ISSN: 2577 - 8005

Original Research

Medical & Clinical Research

Efficacy of adjuvant phosphatidylcholine in the management of egyptian patients with non alcoholic fatty liver disease (NAFLD)

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Submitted: 04 Aug 2021; **Accepted**: 10 Aug 2021; **Published**: 19 Aug 2021

Citation: *Reda Elwakil, Azza El Sayed Mansy, Sarah Mahmoud Shaheen, Alaa Barakat, Nehal Mohamed Nageeb Abou-Seada, Manal El Hamamsy (2021) Efficacy of adjuvant phosphatidylcholine in the management of egyptian patients with non alcoholic fatty liver disease (NAFLD). Medical & Clinical Research 6(8): 669-677.*

Abstract

Background and Aim: *Non-Alcoholic Fatty Liver Disease (NAFLD) has become the most common liver disorder with increased liver related and non-related complications and mortality as a result of increasing obesity, type 2 diabetes and metabolic syndrome (MetS). The current study aims to evaluate the efficacy of adjuvant phosphatidylcholine in treating patients with NAFLD.*

Methods: *This interventional randomized controlled study recruited 100 patients with NAFLD and MetS randomized into: a control group (n=50) that received standard care of life style modifications and an intervention group (n=50) that received phosphatidylcholine (2100 gm/day) plus standard care. Both groups received health education through clinical pharmacist for achieving sustainable weight loss for 6 months. Body mass index (BMI), waist and hip circumference, liver function, lipid profile, homeostasis model of assessment-insulin resistance (HOMA-IR) score, NAFLD-fibrosis score, steatosis score and liver stiffness measurement by transient elastography were recorded at baseline, 3 and 6 months.*

Results: *Intervention group showed significantly ($p<0.05$) higher number with normalized; alanine aminotransferase, total cholesterol and low density lipoprotein at midpoint and endpoint, aspartate amiontransferase at midpoint and high density lipoproteins and malondaldehyde at endpoint. Intervention group showed a significantly higher participants' number who shifted to more favorable category of NAFLD-fibrosis score ($p=0.02$), radiological fibrosis stage ($p=0.015$) at endpoint, radiological steatosis grades and HOMA-IR score at midpoint and endpoint ($p<0.05$). Additionally, significant number of participants in intervention group (34%) lost MetS components compared to (10%) in control group at endpoint ($p=0.004$).*

Conclusion: *Adjuvant phosphatidylcholine has shown laboratory, radiological and clinical benefits in the management of Egyptian patients with NAFLD and ameliorate MetS parameters.*

Keywords: NAFLD, Phosphatidylcholine, Metabolic Syndrome, Oxidative Stress, Liver Fibrosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is considered the most common hepatic disorder affecting 20-30% of adults worldwide [1]. One form of NAFLD is non-alcoholic steatohepatitis (NASH) with hepatocytes ballooning and inflammation with or without fibrosis [2] leading to cirrhosis and possibly hepatocellular carcinoma (HCC) increasing liver related mortality [3]. NAFLD tends to be the most common indication for liver transplantation with high rates of complications due to prevalent comorbidities including diabetes and obesity. NASH is also associated with an increased mortality due to cardiovascular and chronic kidney diseases[4].

Definitive NAFLD diagnosis can be done only by invasive liver biopsy[5],yet with associated morbidity and mortality and unnecessary risk for those with non-progressive conditions[3]. Thus the non-invasive, inexpensive, readily available ultrasound-based elastographic technique is successively used in NAFLD diagnosing (along with raised liver functions), severity grading, therapeutic deciding and monitoring [6]. Several scores, combining serum biomarkers and metabolic parameters, are used to diagnose liver fibrosis including NAFLD fibrosis score that is based on platelet count, serum aspartate / alanine aminotransferase and albumin. Ideal diagnostic panel should combine imaging and scoring modalities [7] Homeostasis model assessment-insulin resistance (HOMA-IR) based on the mathematical transformations of fasting blood glucose (FBG) and fasting blood insulin (FBI) [8] is a useful noninvasive marker for predicting fibrosis in patients with NASH [9].

There is no licensed pharmacotherapy for NAFLD, the cornerstone of management is lifestyle dietary and exercise interventions [10] and bariatric surgery or liver transplantation for some cases [5]. Antidiabetics, antioxidants, prebiotics, drugs acting on bile, lipid-lowering therapies, and weight loss medications have been tested in NAFLD therapy with conflicting results [1,5] .

Adjuvant essential phospholipid (EPL) is one of the medications currently under investigation relating to its potential positive effects on NAFLD and NASH because of its membrane repairing, antioxidative, and antifibrotic effects and high biocompatibility [11]. They are effective in reducing serum and hepatic cholesterol and triglycerides (TG) and increasing high density lipoproteins cholesterol (HDL-C) and apolipoprotein A-I (apo A-I) levels [5,12]. The difficulty of adherence to lifestyle recommendations and the noxious adverse drug events, employ health care professionals, especially pharmacists, to take an active role in rationale selection of pharmacotherapy for NAFLD [13]. Adult obesity is prevalent in Egypt in addition to type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) that is defined by the presence of any 3 out of the following:

1. Waist circumference ≥ 102 cm in men or ≥ 88 cm in women
2. Triglycerides ≥ 150 mg/dL
3. High density lipoprotein-cholesterol <40 mg/dL in men or <50 mg/dL in women
4. Blood pressure ≥ 130/85 mm Hg 5. blood glucose>110 mg/dL (including diabetes) [14].

Since NAFLD affects 65.3% of overweight and obese Egyptian adult, this calls for effective searching for more targeted preventive and therapeutic modalities for NAFLD [15]. Accordingly, this study seeks to evaluate the efficacy of EPL on improving clinical, biochemical and radiological parameters of NAFLD as well as the scores, combining these parameters in Egyptians with simple NAFLD or NASH associated with metabolic comorbidities.

Aim of the work

The current study aims to evaluate the efficacy of adjuvant phosphatidylcholine in the management Egyptian patients with NAFLD.

Methods

Trial design

The current, prospective open labeled, randomized controlled study was conducted on patients (n=100) with NAFLD who were provided adequate dietary and life style advice, and were randomized into two study groups (each; n=50); group I (Control group; CG) without Phosphatidylcholine and Group II (Intervention Group, IG) receiving oral Phosphatidylcholine.

Randomization

Simple randomization was performed by allocating the computer generated random numbers of participants.
Allocation: odd numbers to group I and even numbers to group II.

Study setting

The current study was conducted in out-patient clinics of the Tropical Medicine department in Ain Shams University Hospitals and Ain Shams Specialized Hospital in Cairo-Egypt between January 2015 and January 2019.

Participants

Included those diagnosed with NAFLD. The following criteria were fulfilled before recruiting all consenting patients aged 18 to 65 years; 1) diagnosis of NAFLD by the evidence of (a) fatty liver upon US with either incidental increased alanine aminotransferase (ALT), presence of risk factors related to NAFLD+ increased ALT, or symptomatic liver disease +/- hepatomegaly +/- increased ALT [16], or (b) (HOMA-IR) score >3 with the presence of liver steatosis, stiffness measured by transient elastography or a NAFLD fibrosis score between -1.455 and 0.675 or more [17]. 2) had at least one of the following metabolic comorbidities: hypertension, T2DM, overweight/obesity {body mass index (BMI) >27 kg/m2}, or total cholesterol of >200 mg/d. Patients were excluded from the study if showing evidence of alcoholic or chronic liver disease, HCC, autoimmune hepatitis, end stage liver disease, previous liver surgery including resection or transplant, treatment with other hepatoprotectors, antioxidants (vitamin E or C, or glutathione), prebiotics, drugs acting on bile, lipid-lowering therapies, weight loss medications or other EPL within 30 days of study initiation, drugs known to produce fatty liver disease (as steroids, estrogens, amiodarone, or tamoxifen), pregnancy, lactation, serum creatinine level >1.5 times the upper limit of normal at screening, or CrCl<60 ml/min, or current dialysis. Other therapies allowed to be taken by participants included antidiabetics and antihypertensives.

After signing an informed consent, 111 participants underwent baseline investigations:

1. Presence and nature of metabolic comorbidities

2. BMI and w/h circumference ratio
3. Liver function tests, lipid profile, FBI, FBG, CBC and CRP
4. Serum oxidative stress markers; MDA and SOD
5. Liver fibrosis and steatosis scores using transient elastography (Fibroscan)
6. NAFLD fibrosis score
7. HOMA IR scores in patients without diabetes

Ethical Concern

The study was approved on 20 August 2014 by the institutional review board of Ain Shams University according to the declaration of Helsinki and has been registered in www.clinicaltrials.gov (NCT04411862). All participants provided informed consent documentation.

Interventions

Dosage and administration of EPL (2100mg/day Phosphatidylcholine; two Essentiale® soft capsules 350mg three times daily) was according Gonciarz et al. [18].

Dietary and life style education sessions to all participants as well as education about EPL benefits, use and risks to intervention group were conducted by a clinical pharmacist. Adherence to pharmacological therapy and dietary and life style recommendations were monitored by pill count and self-reporting respectively during follow up. Participants' recruitment, grouping, intervention and follow up are summarized in Figure 1.

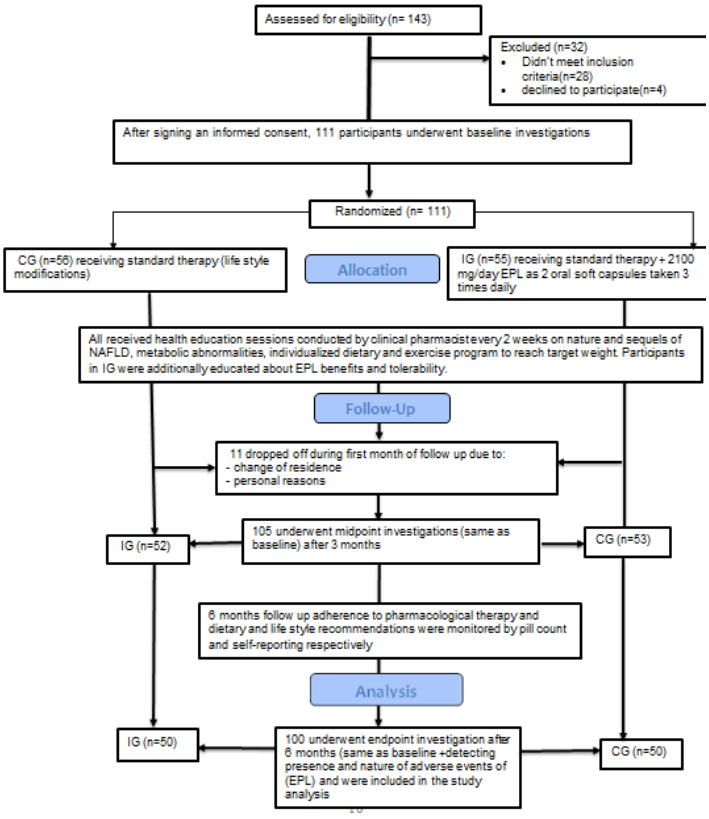

Figure 1: Consort flow diagram.

BMI:Body Mass Index;CG: Control Group; CBC:Complete Blood Count; CRP; C-reactive protein; EPL:Essential Phospholipids; FBG: Fasting Blood Glucose; FBI:Fasting Blood Insulin; HOMA-IR:Homeostasis Model of Assessment-Insulin Resistance; IG:Intervention Group; MDA:Malondialdehyde.

Study Outcomes

The Primary Outcomes of the study included the shift to more favorable

a) liver fibrosis stages; F0: no fibrosis, F1: portal fibrosis without septa, F2: portal fibrosis with few septa, F3: numerous septa without cirrhosis and F4: cirrhosis [6] b) liver steatosis grades:S0 at 180-223 controlled attenuation parameter [CAP] dB/m indicating <5% steatosis ; S1 at 223-268 CAP dB/m indicating 5%-33% steatosis, S2 at 268-301 CAP dB/m indicating 33%-66% steatosis and S3 at 301-346 CAP dB/m; indicating >66% steatosis [7] c) HOMA-IR score categories; normal (<3), moderate (3-5) and severe (>5) [8] and d) NAFLD fibrosis score categories: low cutoffs (<-1.455); indicating a fibrosis level of F0-F2, indeterminate cutoffs (-1.455 to 0.675), and high cutoffs (>0.675) indicating a fibrosis level of F3-F4 [19] as well as loss of metabolic syndrome criteria.

The secondary outcomes included the favorable change in participants, anthropometric measures, loss of signs and symptoms of NAFLD and normalization of disease laboratory indicators. Only 10% of needed weight loss was targeted during the 6 months to avoid rapid weight loss that could increase NAFLD progression [20]. All outcomes were monitored at a midpoint (3 months) and endpoint (6 months) of the study.

Sample size determination

Was expressed as a maximum number needed because there were multiple primary outcomes. 50 patients for each arm were considered sufficient to achieve an alpha error of 5% and a beta error of 1%.

Statistical Analysis

Quantitative data were presented as means, standard deviations and ranges for parametric data and as medians with interquartile ranges for non-parametric data. Comparisons between correlated parametric quantitative data were performed using paired t-test, while similar non-parametric data was compared using Mann-Whitney test. Comparing quantitative parametric independent data was performed using independent t-test, while similar non-parametric data were compared using Wilcoxon Rank test. Comparisons of non-parametric categorical data were performed using Chi-square test. Confidence interval was set to 95%. Thus, P value was considered significant at $P<0.05$. Statistical analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS® 23).

Results

Participant characteristics

There were no statistically significant differences ($p\leq0.05$) between groups at baseline regarding; age, distribution of gender, anthropometric data, presence of MetS; use of antidiabetics, liver functions, lipid profile, glucose metabolic parameters, oxidative stress markers, fibroscan scores, and NAFLD fibrosis score (Table 1).

Table 1: Demographics and selected baseline characteristics of studied groups.

Parameter (unit; description)	CG (N=50)	IG (N=50)	p-value
Age (year; mean ± SD) Range	(51.66±7.2) 38-67	(51.54±7.6) 33-64	0.914
Female; number (%)	43 (86%)	42 (82%)	0.779
Male; number (%)	7(14%)	8 (16%)	0.779
Diabetes Mellitus; number (%)	23 (46%)	24 (48%)	0.84
Use of antidiabetic classes; number (%)	23(46%)	24 (48%)	0.84
Hypertension; number (%)	24(48%)	24 (48%)	1
Presence of MetS; number (%)	28(56%)	31 (62%)	0.54
Body mass index, (kg/m2;mean ± SD) Range	(36.66±3.46) 27.3-44.2	(36.71 ± 4.44) 26.4-48	0.91
Waist to hip ratio; median (IQR) Range	0.95 (0.15) 0.78-1.06	0.9 (0.13) 0.76-1.06	0.16
Platelet count (10^3u/L ;mean ± SD) Range	296.5±79.3 130-439	277.34 ± 58.6 162-411	0.052
Fasting blood glucose (mg/dl; median (IQR) Range	114.5(71.3) 65-260	102.15 (50.4) 65-263	0.13
Fasting blood insulin (U/ml; mean ± SD) Range	13.6±2.7 8-20	12.5 ± 3.6 6-20	0.055
HOMA-IR score; median (IQR) Range	3.5 (3.2) 1.6-10.27	3.2 (2.4) 1-11.7	0.061
Serum triglycerides (mg/dl;mean ± SD) Range	158.5±44.9 55-280	149 ± 53 50-299	0.34
Total Cholesterol (mg/dl ;mean ± SD) Range	196.6±27.6 125-264	206.5 ± 26.6 145-253	0.07
LDL cholesterol (mg/dl; median (IQR)) Range	131 (42) 91-179	144 (45) 67-181	0.096
HDL cholesterol (mg/dl; mean ± SD) Range	42.3±6.3 31-61	41.5 ± 6.9 25-62	0.53
VLDL cholesterol {mg/dl; median (IQR)} Range	20.5 15) 10-54	26 (13) 8-51	0.18
AST {U/ L; median (IQR)} Range	43 (22.3) 15-130	37 (20.38) 10-78	0.152
ALT (U /L; median (IQR)) Range	72 (68) 18-161	63 (54.5) 18-151	0.18
Serum albumin (g/L (mean ± SD)) Range	4.08 ± 0.46 3.2-5.1	4.12 ± 0.56 3.2-5.3	0.381

Serum MDA (nmol/l; mean ± SD) Range	496.4 ±74.8 15.5-39.16	496.8 ± 78.7 320-626	0.980
Serum SOD (U/ml; mean ± SD) Range	7.73 ± 0.34 7.1-8.5	7.64 ± 0.4 7.1-8.6	0.211
CRP (mg/dl; mean ± SD) Range	0.54 ± 0.09 0.37-0.7	0.53 ± 0.1 0.36-0.69	0.539
NAFLD fibrosis score (mean ± SD) Range	0.31 ± 1.35 -2.0 to 3.07	0.44 ± 1.31 -1.465 to 3.659	0.465
p-value>0.05: Non-significant CG:Control Group; IG: Intervention Group; MetS:Metabolic Syndrome; HOMA-IR score:Homeostasis Model of Assessment-Insulin Resistance Score; AST:Aspartate Aminotransferase ALT:Alanine Aminotransferase; LDL:Low Density Lipoprotein; HDL:High density Lipoprotein; VLDL: Very Low Density Lipoproteins; MDA:Malondaldehyde;SOD:Superoxide Dismutase; CRP:C-reactive Protein; NAFLD:Non Alcoholic Fatty Liver Disease.			

Biochemical parameters/scores

In accordance with the significant decrease in FBG and FBI in both groups by time, HOMA-IR scores decreased significantly over time with significantly lower levels of FBI and HOMA-IR scores in IG at midpoint and endpoint and significantly lower level of FBG in IG only at endpoint ($p<0.05$). Both groups showed a highly significant drop in AST and ALT and a highly significant increase in serum albumin over time ($p<0.001$) with significant lower levels of AST and ALT at midpoint and endpoint, and a higher level of serum albumin only at endpoint in IG ($p<0.05$). At the end of the study, both groups showed a significant drop in TG, TC, high density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels and significant increases in HDL ($p<0.05$). TG and TC showed significant ($p<0.05$) lower levels in IG at the midpoint and endpoint of the study, while LDL and VLDL showed significant lower levels in IG at the endpoint only ($p<0.05$). HDL showed significant ($p=0.002$) higher levels in IG at the endpoint. Both groups showed highly significant decrease in malondialdehyde (MDA) and C-reactive protein (CRP) ($p<0.001$) and a high significant increase in super oxide dismutase (SOD) ($p<0.001$) over time. In CG, there were significantly higher levels of MDA ($p=0.035$) and CRP ($p=0.022$) and significantly lower levels of SOD ($p<0.001$) only at the end of the study. Both groups showed a significant decrease in NAFLD fibrosis score over time of the study with IG showing a more significant reduction at the endpoint ($p<0.05$).

As demonstrated in Figure 2, IG showed significantly ($p<0.05$) higher number of participants that achieved normalization of laboratory indicators of NAFLD and associated Mets at either or both the midpoint and endpoint of the study.

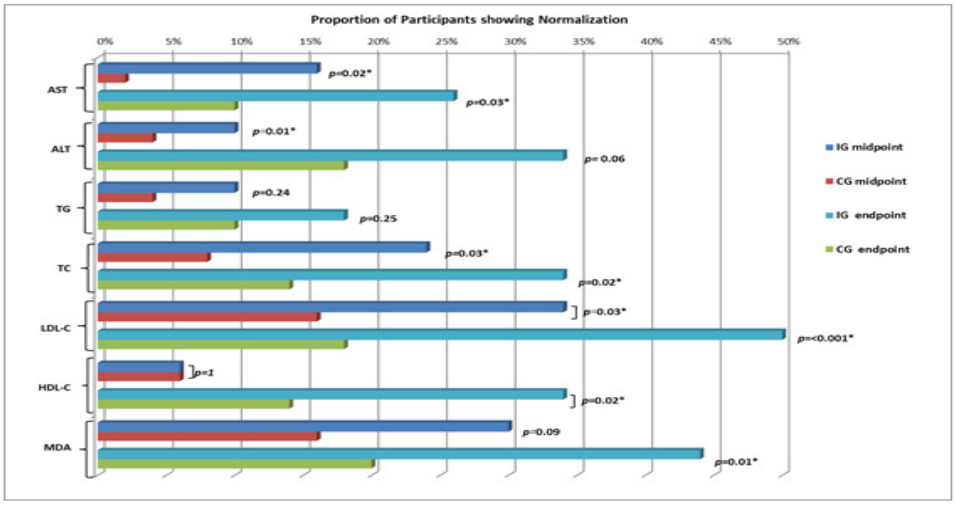

Figure 2: Comparing laboratory indicators normalization between groups over time.

*Statistically significant difference at $p\leq0.05$;ALT:Alanine Aminotransferase; AST:Aspartate Aminotransferase; CG:Control Group; IG:Intervention Group; HDL-C:High density Lipoproteins-cholesterol; LDL-C:Low Density Lipoproteins-cholesterol;MDA:Malo ndaldehyde;TC:Total Cholesterol;TG:Triglycerides; Normal range: AST:10-42 U/L; ALT:10-45U/L; TC<200 mg/dl;TG<150mg/dl; LDL-C<130 mg/dl; HDL-C>50 mg/ dl; MDA:270-450 nmol/ml.

Clinical parameters and scores

There was a highly significant drop in BMI of both groups over time ($p<0.001$) with no significant difference between them ($p>0.05$). The w/h ratio was reduced significantly among both groups over time ($p<0.001$) with IG showing significantly lower values after treatment ($p<0.05$). The presence of MetS showed a highly significant drop in both groups ($p<0.001$) over time with a significantly greater reduction in IG ($p<0.05$).

Radiologically evident liver fibrosis stages and liver steatosis categories have dropped significantly in both groups along study duration ($p<0.001$). At the midpoint and endpoint of the study, greater number of participants in IG (34% and 54.0% respectively) shifted to a less severe fibrosis stage than CG (22% and 30.0% respectively); without significant difference between groups at midpoint and a significantly higher number at endpoint ($p=0.181$ and 0.015 respectively). Similarly, at midpoint and endpoint of the study, greater number of participants in IG (48% and 76% respectively) shifted to improved liver steatosis score grades [14] than CG (28% and 44.% respectively) with a significant difference at the two time points ($p<0.05$) (Figure 3).

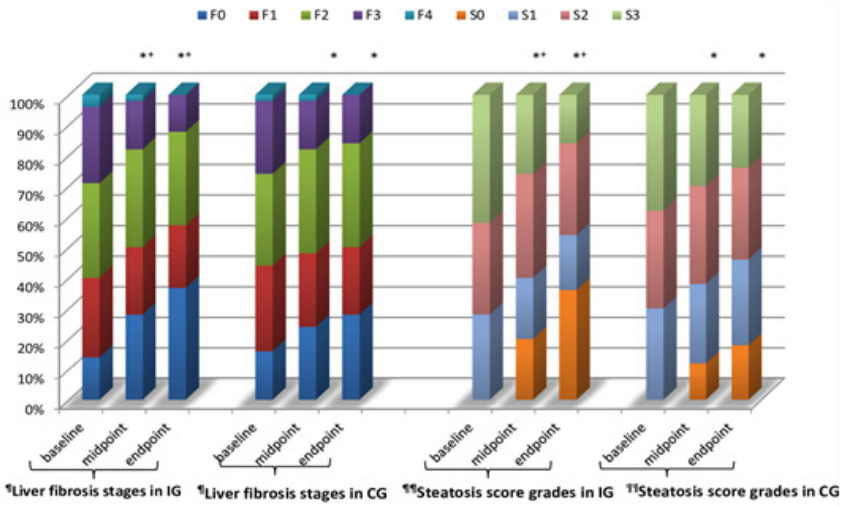

Figure 3: Comparing proportion of participants in different stages of liver fibrosis and steatosis grades in groups over time.

CG:Control Group; IG:Intervention Group;*Statistically significant difference compared to baseline; + Statistically significant difference compared to CG group.

Dyspeptic symptoms, hepatomegaly and pain in the right hypochondrium have significantly improved in both groups over time ($p<0.001$). Shifting to better NAFLD fibrosis score categories and lost criteria for Mets diagnosis were significantly higher in IG at end point ($p<0.05$). Symptoms that improved more significantly in IG are hepatomegaly at midpoint ($p=0.026$) and endpoint ($p<0.001$), dyspeptic symptoms at midpoint and the pain in right hypochondrium at endpoint ($p<0.05$). Although there was a higher number of patients who shifted to an improved or normal HOMA-IR score category [8] in IG at midpoint and endpoint, the differences were insignificant (Figure 4).

Only one patient in IG suffered from diarrhea, managed with anti-diarrhea and didn't need to interrupt treatment.

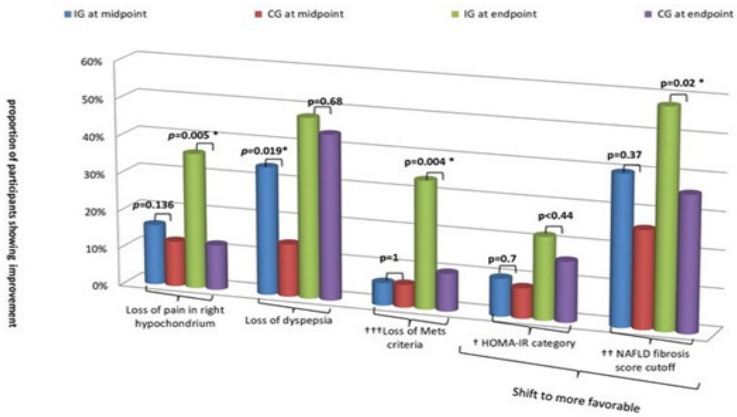

Figure 4: Comparing percentage of improvement of clinical parameters and scores between groups.

*Statistically significant comparison with $p\leq0.05$; CG:Control Group; IG:Intervention Group; Mets:Metabolic Syndrome; NAFLD:Non Alcoholic Fatty Liver Disease.

Discussion

NAFLD is a worldwide condition with multifactorial pathogenesis linking genetic, environmental and metabolic factors. Better characterization of NAFLD development and progression enables identification of those at higher risk of metabolic, cardiovascular and neoplastic complications and updating diagnostic and therapeutic implements. Life style modifications including hypocaloric regimens, physical exercise and controlling hyperglycemia with diet, insulin, or oral hypoglycemic are the only approved therapeutic options so far. Additive pharmacological agents should focus on reverting or improving components of Mets, the main accusation in developing NAFLD [2].

The findings of the current study with NAFLD and metabolic comorbidities showed that adjunctive EPLs had significant favorable effects on Mets attributes including w/h ratio, TG, TC, VLDL, LDL, HDL, and HOMA-IR score at endpoint of the study ($p<0.05$). The suggested mechanisms for these achievements can be in accordance with those of Sahebkar, et al. [12] who proved that EPLs administration increases plasma apo A-I, thus stimulating reverse cholesterol transport from peripheral tissues to liver and that they can reduce hepatic fat content through suppression of lipogenic enzymes, induction of fatty acid β-oxidation and augmentation of biliary cholesterol excretion. Also EPLs increase serum HDL-C while reducing serum TG via inhibition of hepatic lipase.Moreover, EPL inhibits lecithin cholesterol acyltransferase [LCAT], acyl cholesterol acyltransferase and lipoprotein lipase in plasma and peripheral tissues causing decreased; cholesterol esterification, TC concentrations and loading of apo-B containing lipoproteins. Reduced TC is also achieved by excess gut phospholipid interaction with micellar absorption of cholesterol by enterocytes. Meanwhile, the enhanced HDL-C serum level is caused by the EPL induced inhibition of cholesteryl ester transfer protein [12].

The beneficial effect of EPL observed in the current study could also be explained by the correction of impaired hepatic phosphatidylcholine (PC) and phosphatidylethanolamine contents that are involved in metabolic disorders including insulin resistance and obesity and have been linked to NAFLD and impaired liver generation [21].

The results of this study are also justified by the observed negative associations of waist circumference, w/h ratio, body fat percentage, and fat mass with the linoleic, oleic, and docosapentaenoic acids content of PC in patients with MetS traits [22].

Insulin resistance has an important role in the pathogenesis of steatosis, and is a significant risk factor for fibrosis together with high serum AST level and older age among Egyptian patients with NASH [23]. In the end of the current study; EPL showed more positive effect on FBG and FBI; thus lowering HOMA-IR score more significantly in IG ($p<0.05$).

Adjuvant EPL in the current study showed more significantly improved AST, ALT, dyspepsia, hepatomegaly and pain in right hypochondrium in the IG at the endpoint ($p\leq0.05$). These results agree with the findings of Dajani, et al. [24], who conducted a randomized multicenter, open label clinical study in Emirates to evaluate adjuvant EPL on 324 patients with primary NAFLD or NAFLD and comorbid disease and led to a significant improvement of symptoms and a significant reduction of ALT and AST ($p<0.01$) [24]. The combined results of current study on lipid profile, liver function and clinical symptoms agree with those of Padma, et al. [25],who undertook an open labeled, nonrandomized, real clinical practice study on adults with NAFLD treated with EPL for 3 months and showed a steady improvement in all signs and symptoms and a significant decrease in liver enzymes, serum bilirubin, TC, LDL cholesterol and TG levels ($p<0.05$) [25]. The change in serum HDL cholesterol and FBG levels were not significant after three months. Similarly, in the current study, IG showed non-significant increase in HDL and decrease in FBG ($p>0.05$) as compared to CG after 3 months, however there were significant favorable differences in both ($p<0.05$) after 6 months in the favor of IG. These positive effects were reproducible by Li, et al. [26], who conducted a randomized double-blind study on 36 obese patients with fatty liver using EPL plus vitamin D versus placebo and vitamin D for 3 months and reported a decreased TC (by 10%), TG (by 9%) and normalized transaminases (87.5% of patients) [26]. Similarly, serum TC in the current study has decreased by 13% and 20% while serum TG decreased by 8.5% and 14% after 3 and 6 months respectively.

Reversing or improving steatosis is a key therapeutic goal for NAFLD patients to prevent progression to NASH with consequently improved long term prognosis. EPL has demonstrated clear positive effects on improving steatosis as evidenced by histology, CT and US dynamic evaluation in several observational and randomized controlled studies [27]. US is the first-line diagnostic procedure for NAFLD while biomarkers and scores of fibrosis, as well as transient elastography, are acceptable non-invasive procedures. Combining biomarkers, scores and transient elastography provides additional diagnostic and monitoring accuracy that could replace invasive liver biopsies [1]. More significantly improved steatosis score grades were evident in IG at midpoint and endpoint of the current study that could be attributed to individualized life style recommendations and patient education received by all patients together with the achieved weight loss in both groups. The findings of the current study were in accordance with those obtained by Vilar-Gomaz et al. [28], who conducted a prospective study on 293 NASH patients who adhered to lifestyle changes to reduce their weight over 52 weeks and reported significantly improved weight loss (≥10%), histologic features of NASH and attained the highest rates of NASH reduction, NASH resolution, and fibrosis regression [28]. The findings of this study agree also with a study in 68 Chinese patients with NAFLD and T2DM who was openly randomized to either metformin or metformin plus EPL for 3 months showing significantly better ultrasonographic appearance ($p<0.05$) in the EPL plus metformin arm compared with the other arm [29]. Moreover, current study results were in line with a long term trial of 215 Russian diabetic patients with NASH randomly allocated to either metformin or metformin plus EPL which showed significant; 6 months improvement of ultrasonography evident hepatic echotexture in 66.4% ($p=0.02$), 7 years decrease in ultrasound signs of fatty liver in 81.6%, slower fibrosis progression ($p=0.03$) and effective control of T2DM in 86.0% of patients in the combined metformin EPL group [30].

Additionally, current study results were in accordance with a review of 25 clinical studies which proved that adjuvant EPL accelerates the improvement or normalization of ultrasonographic features of NAFLD. This evidenced improvement of NAFLD liver features can be referred to the ability of EPL to be incorporated into damaged parts of cellular membranes of hepatocytes thus improving hepatic regeneration and replacing endogenous less unsaturated hepatic phosphatidylcholine molecules and to its ability to increase membrane fluidity eventually increasing its functioning including cell signaling and receptor influencing. Additionally, these positive effects are mediated by the anti-inflammatory, ant-fibrotic and apoptosis modulation properties of EPL [11].

In the Emirati study by Dajani, et al. [24], abdominal ultrasonography indicated normalization in 4.6% and a shift from grade II to grade I in 24% of patients. Liver stiffness measurement showed an improvement in 21.1%, of patients with a mean reduction in the liver stiffness measurement of 3.1 K Pascal/ patient [24]. Similarly; in the current study 54% of patients in the IG improved by shifting to a less severe fibrosis stage ($p=0.015$). This result could be explained by the ability of EPLs, with their polyenylphosphatidylcholine to induce a noticeable deactivation of pro-fibrogenic hepatic stellate cells, involved in the progression of liver fibrosis as stated by a recent study of Valentino, et al. [31], making EPL effective in managing hepatic fibrosis [31].

The oxidant, antioxidant and lipid patterns in NASH were evaluated by Stiuso, et al. [32], who treated these patients with a combination of silybin, phosphatidylcholine, and vitamin E for 12 months. In patient with NAS scores=3.8±1.5; there was a significant decrease of serum lipid peroxidation and a significant increase of SOD activity ($p=0.01$) [32]. In the current study; the IG mean percent change of MDA showed a significant more drop at the midpoint (8.4% compared to 3.8% in CG; $p<0.001$) and the endpoint of study (15.6% compared to 8.6% in CG; $p=0.001$). Similarly; the IG mean percent change of SOD showed a significantly more elevation at the midpoint (3.8% compared to 1.2% in CG) and the endpoint of study (7.1% compared to 3.5% in CG) ($p<0.001$).

Study Limitations

The limited number of enrolled subjects, and non-blindness are the major limitations of the current study, although non-blindness allow discontinuation of treatment in case of serious adverse reactions and targeted drug education for intervention group. Small sample size was the result of multiple exclusions to overcome heterogeneity of affected populations.

Study Conclusion

The present study proved that adding EPL to life style modifications and health education has significantly improved the clinical, laboratory and radiological outcomes in Egyptian NAFLD patients with metabolic comorbidities.

Funding

None.

Conflicts of Interest/Competing Interests

The authors declare that there is no conflict of interests related to this manuscript.

Ethics Approval

Study design and procedures were approved by the institutional review board of Ain Shams University hospital on 20 August 2014 (232326). According to the declaration of Helsinki and have been registered in www.clinicaltrials.gov under the identifier; NCT04411862.

Consent to Participate

All participants in the submitted study gave their written informed consent prior to their inclusion in the study.

Consent for Publication

All authors consented to publication.

Availability of Data and Material

Are available upon request.

Acknowledgement

The authors are grateful to Dr. Gihan Rabeh Clinical Nutrition Specialist for providing proper Nutrition plans for NAFLD patient.

References

1. European Association for the Study of the, Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity, EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016. 64(6):1388-402.
2. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, et al. (2018) The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 67(1):328-357.
3. Kyu HH (2018) Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392(10159):1859-1922.
4. Adams LA, Anstee QM, Tilg H, Targher G (2017) Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut 66(6):1138-1153.
5. Marchisello S, Di Pino A, Scicali R, Urbano F, Piro S,et al. (2019) Pathophysiological, Molecular and Therapeutic Issues of Nonalcoholic Fatty Liver Disease: An Overview. Int J Mol Sci 20(8):1948.
6. Manning, DS, Afdhal NH (2008) Diagnosis and quantitation of fibrosis. Gastroenterology134(6): 1670-1681.
7. Petta S, Wong VW, Cammà C, Hiriart JB, Wong GL, et al. (2017) Serial combination of non-invasive tools improves the diagnostic accuracy of severe liver fibrosis in patients with NAFLD. Aliment Pharmacol Ther 46(6):617-627.
8. Muniyappa R, Lee S, Chen H, Quon MJ (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 294(1):E15-26.
9. Mahmoud AA, Bakir AS, Shabana SS (2012) Serum TGF-β,

Serum MMP-1, and HOMA-IR as non-invasive predictors of fibrosis in Egyptian patients with NAFLD. Saudi J Gastroenterol 18(5):327.
10. Lonardo A (2017) AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. Dig Liver Dis 49(5):471-483.
11. Gundermann KJ, Gundermann S, Drozdzik M, Mohan Prasad VG (2016) Essential phospholipids in fatty liver: a scientific update. Clin Exp Gastroenterol 9:105-117.
12. Sahebkar A (2013) Fat lowers fat: purified phospholipids as emerging therapies for dyslipidemia. Biochim Biophys Acta 1831(4):887-893.
13. Malinowski SS, Byrd JS, Bell AM, Wofford MR, Riche DM (2013) Pharmacologic therapy for nonalcoholic fatty liver disease in adults. Pharmacotherapy: Pharmacotherapy 33(2):223-242.
14. Association AH (2001) Executive Summary of Third report of the National Cholesterol Education Program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults-Adult Treatment Panel III (NCEP-ATP III). Final Report. J American Medical Association- 285:2486-2497.
15. Wafaa M Ezzat, Shadia Ragab, Nagwa Abdallah Ismail, Yasser A Elhosary, Abeer M Nour Eldin Abd ElBaky, et al. (2012) Frequency of non-alcoholic fatty liver disease in overweight/ obese children and adults: Clinical, sonographic picture and biochemical assessment. J Genetic Engineering Biotechnol 10(2):221-227.
16. Nichols T (2014) A Review of Fatty Liver/NASH and Liver Cirrhosis: Genetics, Prevention, Nutritional, Behavioral Modification, Exercise, Pharmaceutical, Biophysics and Biotech Therapy. J Liver 2014/01/013.
17. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, et al. (2012) The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 55(6):2005-2023.
18. Gonciarz Z, Besser P, Lelek E, Gundermann Karl-Josef, Johannes KJ (1988) Randomised placebo-controlled double blind trial on essential phospholipids in the treatment of fatty liver associated with diabeteS. Med Chir Dig 17:61-65.
19. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, et al. (2007) The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 45(4):846-854
20. Promrat K, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, et al. (2010) Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. Hepatology 51(1):121-129.
21. van der Veen JN, Kennelly JP, Wan S, Vance JE, Vance DE, et al. (2017) The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochim Biophys Acta Biomembr 1859(9 Pt B):1558-1572.
22. Zeman M, Vecka M, Burda M, Tvrzická E, Staňková B, et al. (2017) Fatty Acid Composition of Plasma Phosphatidylcholine Determines Body Fat Parameters in Subjects with Metabolic Syndrome-Related Traits. Metab Syndr Relat Disord 15(7):371-378.
23. Ahmed AM, Hassan MS, Abd-Elsayed A, Hassan H, Hasanain AF, et al.(2011) Insulin resistance, steatosis, and fibrosis in Egyptian patients with chronic Hepatitis C virus infection. Saudi J Gastroenterol 17(4):245.
24. Dajani AI, Abu Hammour AM, Zakaria MA, Al Jaberi MR, Nounou MA, et al. (2015) Essential phospholipids as a supportive adjunct in the management of patients with NAFLD. Arab J Gastroenterol 16(3-4): 99-104.
25. Padma L, Mukaddam Q, Trailokya A (2013) An observational study of Essentiale-L in the treatment of patients with fatty liver disease. Indian J Clin Pract 3(11):735-739.
26. Li JH, Chen XY, Zhong CF, Min J (200) A randomized controlled study of essential phospholipids (Essentiale capsules) in the treatment of fatty liver. Infect Dis Info 13(4):180-181.=
27. Ivashkin VT, Mayevskaya MV, Pavlov ChS, Tikhonov IN, Shirokova YN, et al. (2016) Diagnostics and treatment of non-alcoholic fatty liver disease: clinical guidelines of the Russian Scientific Liver Society and the Russian gastroenterological association. Rus J Gastroenterol Hepatol Coloproctol 26(2):24-42.
28. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, et al. (2015) Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. Gastroenterology 149(2):367-378.
29. Sun C, Zheng X, Tan Z, Cui F, Zhang R (2008) Clinical observation on polyene phosphatidyl choline and metformin in the treatment of type 2 diabetes and non-alcoholic fatty liver disease. Clin Focus 23(17):1272-1273.
30. Sas E, Grinevich V, Efimov O,,, et al. (2013) 1366 beneficial influence of polyunsaturated phosphatidylcholine enhances functional liver condition and liver structure in patients with nonalcoholic steatohepatitis. Results of prolonged randomized blinded prospective clinical study. J Hepatol 58:549.
31. Valentino G, Zivko C, Weber F, Brülisauer L, Luciani P (2019) Synergy of Phospholipid-Drug Formulations Significantly Deactivates Profibrogenic Human Hepatic Stellate Cells. Pharmaceutics 11(12): 676.
32. Stiuso P, Scognamiglio I, Murolo M, Ferranti P, De Simone C, et al. (2014) Serum oxidative stress markers and lipidomic profile to detect NASH patients responsive to an antioxidant treatment: a pilot study. Oxid Med Cell Longev 2014:169216.