD.

Methods

This was a retrospective comparative review of data collected during the period July 1996 to June 2004. The study protocol was cleared by the institute's ethics committee.

Patients aged 18-80 years, who had raised ALT at least one and a half times upper limit of normal for at least six months before inclusion and who had biopsy-proven NAFLD¹⁴ were included. Patients who had positive markers for other liver diseases (viral, metabolic, genetic) or who had history of alcohol intake in excess of 20 grams per day were excluded.

Work up

At baseline, all patients had clinical evaluation including measurement of body mass index (weight/height²) along with standard hematological and biochemical tests. These included complete blood counts,

prothrombin time, serum bilirubin, alanine and aspartate aminotransferase, alkaline phosphatase, serum proteins and albumin, fasting blood sugar and serum lipid profile.

Anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-liver kidney microsomal antibodies (ALKM1) were done in all patients using the standard immunofluorescence technique. Eye examination was done to look for KF rings, along with 24 hours' urinary copper estimation¹⁵ to rule out Wilson's disease. Serum ferritin was measured to screen for the presence of iron overload.¹⁶ Viral serology included HBsAg and total anti-HBc (Organon Technika, Boxtel, the Netherlands), and anti-HCV (in-house developed ELISA).¹⁷ Hepatitis C virus (HCV) RNA was tested by reverse transcription-polymerase chain reaction (rt-PCR) technique, also developed at our own laboratory.¹⁷

Homeostasis model assessment for insulin resistance (HOMA-IR) was measured using the formula: Fasting serum insulin (mIU/L) x Fasting plasma glucose (mg/dL) / (22.5 x 18).

The diagnosis of NAFLD was confirmed after exclusion of other causes and by liver biopsy showing characteristic diagnostic features. Liver biopsy was done with 18G Menghini's needle after informed consent. Liver histology was graded and staged using Brunt's classification.¹⁴

Therapeutic protocol

Patients in group I were asked to undergo weight and diet management. They were asked to follow the therapeutic lifestyle changes (TLC) diet¹⁸ and were encouraged to do regular exercises (either jogging or brisk walking for at least 30 minutes each day). The target was to achieve 10% reduction in body weight over 6 months. Patients in group II received in addition to weight management, tablet UDCA in a dose of 600 mg per day orally in two divided doses. Patients in group III received vitamin E in a dose of 400 mg per day orally in two divided doses in addition to UDCA in the above mentioned dose, along with weight management. Data were analyzed in patients who completed at least 6 months of therapy. The patients received the different therapeutic regimens at different time points.

Monitoring

Each patient attended the liver clinic at monthly intervals, when body weight was recorded and blood sample was collected for liver function tests. The

primary outcome measure was normalization of ALT, defined as a value of less than 50 IU/L (upper limit of normal in our laboratory).

Statistical analysis

The continuous variables were expressed as mean (SD) or median (range) and the categorical variables as frequencies and percentages. Continuous variables between the three groups were compared using non-parametric (Kruskal Wallis) test and categorical variables were compared using chi-square test. Within each group, post-treatment AST and ALT were compared using Wilcoxon's sign rank test for paired observations. p value <0.05 was considered significant. The data were analyzed using SAS 8.0 software (SAS Institute, Carry, NC, USA).

Results

A total of 57 NAFLD patients were registered in our liver clinic, of whom 42 patients were analyzed. Thirteen patients did not have liver biopsy done and two did not complete the 6-month therapy. There were 18 patients in group I, 12 in group II and 12 in group III. The median (range) duration of treatment for the three groups was 6 (6-18), 6 (6-8) and 6.5 (6-9) months (p=0.8), respectively. Analysis was done at the completion of six months of therapy. The baseline characteristics in the three groups were not significantly different except for higher median serum triglycerides in group III (Table 1).

Effect of therapy on transaminases

Significantly higher proportion of patients had normalization of transaminases in group III compared to groups II and I (Table 2). There was significant reduction in median ALT levels from baseline after therapy in all the three groups (group I p=0.001, group II p=0.001 and group III p=0.0001). Median post treatment ALT levels were lowest in group III. However in the post-hoc analysis, the difference in ALT levels was significant only between groups I and III (p=0.01).

Thirty patients (71.4%) were successful in achieving weight reduction, but the total weight loss achieved was only modest (2 [0-15] Kg). The median amount of weight reduction achieved in each group was similar (2 [0-8] Kg in group I, 1.5 [0-9] Kg in group II, 2 [0-15] Kg in group III; p=0.7) and the number of patients who were successful in achieving weight reduction was also similar in the three groups. Only 7 (16%) patients achieved the target weight loss (reduction by 10%

Table 1: Baseline characteristics of the three groups

| Parameters | Group I (n=18) | Group II (n=12) | Group III (n=12) | p value |
|---|-------------------|--------------------|---------------------|---------|
| Age (y) | 30.5 (22-42) | 34.5 (25-50) | 35 (26-58) | 0.1 |
| BMI (Kg m ⁻²) | 26.5 (21.3-32.1) | 27.8 (22.5-31.6) | 26.7 (22.1-32.5) | 0.5 |
| Sex (males) | 16 | 10 | 11 | 0.8 |
| Bilirubin (mg/dL) | 0.7 (0.5-2.7) | 0.8 (0.3-1.6) | 0.9 (0.3-2.7) | 0.5 |
| AST (IU/L) | 61 (39-229) | 61.5 (51-115) | 78.5 (29-230) | 0.5 |
| ALT (IU/L) | 95 (56-308) | 111.5 (72-175) | 91.5 (57-162) | 0.7 |
| ALP (IU/L) | 162.5 (87-275) | 165 (108-335) | 145 (98-196) | 0.4 |
| Albumin (g/dL) | 4.6 (4-5.3) | 4.6 (3.6-5.3) | 4.5 (3.0-5.1) | 0.6 |
| Globulin (g/dL) | 3.4 (2.3-4.2) | 3.1 (1.2-3.6) | 3.2 (2.6-3.7) | 0.1 |
| Prothrombin time (seconds above control) | 1.5 (0-3.0) | 1.0 (0-2.0) | 1.5 (0-4.0) | 0.7 |
| Sugar (mg/dL) | 91 (81-122) | 90 (70-140) | 86 (74-150) | 0.3 |
| Cholesterol (mg/dL) | 184 (100-220) | 196 (96-323) | 179 (112-248) | 0.5 |
| Triglycerides (mg/dL) | 125 (67-226) | 155 (75-365) | 195 (138-322) | 0.01 |
| HDL (mg/dL) | 40.5 (28-47) | 42 (37-60) | 41 (36.6-65) | 0.5 |
| Insulin (mIU L ⁻¹) | 10.7 (5-27.5) | 8.3 (2.5-28.4) | 9.2 (2-24) | 0.6 |
| HOMA-IR | 2.6 (1.1-6) | 2.1 (0.5-5.2) | 1.96 (0.4-5.1) | 0.7 |
| Histology | | | | |
| Grade (1/2/3) [n/n/n] | 11/6/1 | 8/3/1 | 7/4/1 | |
| Stage (0/1/2/3) [n/n/n/n] | 9/8/1/0 | 8/3/1/0 | 6/2/4/0 | |

Group I – Weight management; Group II – UDCA + weight management; Group III – UDCA + vitamin E + weight management. Continuous variables expressed as median (range). Categorical variables expressed as frequencies

of body weight over six months): 2 in group I, 2 in group II and 3 in group III.

The number of patients who achieved normalization of transaminases did not differ between those who did (19/30) and who did not (7/12; p=1.0) lose weight and between those who achieved target weight reduction (5/7) and who did not (14/23; p=1.0).

Factors associated with ALT normalization

On univariate analysis, the only factor significantly associated with normalization of ALT was treatment with vitamin E-based therapy. Among patients who had normalization of ALT after treatment, significantly higher number of patients received vitamin E-based therapy than those whose ALT did not normalize after therapy (12/25 in normal ALT group and 0/17 in high ALT group; p=0.0001) (Table 3).

Table 2: Effect of therapy in the three groups

| Parameters | Group I (n=18) | Group II (n=12) | Group III (n=12) | p value |
|---|-------------------|--------------------|---------------------|---------|
| Post treatment AST (IU/L) | 34 (15-117) | 37 (11-57) | 24.5 (15-41) | 0.08 |
| Post treatment ALT (IU/L) | 49 (24-145) | 48 (8-121) | 25.5 (15-46) | 0.007 |
| Number of patients who normalized ALT n (%) | 8 (44.4%) | 5 (50%) | 12 (100%) | 0.003 |
| AST, ALT values as median (range) | | | | |

Discussion

Weight reduction with dietary control, UDCA and vitamin E are the most frequently used therapies among patients with NAFLD. However, data on comparison of efficacy of these three agents in NAFLD and the effect of combination therapy using these agents are scarce in English literature. The present study documented the efficacy of a combination of UDCA, vitamin E and weight management program in normalization of ALT among patients

Table 3: Factors associated with normalization of ALT

| ALT normalization | Yes (n=26) | No (n=16) | p value |
|---------------------------------|---------------|--------------|---------|
| Age (years); (mean[SD]) | 34.6 (8.8) | 32.4 (6.8) | 0.39 |
| Sex (male) | 23 (88.4%) | 14 (87.5%) | 1.0 |
| BMI (Kg/m ²) | | | |
| mean (SD) | 26.7 (3.0) | 26.9 (2.8) | 0.57 |
| BMI <23 | 4 (15.3%) | 2 (12.5%) | 1.0 |
| Therapy group (n) | | | |
| Group I/II/III | 8/6/12 | 10/6/0 | 0.005 |
| Weight reduction | 19 (73%) | 11 (68.7%) | 1.0 |
| Amount of weight reduction (Kg) | | | |
| median (range) | 2 (0-15) | 2 (0-8) | 0.99 |
| Mild histological grade | 16 (61.5%) | 10 (62.5%) | 0.95 |
| Mild histological stage | 22 (84.6%) | 14 (87.5%) | 1.0 |
| Cholesterol (mg/dL) | | | |
| mean (SD) | 176.5 (45.5) | 196.5 (44.5) | 0.17 |
| Triglycerides (mg/dL) | | | |
| mean (SD) | 176.7 (63.8) | 159.6 (92.2) | 0.49 |
| HOMA-IR (mean [SD]) | 2.6 (1.5) | 2.6 (1.1) | 0.93 |

with NAFLD. On univariate analysis, only vitamin E-based therapy was associated with normalization of transaminases.

Efficacy of vitamin E in NAFLD has also been documented by others, albeit in small uncontrolled studies.^{19,20,21} In these studies, the dose of vitamin E used was 300-1200 mg per day. In one study 300 mg vitamin E per day was associated with fibrosis reversal.¹⁸ The useful effect of vitamin E on inflammation and fibrosis among patients with NASH has been attributed to its potent antioxidant action. Oxidant stress has been cited as an important second hit in the pathogenesis of NASH, and obese children have been demonstrated to have significantly decreased serum levels of α -tocopherol.²² The resultant effect on hepatic necroinflammation is reflected in the significant reduction in plasma TGF- β 1 (a potent pro-fibrogenic cytokine) levels in patients who were administered vitamin E as therapy for NASH. Even other potent antioxidant drugs such as betaine,¹³ vitamin C²³ and probucol²⁴ have been found to be useful in reducing necroinflammation in patients with NAFLD. However in a recent randomized controlled trial, metformin was found to be superior to vitamin E in terms of normalization of ALT.²⁵ The poor response to vitamin E in this study may be related to the use of vitamin E alone without the use of prescriptive diet, unlike in our study.

UDCA along with exercise was not found superior to exercise alone in the present study. A recent large multicenter trial could not document any therapeutic benefit of UDCA among patients with NAFLD.¹² This study also documented inadequacy of lifestyle modifications and exercise. While efforts were made to emphasize the importance of this latter mode of therapy to patients, in our study only a modest median weight loss of 2 Kg could be achieved among 70% of patients. Only 7 (16%) patients could achieve target weight loss. Even among those who could not lose weight, ALT normalization occurred in 58% (7/12), probably because of concomitant drug therapy.

A number of studies have documented significant improvement in liver biochemistry as well as liver histology among NAFLD patients who have been prescribed lifestyle modifications.^{11,26,27} A recent report suggested that patients maintained normal ALT only as long as they maintained a weight that was about 10% less than the original weight in obese individuals.¹¹ In all the three groups in our study there was significant reduction in ALT levels

as compared to pretreatment values, and the only common factor in the three groups was a diet and exercise plan. In the subgroup who achieved target weight reduction, more than 70% had normalized their ALT. Therefore, a longer trial of lifestyle measures may be required to achieve the desired effect.

The major drawbacks in the present study are the retrospective design and absence of demonstration of histological improvement after therapy. It is known that patients with normal liver enzymes may harbor advanced stages of NAFLD; on the other hand, a decline in ALT levels may reflect improvement in histology.^{11,28} Further the small number of patients in each group may have resulted in lack of significance. A larger prospective study using similar groups and with vitamin E alone in one of the groups would be able to elaborate the individual effects of vitamin E and UDCA. It would also be important to have both baseline and post-treatment liver biopsy in such a study.

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Correspondence to: Professor Acharya, Head of Department. Fax: (11) 2658 8641, 2658 8663. E-mail: subratacharya2004@yahoo.com

Received March 24, 2005. Received in final revised form October 3, 2005. Accepted October 10, 2005