EFFECT OF ESSENTIALE IN DIABETIC SUBJECTS WITH NON - ALCOHOLIC FATTY LIVER*

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ABSTRACT

Nonalcoholic fatty liver (NAFL) has been reported to be common among subjects with diabetes. However, there are not much therapeutic options for NAFL. In this open labeled clinical trial we studied the effect of Essentiale in diabetic subjects with NAFL. Twenty-eight type 2 diabetic patients attending the out-patient division of M.V. Diabetes Specialities Centre, Chennai and satisfying the inclusion criteria were recruited for the study. High resolution B mode ultrasonography was carried out for diagnosis of NAFL. Liver function markers [Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma Glutamyl transferase (GGT)] were measured. 22 out of the 28 patients (78.5%) were available for follow up. The mean age of the study subjects was 41±8 years and 50% were males. A significant reduction in all the liver enzymes were observed after Essentiale treatment (baseline vs. six months after treatment: ALT: 54.5± 29.6 IU/L vs. 37.1±18.7 IU/L, p< 0.05, AST: 38.0±18.0 IU/L vs. 27.6±12.4 IU/L, p< 0.05, GGT: 38.7±27.5 IU/L vs. 29.6±13.8 IU/L, p< 0.05). Ultrasound studies revealed that the hepatic echotexture improved after *Essentiale* treatment in 12/22 (54.5%) of the study subjects, while there was no change in 9/22 (40.9%), and it worsened in only one patient (4.5%). The study results suggest that Essentiale protects and improves liver function in diabetic subjects with NAFL. Prospective, blinded clinical trials are required to confirm these findings.

KEY WORDS: Non-alcoholic fatty liver (NAFL); Essentiale; Diabetes; Asian Indians.

INTRODUCTION

The liver plays a major role in the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and also synthesize glucose from non-carbohydrate sources. This key function of liver makes it vulnerable to diseases in subjects with metabolic disorders, particularly diabetes (1). Nearly 70-80% of the diabetic subjects have been reported to have hepatic fat accumulation, referred to as is nonalcoholic fatty liver (NAFL) (2). NAFL leads to nonalcoholic steatohepatitis (NASH), a progressive fibrotic disease, which can result in cirrhosis or liver related death (3,4).

Nonalcoholic fatty liver (NAFL) was first reported in 1980's in obese females with diabetes. There is renewed interest recently because of the increased prevalence of NAFL in diabetes and as it has been shown to be a predisposing factor for insulin resistance and hyperinsulinemia (5). Further proof for the association of liver disease with diabetes comes from the Insulin Resistance Atherosclerosis Study (IRAS), which showed that liver function markers like the Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are predictors of incident diabetes (6).

Presently there are not much therapeutic options for nonalcoholic fatty liver except correction of obesity with hypocaloric diets and physical exercise and controlling hyperglycemia with diet, insulin, or oral hypoglycemic agents (7). Any therapeutic intervention, which can target fat accumulation and ameliorate hepatic histology, would be of great benefit. *Essentiale* is prepared from Soya beans, and has phosphatidylcholine as its active ingredient. Phosphatidylcholine (the main component of lecithin) is an integral part of cell membranes and is essential for structural and functional integrity of the cell. It also enhances cell membrane function throughout the body and facilitates the movement of fats in and out of the cells. Though *Essentiale* is routinely used in treatment of liver disorders irrespective of the etiological factor, it's effects on liver function and ultrasound appearance of hepatic echotexture in subjects with diabetes and nonalcoholic fatty liver has not been explored. This forms the basis of this open labeled clinical trial in which the effect of Essentiale was studied in diabetic subjects with nonalcoholic fatty liver.

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METHODS

Type 2 diabetic patients (n = 28) attending the out-patient division of Dr. Mohans' M.V. Diabetes Specialities Centre, Chennai and satisfying the inclusion criteria were recruited for the study. The ethical committee of the hospital approved the study and informed consent was obtained from all the study subjects. The inclusion criteria for the study was type 2 diabetic patients within the age range of 25 – 60 years, with NAFL. Patients with liver cirrhosis, diabetic complication including diabetic ketoacidosis and history of regular alcohol consumption were excluded from the study.

Diabetes was diagnosed based on drug treatment for diabetes (insulin or oral hypoglycemic agents) and/ or criteria laid by the WHO Consultation Group report i.e. fasting plasma glucose (FPG) ³ 126 mg/dl or 2 hr post glucose value ³ 200 mg/dl (8).

High resolution B mode ultrasonography (Logic 400 GE, Milwaukee, U.S.A.) was carried out for diagnosis of NAFL by a well trained ultrasonologist who was masked to the patient status or the indication for the ultrasound. Liver texture was graded as follows:

Grade 1**:** a slight diffuse increase in fine echoes in the hepatic parenchyma with normal visualization of the diaphragm and intrahepatic vessel borders.

Grade 2: a moderate diffuse increase in fine echoes with slightly impaired visualization of the intrahepatic vessels and diaphragm

Grade 3: a marked increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm and posterior portion of the right lobe of the liver (9).

In order to assess reproducibility of the grading the ultrasound was repeated within a week in 6 subjects. The kappa value between the two tests was 0.75 indicating excellent reproducibility.

28 type 2 diabetic patients who satisfied the inclusion and exclusion criteria were taken up for the study. They were initiated on *Essentiale* therapy (2 tablets of 350 mg – three times a day). Patients received a standard diabetic diet of High Carbohydrate High Fibre (HCHF) as described earlier (10). The diet was kept constant throughout the study. A dietitian checked the adherence to the diet at each visit to ensure that it was kept constant throughout the study. Subjects were provided the antidiabetic treatment depending upon the severity of the disease (glycemic level). If patient was on fibrates or statins, the dose of these was kept constant throughout the study. All subjects were requested to undertake a battery of tests during each follow up visit (once in two months) for a total period of six months.

Patient's clinical history was recorded at baseline. At each follow-up visit, the blood pressure was recorded. Fasting plasma glucose (glucose oxidase-peroxidase method, Roche Diagnostics, Mannheim, Germany) serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method, Roche Diagnostics, Mannheim, Germany), serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method, Roche Diagnostics,Mannheim, Germany) and HDL cholesterol (direct method–polyethylene glycol-pretreated enzymes, Roche Diagnostics, Mannheim, Germany) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra and inter assay co-efficient of variation for the biochemical assays ranged from 3.1% to 7.6%. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (11). Glycated hemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, USA). The intra and inter assay co-efficient of variation of HbA1c was <10%.

Liver function markers measured were Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Gamma Glutamyl transferase (GGT) was estimated in Hitachi 912 Autoanalyser (Hitachi, Mannheim, Germany). The intra and inter assay precision of these assays ranged from 3.6% to 7.5%.

Ultrasound assessment of hepatic echotexture was done at baseline and six months after treatment.

Statistical Analysis: Data were computed in MS excel data sheet. Paired 't' test was used to compare the data among different visits. All analyses were done using Windows based SPSS statistical package (Version 10.0, Chicago) and p values <0.05 were taken as significant.

RESULTS

22 out of the 28 patients (78.5%) were available for follow up. 6 patients dropped out for various reasons (3 patients changed their residence, 3 were unable to come for the last visit for personal reasons). Of the 22 patients, 5 (22.7%) were on metformin therapy, 5 (22.7%) were on a sulphonylurea and 10 (45.5%) were on combination of both, while 2 (9.0%) were on insulin therapy. 4 (16.6%) patients were on a statin and 2

(8.3%) on fibrate. Step up or anti-diabetic drug doses were done in 6 (27.3%) patients to control hyperglycemia, but the drugs were not changed.

The mean age of the study subjects was 41±8 years. There were 11 (50.0%) males and the mean body mass index was 28.2 kg/m^2. Table 1 shows the clinical characteristics of the study subjects before and after treatment. Significant decrease in fasting plasma glucose (p = 0.007), HbA_{1c} (p = 0.001) and total cholesterol (p = 0.046) were observed after the treatment.

Table 1: Clinical and Biochemical Characteristics of the Study Subjects Before and After Treatment

Parameters	Before Treatment (n = 22)	After Treatment (n = 22)	p value
Systolic blood pressure (mm Hg)	124 ± 12	119 ± 11	0.070
Diastolic blood pressure (mm Hg)	79 ± 8	74 ± 8	0.003
Fasting plasma glucose (mg/dl)	164 ± 77	121 ± 25	0.007
Glycated hemoglobin (%)	8.5 ± 2.6	6.7 ± 0.9	0.001
Total cholesterol (mg/dl)	191 ± 42	173 ± 36	0.046
Serum triglycerides (mg/dl)	217 ± 151	169 ± 87	0.086
LDL cholesterol (mg/dl)	112 ± 34	99 ± 28	0.057

Fig 1 presents the results of ultrasound hepatic echotexture of the study subjects before and after treatment. At baseline, 18.2% (4/22) were diagnosed as Grade 1, 45.5% (10/22) as Grade 2 and 36.4% (8/22) as Grade 3. Among those with Grade 1, 50% (2/4) showed an improvement in echotexture, while 25% (1/4) showed no change and in 25% (1/4), it worsened**.** Among subjects with Grades 2 and 3, 41.7% (5/12) and 83.3% (5/6) respectively showed improvement. Overall 54.5% (12/22) showed improvement, 40.9% (9/22) of the study subjects showed no change in hepatic echotexture, and in only one patient (4.5%), it worsened.

Fig 1: Effect of Essentiale on Hepatic Echotexture

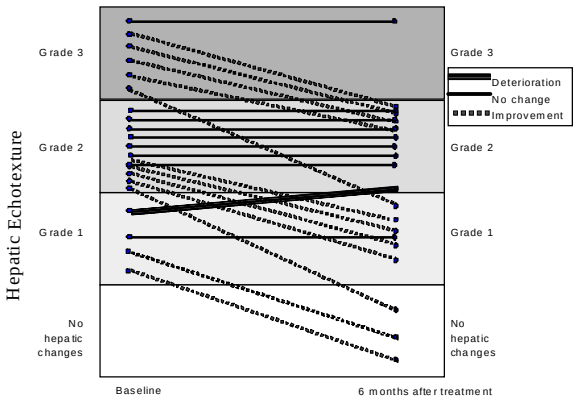
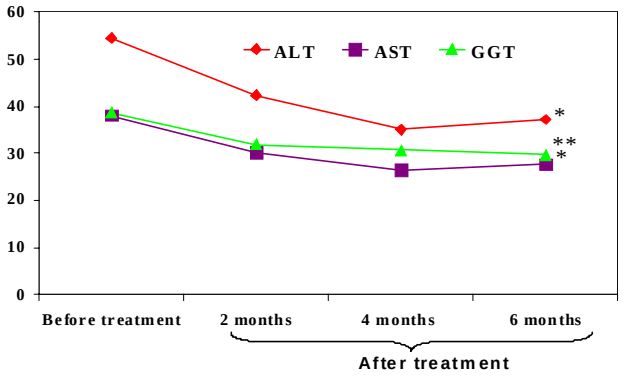


Fig 2 shows the liver function markers in the study subjects during the various visits. As observed from the graph, there was a significant reduction in all the liver enzymes after *Essentiale* treatment

Fig 2: Mean Levels of ALT, AST and GGT in the Study Subjects



** p < 0.05 for all visits compared to before treatment*
*** p < 0.05 for the visit 6 months after treatment compared to before treatment*

Table 2 presents the liver enzyme levels according to different grades of baseline hepatic echotexture of the study subjects. In the total study subjects, significant decrease was observed in ALT (p = 0.007), AST (p = 0.004), and GGT (p = 0.024) values, six months after treatment with *Essentiale.* Liver function markers did not show any alteration in Grade 1 subjects while those in Grades 2 and 3 all hepatic enzymes showed a marked reduction after treatment. However, the differences did not reach statistical significance owing to small sample size. Liver function markers were also compared according to the changes in hepatic echotexture after treatment. Even among subjects who did not show any change in echotexture, a decrease in all enzymes was observed. None of the study subjects had any adverse reactions to the drug.

DISCUSSION

NAFL and NASH are reported to be the most common cause of chronic liver disease in the U.S. with a prevalence rate ranging between 10 – 24%. NAFL is characterized by steatosis, periportal and lobular inflammation (7). Most often, NAFL remains asymptomatic. It is twice as common among diabetic subjects compared to non-diabetic subjects. An Italian

Table 2: ALT, AST and GGT Levels Before and After Treatment in the Study Subjects

Parameters	ALT		AST		GGT	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Overall	54.5 ± 29.6	37.1 ± 18.7 **	38.0 ± 18.0	27.6 ± 12.4 **	38.7 ± 27.5	29.6 ± 13.8 **
Categorized According to Baseline Ultrasound Hepatic Echotexture						
Grade 1	37.8 ± 11.0	37.8 ± 15.2	28.3 ± 8.6	28.0 ± 15.0	24.5 ± 7.6	26.0 ± 2.9
Grade 2	58.5 ± 35.4	39.6 ± 23.1	40.5 ± 20.6	28.8 ± 12.5 *	43.1 ± 35.4	31.7 ± 17.5
Grade 3	57.5 ± 23.9	31.8 ± 10.5	39.3 ± 17.0	24.8 ± 12.5	39.5 ± 12.9	27.8 ± 9.7
Categorized According to Changes in Ultrasound Hepatic Echotexture After Treatment						
Improved	47.9 ± 28.0	24.8 ± 6.7 **	32.4 ± 16.3	19.8 ± 2.8 *	29.2 ± 15.2	22.8 ± 8.2
No Change	65.9 ± 30.6	53.2 ± 18.4	47.1 ± 17.8	38.4 ± 12.9	53.8 ± 34.9	38.7 ± 15.7

** p < 0.05, ** p<0.01 compared to before treatment*

study showed that 10.5% of the subjects who had elevated AST and ALT had diabetes (12). A similar study in Cleveland showed that nearly 33% of subjects with NASH had diabetes (13).

NAFL is usually innocuous but in some patients it could result in cirrhosis, liver failure and cancer (7). Currently, there have been no well-proven therapies for treatment of NASH. However, some studies have explored the efficacy of glitazones, vitamin E, probucol, atorvastatin and alternative therapies like betaine, and have shown some beneficial results (14 - 17).

In this study, *Essentilae*, which essentially contains phosphatidylcholine, seems to be effective in reducing the liver function markers and altering the hepatic echotexture favorably in diabetic subjects with non-alcoholic fatty liver.

Fasting plasma glucose and glycated hemoglobin were significantly reduced in the study subjects after treatment. This is probably due to the step up in doses of anti-diabetic therapy. Liver function markers, particularly ALT and AST showed a significant reduction even within two months of treatment (Fig 2), while GGT showed a significant reduction only after six months of treatment.

Ultrasound assessment of hepatic echotexture showed favorable results with nearly 50% of subjects showing improvement in hepatic echotexture. Subjects with more severe hepatic changes benefited more with Essentiale treatment as more than 80% of subjects with grade 3 showed an improvement in hepatic echotexture while among grade 1 subjects only 50% showed favorable changes.

It is of interest that even among subjects who failed to show any change in the echotexture after treatment, the liver function markers showed a significant decrease indicating that *Essentiale* improves liver function even in those without obvious structural improvement. Another interesting observation in this study was that subjects with more severe grades of NAFL as diagnosed by ultrasound had marked reduction in liver enzymes than those with lesser grading. These data suggest that *Essentiale* may in fact offer more benefit to diabetic subjects with severe grades of NAFL.

Earlier, in an open label study in children below 16 years with non-alcoholic fatty liver, Vitamin E was shown to normalize ALT and alkaline phosphatases (14). A recent prospective double-blind study showed that both vitamin E and C were effective in improving hepatic fibrosis scores (18). Metformin therapy transiently improved liver function markers in a open labeled trial while a randomized clinical trial showed

that improvement of insulin sensitivity occurs with improvement in the liver function in subjects with NAFL (19, 20).

Choline deficient diet has been observed to cause hepatitis in mice, which resembles that in humans (21). It is more reasonable to presume that phosphatidylcholine supplementation could be of benefit to subjects with hepatitis. Polyenephosphatidyl choline extract from soyabean has been approved for the treatment of chronic liver diseases in many European countries (http://www.lef.org/magazine/mag2004/feb2004_report_liver_02.htm) Phosphatidylcholine gets incorporated into the cell membrane of both normal and damaged liver cell in animal models (21), the end result being an increase in membrane fluidity and active transport across the membrane. Similarly, it has been suggested that incorporation of phosphatidylcholine into blood lipoprotein results in lipid-lowering properties. In the present study, a significant reduction in LDL cholesterol was observed after treatment with Essentiale. This is in agreement with a clinical trial in Russia where supplement of phosphatidylcholine lowered total and LDL cholesterol by about 15%, decreased triglyceride levels by 32%, and raised levels of HDL cholesterol by 10% (22). However, in the present study one cannot rule out the fact that improved glycemic control as observed from HbA_{1c} reduction could have caused the improvement in lipid levels.

In addition, like vitamin E and C, phosphatidylcholine also appears to have antioxidant properties, which means it may effectively reduce the oxidative stress shown to be a contributing factor in the inflammation and scarring of nonalcoholic steatohepatitis (23).

To conclude, the data suggests that Essentiale protects and improves liver function in diabetic subjects with NAFL. In order to confirm these findings and to determine its mechanism of action, prospective, randomized blinded clinical trials are needed.

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