

Original Article

Essential phospholipids as a supportive adjunct in the management of patients with NAFLD



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ABSTRACT

Background and study aims: Treatment of nonalcoholic fatty liver (NAFLD) is important because NAFLD patients have a 1.7-fold increase in standardised age and gender matched mortality. Currently treatment is based on life style modification and managing comorbid associating disease. Other medications remain experimental. Essential phospholipid (EPL) is a nutrient for the liver, helping to maintain vitality of cell membranes where the vast majority of liver activities are regulated.

We performed a randomised open label study to evaluate EPL as an adjuvant nutrient to the treatment of primary NAFLD or NAFLD with comorbid disease.

Patients and method: Three groups of NAFLD patients were recruited: lone ($n = 113$), diabetes mellitus type 2 ($n = 107$) and mixed hyperlipidaemia ($n = 104$). Diagnosis was established by excluding other chronic liver diseases. A standard diet and physical activity plan were advised to all patients. 1800 mg of EPL a day was given for 24 weeks, followed by 900 mg for 48 weeks.

Results: Essential phospholipid EPL led to a significant improvement of symptoms and a mean reduction of ALT of 50.8 IU and AST of 46.1 IU per patient ($p < 0.01$). Abdominal ultrasonography indicated normalisation in 4.6% and a shift from grade II to grade I in 24% of patients. Liver stiffness measurement indicated an improvement in 21.1%, with a mean reduction in the LSM of 3.1 K Pascal/patient. Reducing the dosage after six months led to a limited relapse in 43.8–63.2% of patients, for lone and NAFLD with co-morbid conditions.

Conclusion: Essential phospholipid (EPL) as a nutritional supplement resulted in a significant improvement in clinical parameters and transaminases for all NAFLD patients. Ultrasound and LSM revealed modest improvement. There is a need for uninterrupted maintenance to avoid relapse.

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Introduction

Fatty liver disease is a pathological condition that develops in the absence of alcohol abuse, or other liver disorders, and is defined as fat accumulation in the liver exceeding 5–10% of the liver weight [1]. It is recognised increasingly as a major health burden affecting 20–30% of the adult population worldwide [2–4]. NAFLD is an acquired metabolic stress-induced liver disease, caused by multifactorial pathogenic mechanisms. Insulin resistance is a leading factor causing accumulation of fatty acids in the liver, which in the presence of oxidant stress, adenosine

triphosphate (ATP) depletion, and mitochondrial dysfunction may be the main cause of hepatocellular injury. Genetic susceptibility and heritability may also play a role in the pathogenesis of NASH with or without cirrhosis. The most consistently replicated genetic risk factor for the NAFLD spectrum is the single-nucleotide polymorphism rs738409 in the gene encoding patatin-like phospholipase domain-containing protein 3 (PNPLA) [3]. Subtle inter-patient genetic variations and the environment interact to determine disease phenotype and influence its progression [5–9]. The spectrum of histologic abnormalities defined by NAFLD includes simple steatosis, NASH, cirrhosis with possible development of hepatocellular carcinoma. Isolated steatosis appears to run a benign course with a minimal but not

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non-existent risk of progression to advanced liver disease. There has been an association of simple steatosis with increased all-cause mortality, liver-related death, cardiovascular disease, diabetes mellitus type 2 and hepatocellular carcinoma without cirrhosis [10–13]. NASH on the other hand, is a more aggressive form of NAFLD which is associated with varying degrees of inflammation, fibrosis and the more serious possible progression to cirrhosis with ensuing end-stage liver disease or the development of hepatocellular carcinoma [14–17].

NAFLD is recognised as the hepatic component of the metabolic syndrome, which includes hyperlipidemia, glucose intolerance, obesity and systemic hypertension. Most patients with NAFLD may have one or more of these risk factors, the risk and severity of NAFLD increase with the number of components of the metabolic syndrome present [18]. Diabetes mellitus type 2 has been described in 20–75% of adult patients which may be an independent predictor of advanced NAFLD, emergence of cirrhosis and hepatocellular carcinoma. The association between diabetes mellitus type 2 and NAFLD appears strongest in the morbidly obese patient. NAFLD however, has been associated with insulin resistance and hyperinsulinemia, even in the lean subjects with normal glucose tolerance [19,20].

Hyperlipidemia is found in a substantial proportion of patients with NAFLD. The severity of ultrasound detected steatosis was positively correlated with BMI, raised ALT, insulin resistance and hypertriglyceridemia [21,22].

There is no standard of care medicine for treatment of steatosis/NASH, the current management focuses on life style modification, medications used for the treatment of a co-morbid disease, and liver protection. The concept of using naturally occurring compounds such as the essential phospholipids as an adjuvant to the treatment or to prevent hepatic steatosis appears a very attractive option for liver protection [23–27]. Metformin and pioglitazone are well known medications for the treatment of diabetes mellitus type 2, being insulin sensitisers either of them when used alone or both of them used together had been recommended to treat NAFLD when diabetes mellitus type 2 coexists with it. Clinical studies had revealed variable results with metformin indicating a temporary improvement of transaminases but the setback for these studies was that no histological evaluation was done in most of them [28–30]. Only pioglitazone was believed to induce a convincing effect, however it should be noted that relapse was common when these medicines were stopped and hence it should be used for a very long period of time which raises the worries with pioglitazone about the risk of cardiovascular events, increase in body weight, increase in fracture risk and increase in the incidence of bladder and prostatic cancer [31–33]. Many open label clinical studies demonstrated that atorvastatin and rosuvastatin have a good effect in treatment of NAFLD as well, more RCTs with histological endpoints to prove their efficacy, are needed. Ezetimibe, which lowers hepatic free and total cholesterol stores appeared in animal studies and few human pilot studies as a promising drug for the treatment of NAFLD [34–39].

In this study we evaluated the nutritional supplement EPL in lone, diabetes mellitus type 2 associated and hyperlipidemia associated NAFLD.

Patients and method

This study is a prospective randomised open label study to evaluate the clinical, biochemical, ultrasonography and liver stiffness measurement response to essential phospholipid (EPL) when given as an adjuvant nutritional supplement to patients with non-alcoholic fatty liver disease (NAFLD). The study included the following patients with NAFLD:

1. Patients with lone NAFLD: no associating co-morbid disease.
2. Non-alcoholic fatty liver disease (NAFLD) patients who had associating diabetes mellitus type 2 and were being treated with metformin, pioglitazone or both for at least six months before enrolment.
3. Non-alcoholic fatty liver disease (NAFLD) patients who had associating mixed type hyper-lipidemia being treated with atorvastatin, ezetimibe or both for at least six months before enrolment.

Inclusion criteria

All consenting patients 14–80 years of age clinically diagnosed to have lone NAFLD, NAFLD with diabetes mellitus type 2 on pioglitazone, metformin or both, NAFLD with hyperlipidemia on a statin, ezetimibe or both, were included in the study. NAFLD diagnosis was established on the basis of clinical history, clinical examination and laboratory tests, supported by imaging, (abdominal ultrasound) and liver stiffness measurement as evaluated by elastography, without the need for liver biopsy. Liver biopsy is currently the gold standard for diagnosing progressive NASH, yet we did not do it for all patients because we were evaluating a nutritional agent, the effect of other medicines used is well documented, the large size of the cohort and the patients refusing to do this invasive procedure. Clinical diagnosis as suggested above is well established besides the fact that liver biopsy has many drawbacks, such as sampling error, cost, and risk of complications. Furthermore, it is not realistic to perform liver biopsies on all NAFLD patients [40].

Other causes of chronic liver disease (viral hepatitis, drug induced hepatitis, haemochromatosis, copper overload, and autoimmune hepatitis) were ruled out. All patients had the liver stiffness measured in K pascals with the equivalent fibrosis score computed by transient elastography (Fibroscan, Echosense).

Exclusion criteria

Standard exclusion criteria were applied to assure the safety of treatment and to rule out other comorbid illnesses that might have a negative impact on the response to the medication. These criteria included exclusion of patients with evidence of intrahepatic cholestasis, obstructive disease or elevated alkaline phosphatase, diabetes mellitus type 1, pregnant and lactating women, history of allergic reactions to used medication, chronic renal or hepatic failure, and neoplastic disease. Patients of diabetes mellitus type 2 and hyperlipidemia who were treated with other medications than assigned or who had to modify their therapy by adding a different medicine than specified in the protocol were excluded from the analysis.

Patients referred to the participating centres with a diagnosis of fatty liver detected by ultrasonography done for any indication were recruited. After thorough clinical examination, when they fulfil the inclusion and exclusion criteria, with laboratory testing including the usual parameters of liver function tests, Hgb A1C, lipid profile, virology study, anti-nuclear antibodies, anti-mitochondrial antibody, anti smooth muscle antibody, LKB antibody, ferritin, serum copper and ceruloplasmin. Ultrasonography, was the main guide for recruiting patients and had to indicate the degree of fatty infiltration of the liver as stage I, II or III based on radio-imaging criteria. A baseline liver stiffness measurement (LSM) was done by transient elastography using the Fibroscan (Echosens, Paris, France). All patients were advised to follow a standard diet that aims at maintaining the BMI within the standard range, after counselling with a nutritionist and to perform a 30 min daily walking exercise at least 5 times a week. Each patient was prescribed essential phospholipid (EPL) 1800 mg 6 capsules per

day in 3 divided doses for 24 weeks followed by EPL 900 mg 3 capsules a day in 3 divided doses for another 48 weeks. Patients of diabetes mellitus type 2 and hyperlipidemia were to take their medications as stated above and adjust dosing as per the results of treatment, the main target was to achieve an HgbA1C <6.5% for diabetics and normalisation of the lipid profile parameters for hyperlipidemia patients. Follow up visits were done every three months. During this consultation patients were subjected to clinical assessment, and to performing the relevant laboratory investigations, ultrasonography and transient elastography. Patients of diabetes mellitus type 2 and hyperlipidemia were to do HgbA1C, and lipid profile respectively in addition. Compliance was considered satisfactory if the patient could achieve at least 80% of consultation and follow up visits, taking at least 80% of medications as prescribed, and doing laboratory work, ultrasonography and Fibro-scanning. The study extended for a 72 week period. Patients who completed the treatment period were kept under observation with no EPL for another 24 weeks.

Statistical analysis was performed using the IBM SPSS 20.0 software. Quantitative variables were given as means \pm SD. A univariate analysis including age, sex, body mass index, ethnicity, baseline disease (diabetes mellitus type 2 and hyperlipidemia), was performed. Variables achieving a *p*-value lower than 0.3 were included in a multivariate analysis performed by logistic regression. *p*-Values lower than 0.05 were considered significant.

Results

A cohort of 324 patients suffering from NAFLD were recruited in six medical centres in the United Arab Emirates and were randomly assigned to one of the three treatment groups.

Tables 1 and 2 indicate that the demographic details and clinical findings were well matched for all parameters in the three treatment groups. All patients had some degree of elevation of the transaminases (ALT and AST); the mean values of these enzymes for each group are shown in Fig. 1. Figs. 2 and 3 show details of the ultrasound and elastography findings at initial evaluation and after treatment.

Complaints that could be related to NAFLD were generally not common amongst patients of the three treatment groups and were reported in 43 subjects of each of the lone and diabetes mellitus type 2 NAFLD groups and in 49 subjects of the hyperlipidemia NAFLD group, representing 38.1%, 40.2% and 47.1% of each respectively. At the end of treatment patients reported remarkable improvement of all clinical parameters: both the general symptoms like weakness, asthenia, irritability and sleep disturbances and the non-specific gastrointestinal symptoms like postprandial distress, flatulence, nausea and right upper quadrant discomfort. Only 18.6%, 20.6% and 23.1% of the lone, diabetes mellitus type 2 and hyperlipidemia

NAFLD patients were still having mild symptoms. This reflects a significant subjective regression of the clinical symptoms presented by the patients treated with EPL ($p < 0.01$) (Table 2).

The transaminase levels dropped markedly over the first six months of treatment for the three groups. This happened in 80.5% of the lone NAFLD patients with a mean drop of ALT of 54.6 IU (range: 11–135 IU) and of AST of 48.7 IU (range: 16–110 IU) per patient. In the group of NAFLD with diabetes mellitus type 2 the transaminases behaved similarly in 84.1% of the diabetic patients with a mean drop of ALT of 44.9 IU (range: 14–141) and AST of 40.5 IU (range: 21–124) per patient. The same was observed in 87.5% of the NAFLD patients with hyperlipidemia revealing a mean drop of 52.9 IU for ALT (range: 21–123 IU) and of 49.2 IU for AST (range: 15–116 IU) per patient. After dose reduction to 900 mg per day there was a slight rise of the transaminases over the next three months but then it steadily decreased and was maintained significantly at a normal level or just above the upper limit of normal ($p < 0.01$). The response of liver enzymes to EPL appeared slightly better in the NAFLD patients with diabetes mellitus type 2 and hyperlipidemia but this was not statistically significant ($p = 0.05$). It is probably attributed to the additive effect of other medications used for the treatment of the co-morbid disease.

Ultrasound examination indicated a slight but appreciable improvement in the three treatment groups. This occurred in 29.2% of the lone NAFLD patients with six patients becoming normal (5.3%) and 27 patients changing from grade II to grade I fatty infiltration. The diabetic patients had a similar observation with 23.4% of them showing an improvement of the ultrasound findings, four patients (3.7%) attained a normal US and 25 patients changed their echography pattern from grade II to grade I fatty infiltration at the end of treatment. The same trend was noted in 20.2% of the patients with hyperlipidemia who had an improvement of the ultrasound findings with five patients 4.8% becoming normal and 11 patients changing from one grade to a better grade of fatty infiltration.

In the lone NAFLD treatment group elastography revealed a change of liver stiffness measurement in 14.2% of the patients with a mean drop of 3.1 K pascal per patient and a range of 0.5–6 K pascal. In the diabetic treatment group however, a change in the liver stiffness occurred in 26.1% of the patients with a mean drop of 3.4 K pascal per patient and a range of 0.3–6.9 K pascal. The patients with hyperlipidemia also revealed a change in the liver stiffness in 20.2% of the patients with a mean drop of 3.1 K pascal per patient and a range of 0.6–5.5 K pascal. The change in liver stiffness was more appreciable in the co-morbid treatment group implicating a possible added effect by the specific treatments taken for these clinical conditions.

The effect of exercise, dietary restriction and weight loss could not be evaluated in this cohort, which is a drawback in this study. This is attributed to the very low compliance rate with the planned instructions designed for each patient. The compliance rates were 35.4%, 35.5% and 34.6% for lone NAFLD, NAFLD with diabetes mellitus type 2 and NAFLD with hyperlipidemia respectively, thus rendering a low number of patients to evaluate which does not allow sound statistical conclusions. An observed trend for a better result was noted though, for those who achieved an effective loss of weight in the three arms of the study. The non-compliance could be due to the lengthy period of the study and to the behavioural attitude of patients in this area who tend to give up easily with weight reduction plans.

Discussion

Most guidelines addressing the treatment of NAFLD unanimously agree on the principle of lifestyle modifications including

Table 1

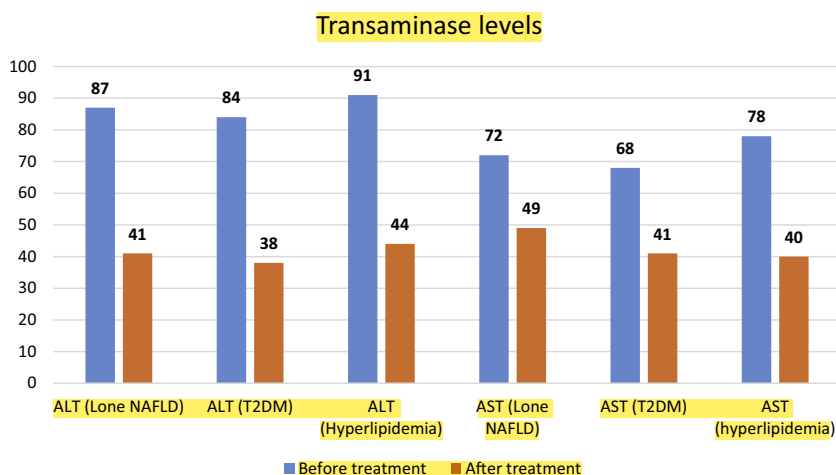
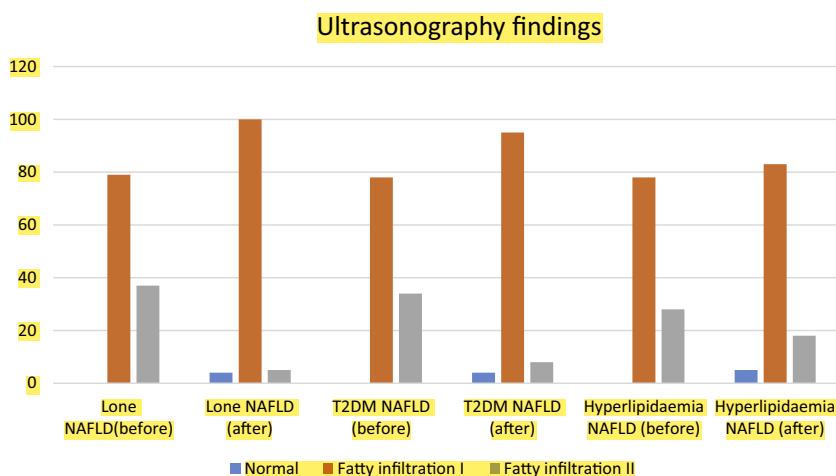
Demographic details indicated that the three groups were almost fully matching as regards age, gender, ethnicity, and BMI. In the three arms two thirds of the patients recruited were either overweighted or frankly obese which fits in the general trend of the population in the area.

	Primary NAFLD	T2DM NAFLD	Hyperlipidemia NAFLD
Number	113	107	104
Male:Female	62:51 (1.2:1)	57:50 (1.14:1)	57:47 (1.2:1)
Age	46.8 (26–69)	42.6 (24–64)	40.9 (21–69)
BMI	29.8 (25–33)	29.6% (21–36)	28.3% (19.8–36.9)
Normal body weight	41 (36.3%)	43 (40.2%)	42 (40.4%)
Overweight	72 (63.7%)	64 (59.8%)	62 (59.6%)
Ethnicity			
Nationals	43 (38.1%)	40 (37.4%)	43 (41.3%)
Expatriates	70 (61.9%)	67 (62.6%)	61 (58.7%)

Table 2

Profile of patients' symptoms for the three arms of the cohort before and after treatment.

	Lone NAFLD		T2DM NAFLD		Hyperlipidemia NAFLD	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Asymptomatic	70 (61.9%)	92 (81.4%)	64 (59.8%)	85 (79.4%)	55 (52.9%)	80 (76.9%)
Symptomatic	43 (38.1%)	21 (18.6%)	43 (40.2%)	22 (20.6%)	49 (47.1%)	24 (23.1%)
<i>General symptoms</i>						
Asthenia	13/43 (30.2%)	3/21 (14.3%)	19/43 (44.2%)	4/22 (18.2%)	19/49 (44.2%)	13/24 (30.2%)
Sleeping disorder	13/43 (30.2%)	2/21 (9.5%)	13/43 (30.2%)	3/22 (13.6%)	13/49 (30.2%)	8/24 (18.6%)
Irritability	14/43 (32.6%)	3/21 (14.3%)	21/43 (48.8%)	6/22 (27.3%)	21/49 (48.8%)	6/24 (13.9%)
<i>GI features</i>						
PP distress	12/43 (27.9%)	4/21 (19%)	21/43 (48.8%)	6/22 (27.3%)	21/49 (42.9%)	9/24 (20.9%)
Flatulence	13/43 (30.2%)	6/21 (28.6%)	21/43 (48.8%)	8/22 (36.4%)	21/49 (42.9%)	18/24 (41.9%)
RUQ pain	9/43 (20.9%)	2/21 (9.5%)	9/43 (20.9%)	4/22 (18.2%)	9/49 (18.3%)	4/24 (9.3%)
Nausea	8/43 (18.6%)	2/21 (9.5%)	8/43 (18.6%)	3/22 (13.6%)	8/49 (16.3%)	7/24 (16.3%)
Heartburn	5/43 (11.6%)	3/21 (14.3%)	5/43 (11.6%)	2/22 (9.1%)	5/49 (10.2%)	2/24 (4.65%)
Jaundice	4/43 (9.3%)	2/21 (9.5%)	5/43 (11.6%)	2/22 (9.1%)	5/49 (10.2%)	3/24 (6.97%)
Hepatomegaly	9/43 (20.9%)	4/21 (19%)	13/43 (30.2%)	2/22 (9.1%)	13/49 (26.5%)	11/24 (25.6%)
		$p < 0.01$		$p < 0.01$		$p < 0.01$

**Fig. 1.** The mean values for the transaminases ALT and AST before and after treatment for the three treatment groups.**Fig. 2.** Ultrasonography findings before and after completion of treatment for the three treatment groups: there is a noticeable reduction in the number of patients with grade II fatty infiltration, thus increasing the number of patients with grade I infiltration and leading to the appearance of few patients with normal post treatment ultrasonography in the three arms.

weight loss by a combination of decreased caloric intake and increased physical activity. The medicinal treatment for NAFLD however, remains speculative with many unmet needs. No specific

drug has proved to be effective in controlling the disease by itself for all patients. Targets of therapy included control of diabetes mellitus type 2, insulin sensitizers, control of blood lipids and

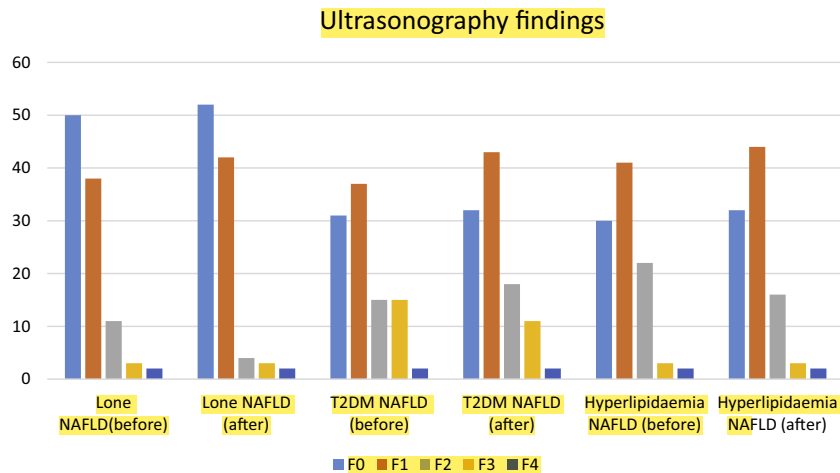


Fig. 3. Elastography findings before and after completion of treatment for the three treatment groups: there is a slight shift in the number of patients who had some fibrotic changes from the higher grades F2 to F1 and few from F1 to F0, F3 and F4 patients remained the same.

hepato-protective agents. Prevention would be a reasonable goal until a proper medicine that addresses all the pathological events in NAFLD is produced. Essential phospholipid is a nutritional supplement that can reduce liver lipids and thus is an attractive agent for prevention. Earlier research on animal studies had documented that the major phospholipid extracted from soya beans, Dilinoleoyl phosphatidyl choline DLPC reduced lipid peroxidation, oxidative stress and hepatic fibrosis and thus helped in protecting against alcoholic liver disease. DLPC would achieve this by virtue of a number of hepato-protective properties including the ability to reduce: alcohol-induced hepatocyte apoptosis, cytochrome P450IIE1 (CYP2E1) induction, mitochondrial respiratory dysfunction, and TNF- α generation by Kupffer cells in addition to inhibition of hepatic stellate cell proliferation and collagen accumulation which lead to fibrosis [41,42]. DLPC exerts its effect mainly in the intestinal lumen, by interfering with neutral sterol absorption. They also can stimulate bile acid and cholesterol secretion. Dietary phospholipids can have a beneficial effect on plasma lipid and lipoprotein levels [43]. Several investigators have described similar effects of DLPC on hepatic steatosis in animal models with nonalcoholic liver disease induced by different mechanisms [44–48]. Controlled human trials are therefore required to substantiate the efficacy of EPL in this approach.

We aimed in this study to evaluate EPL as a supportive agent in the treatment of NAFLD. Most of the patients who were treated had a satisfactory clinical response with 81%, 79.4%, and 76% of the patients in the lone NAFLD, NAFLD with diabetes mellitus type 2 and NAFLD with hyperlipidemia respectively reporting no clinical symptoms while on treatment with a statistically significant change as compared to the symptomatic patients before treatment ($p < 0.01$). This subjective change in the clinical symptoms is an indicator of improvement and reflects the positive impact of EPL on the quality of life of NAFLD patients. Quantitative improvement was concluded from other common surrogate markers which included the change in values of aminotransferases, hepatic injury as detected by the noninvasive ultrasonographic imaging and liver stiffness measurement detected by transient elastography.

The transaminase levels were reduced after treatment in 80%, 84% and 87% of patients in the three arms respectively ($p < 0.001$) this finding pointed at an impressive significant support to the liver by EPL. Ultrasonography cannot quantify the amount of triglycerides deposited in the liver, it is not a sensitive indicator in this context; however it reflects the trend of change in radio-imaging pattern in every patient, and would undoubtedly be of

value when it shows regression of findings to the normal situation. Eight patients were reported to have normal ultrasonography findings after treatment (2.5%) whereas 46 patients (14.2%) were said to have a changed pattern of fatty infiltration from grade II to grade I after treatment indicating a partial response. Findings of transient elastography that indicate complete regression of fibrosis complicating the disease took place in eight patients only (2.5%) who were the same patients to have normal ultrasonography as well, with the LSM becoming normal (F0). In another 21 patients (6.5%) a partial regression was noted with change of the fibrosis score from a higher (mainly F2) to a lower grade (F1). Liver stiffness in patients with F3 and F4 equivalent metavir score did not show any remarkable response. Although this observation of quantitative improvement does not carry a statistical value, it seems to be promising and gives hope to a subset of patients who might respond to this simple nutritional support. We assume that upregulating the dosage and extending the period of treatment at such a dose might evolve into better results, this theory however, has to be validated. In this cohort the improvement in the values of transaminases, ultrasound and elastography findings seem to be genuinely induced by EPL regardless of the presence of comorbid conditions or not. Patients of diabetes mellitus type 2 on metformin and/or pioglitazone and patients of hyperlipidaemia on atorvastatin and/or ezetimibe were actually on this treatment for at least 6 months prior to enrolment and starting EPL, yet they had almost the same changes as in the lone NAFLD cases with only minor insignificant differences. Weight loss and exercise have a very important role in the management of NAFLD, however they did not seem to affect the results in this study because the majority of patients could not comply with the instructions and either remained with the same weight or had gained weight.

We can conclude, that essential phospholipid (EPL) when used as an adjunctive support treatment for NAFLD patients had led to a significant clinical response as indicated from improvement of all symptoms whether they were general, or GI related. Transaminases revealed a significant reduction post treatment, ($p = 0.001$). Improvement of ultrasonography findings (complete or partial) occurred in 20–26% of patients ($p = 0.07$). Elastography indicated a similar trend with a favourable mean reduction in the liver stiffness measurement from 3.1 to 3.4 K Pascal/patient regardless of the result of treatment.

Relapse, appeared to be common after early stoppage or reduction of the treatment, and is more likely to happen with the primary NAFLD group. Extension of the treatment to longer periods

had stabilised patients' response. Unfortunately the importance of body weight reduction and exercise could not be concluded from this work due to non-compliance. **These findings appear to be encouraging yet, should be considered as a primer for larger scale multicentre studies that could provide an effective, safe and simple nutritional support that could help protect the liver.**

Conflict of interest

The authors declared that there was no conflict of interest.

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