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A randomized controlled trial comparing the effects of Vitamin E, Ursodeoxycholic acid and Pentoxifylline on Egyptian non-alcoholic steatohepatitis patients

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Abstract. – OBJECTIVE: Currently, no NASH-specific therapies are approved by the US Food and Drug Administration. This study aimed to compare the clinical effect of vitamin E (Vit. E), Ursodeoxycholic Acid (UDCA) and pentoxifylline (PTX) on Egyptian patients with NASH with exploration of their possible roles on inflammatory cytokines and chemokines mainly Interleukin 6 (IL6) and Monocyte Chemoattractant Protein-1 (CCL2/MCP-1).

PATIENTS AND METHODS: We conducted a 3-month, randomized, single-blind study in 102 Egyptian NASH patients who were divided into three groups; group 1 received Vit. E 400 mg twice a day, group 2 received UDCA 250 mg twice a day and group 3 received PTX 400 mg twice daily. Liver aminotransferases (AST, ALT), IL6, CCL2/MCP-1, albumin, bilirubin, and lipid panel were measured both before and after intervention intake.

RESULTS: A significant decrease was found in liver aminotransferases, serum cytokine and chemokine in participants after Vit. E, UDCA or PTX intake. Compared to the UDCA and PTX groups, liver aminotransferases, serum cytokine and chemokine showed a more statistically significant reduction after Vit. E administration (50%, 43%, 57% and 55% for ALT, AST, IL6 and CCL2/MCP-1, respectively). In contrast, other biochemical tests showed non-significant change after any drug intake. None of the tested drugs showed significant safety issues in this population.

CONCLUSIONS: Treatment with Vit. E, UDCA and PTX was both safe and effective in improving hepatic aminotransferases and inflammatory markers in Egyptian NASH patients. The superior effect of Vit. E compared to UDCA and PTX may suggest that oxidative stress plays a key role in disease progression of NASH patients. Moreover, IL6 and CCL2/MCP-1 may be used with or without ALT for treatment evaluation of NASH people.

Key Words:
Vitamin E, Ursodeoxycholic acid, Pentoxifylline, NASH, CCL2/MCP-1, IL6, ALT.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease with a global prevalence of 25% in adults. Currently, it is the second leading cause of liver transplantations in the United States[1]. Nonalcoholic steatohepatitis (NASH) is regarded as the inflammatory subtype of NAFLD, with steatosis in addition to evidence of hepatocyte injury and inflammation, with or without fibrosis[2]. With time, NASH can progress to cirrhosis, end-stage liver disease, or liver transplantation[3-5].

Although the pathogenesis and progression of NAFLD and NASH are very complex, most recent research indicates that multiple pathways are involved. Abnormalities in inflammatory cytokines and oxidative stress have been reported in NASH patients[6,7]. Cytokines like TNF-α, Interleukin 6

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(IL6), and chemokines like Monocyte Chemoattractant Protein-1 (CCL2/MCP-1) with oxidative stress and lipid peroxidation have been postulated to play an important role in the induction of steatohepatitis[6-8]. Recent studies indicated increasing levels of chemotactic cytokines CCL2/MCP-1 and IL6 in NASH[9] suggesting a possible noninvasive tool for diagnosis and treatment evaluations on NAFLD despite limited data is available.

Up till now, no pharmacological therapies, targeting pathophysiological disease modalities, are approved for treatment of NAFLD or NASH[1]. A variety of drugs have been tried for treatment of NASH, including promising drugs like vitamin E (Vit. E), pentoxifylline (PTX), and Ursodeoxycholic acid (UDCA). Vitamin E is one of the body's most effective chain-breaking antioxidants that has shown to delay the pathogenesis of NASH[10,11]. Vitamin E can suppress peroxidation and limit the expression of transforming growth factor-beta, which has been linked to hepatic fibrosis and hepatocyte apoptosis by stimulating hepatic stellate cells[12]. Additionally, Vit. E has other therapeutic effects that can delay hepatic fibrosis and possibly prevent cirrhosis by modulating inflammatory response, cell injury, cellular signaling, and cellular proliferation[13,14]. Moreover, Vit. E can reduce the inflammatory response in NAFLD by reducing the expression of cytokines, such as TNF-α, IL1, IL2, IL4, IL6, and IL8[15-17]. Regarding Ursodeoxycholic acid (UDCA), it is a metabolic by-product of intestinal bacteria and has been proven to be useful in the non-surgical treatment of cholesterol gallstones and primary biliary cirrhosis (PBC)[18]. The clinical features of UDCA include anti-apoptotic effects, reduction of serum TNF-α concentrations, and improvement of hepatic insulin sensitivity[19]. Concerning Pentoxifylline (PTX), it is a well-tolerated medication that improves blood viscosity and erythrocyte rheological characteristics in individuals with peripheral vascular disease[20]. In addition, PTX is a nonspecific phosphodiesterase inhibitor that increases cyclic adenosine monophosphate (cAMP) levels while decreasing TNF-α gene transcription[21,22]. Therefore, pentoxifylline (PTX) has a potent inhibitory effect on both proinflammatory and anti-inflammatory cytokines[23].

Despite the promising role of Vit. E, PTX, and UDCA, there is no clinical study to compare the efficacy and safety of these drugs in NASH populations. Therefore, this study aimed to compare the efficacy and toxicity of these promising drugs on Egyptian patients with NASH with exploration of their possible roles on inflammatory cytokines and chemokines mainly IL6 and CCL2/MCP-1 respectively.

Patients and Methods

Study Design and Patients

This was a 3-month, prospective, randomized, parallel, and single blind study. Participants were recruited from the outpatient clinic and hepatology, gastroenterology and infectious diseases department at Kafrelsheikh University Hospital between February 2020 and January 2021. Two-hundred and five Egyptian patients diagnosed with NAFLD with potential clinical inclusion criteria were invited for a screening session to determine their eligibility for the study. Patients were enrolled in the study if they are ≥ 18 years old, had evidence for NASH; persistently elevated alanine aminotransferase (ALT) >1.5 times the upper limit of normal), imaging (ultrasound) showing fatty infiltration, and histological evidence of NASH after biopsy (macrovascular steatosis, ballooning degeneration of hepatocytes, scattered lobular inflammation and apoptotic bodies).

Patients were excluded if they had a history of alcohol dependence, treatment with drugs known to induce NASH (e.g., amiodarone, calcium channel blocker, tamoxifen, oral anticoagulation, methotrexate, steroids and estrogen), positive serologic markers for known chronic liver diseases (hepatitis B surface antigen, anti-hepatitis C virus antibody antinuclear antibody), human immunodeficiency virus (HIV) infection, diabetes and decompensated liver disease defined as serum bilirubin level >1 mg/dL, albumin level <3.5 g/dL, and international normalized ratio (INR) ≥1.7.

All procedures were performed according to the ethical standards of the National Research Committee (Kafrelsheikh University Ethical Committee) and in compliance with the Declaration of Helsinki[24]. Each patient gave a written informed consent for the inclusion in this study.

One hundred and forty-six patients were excluded from the study either because they did not meet the inclusion criteria (n = 135) or refused to enroll in the study (n =11). Qualifying participants (n = 102) were randomly assigned to one of the three treatment groups using a computer-generated randomization sequence. Group I (n = 34) received 400 IU Vitamin E (Vitamin

E 400 IU®, MEPACO Pharmaceutical Company, Enshas El Raml, Ash Sharqia, Egypt) twice daily for 3 months. Group II (n =34) received 250 mg Ursodeoxycholic acid (Ursofalk 250 mg®, MINAPHARM Pharmaceutical Company, Takseem Asmaa Fahmy, Ard El Golf, Heliopolis, Cairo, Egypt) twice daily for 3 months. Group III (n = 34) received 400 mg sustained release (SR) Film-Coated Tablets of pentoxifylline (Trental 400 mg®, SANOFI Pharmaceutical Company, St., Zeitoun, EL Massaneh, Cairo, Egypt) twice daily for 3 months. Patients were followed up every week to ensure compliance, drug adherence and to report side effects or drop out from the study.

Biochemical Assays

The study period was 3 months, and the patients have been evaluated both at baseline (before treatment) and at the end of the study duration. At each time point, serum samples were immediately prepared using approximately 3 mL blood collected after 8-10 hours of fasting, then aliquots were frozen and stored at -80°C until analysis. Aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), bilirubin and serum albumin were determined by enzymatic colorimetric assay on automated chemistry analyzer (Pentra C 400-Horiba, 34184 Montpellier, Cedex 4 -France). Serum IL6 and CCl2/MCP-1 were determined by using Enzyme-Linked Immunosorbent Assay Kits (My BioSource, San Diego, CA, USA). Complete blood picture, including hemoglobin, platelets and total leucocytes count, were measured using an automated cell counter (Pentra XL80, Horiba, 34184 Montpellier, Cedex 4, France). Lipid blood profile, including total cholesterol, triglycerides, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were determined by enzymatic colorimetric assay using Indiko™ Plus Clinical Chemistry Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Clinical Symptoms

Patients have been evaluated both at baseline (before treatment), every two weeks (follow up) and at the end of the study duration for any reported symptoms of NASH (mainly malaise, abdominal pain) or any reported side effects of therapeutic intervention. The primary study endpoint was normalization of ALT levels and resolution of NASH. The secondary endpoints were normalization of serum levels of cytokine and chemokine.

Statistical Analysis

The sample size of each group was calculated from Cohen's d tables using preliminary data obtained of liver enzyme changes (primary end point) according to the desired study power (90%), and the significance level (0.05). Values were presented as the mean ± SD using IBM SPSS Statistics 24.0 per Windows (Armonk, NY, USA). Paired *t*-test was used to assess any significant difference between each group before and after treatment course. One-way analysis of variance test was used to assess any significant difference among the three groups and two-sample *t*-test was used to assess any significant difference between each two groups after treatment. A $p < 0.05$ was considered statistically significant. Chi square tests was used to compare the clinical symptoms in the three groups. Pearson's correlation coefficient was used for correlation analysis.

Results

The study enrolled 205 NAFLD Egyptian patients who were evaluated by a liver function test, and a liver ultrasonography examination. According to the inclusion criteria, 102 patients were included and randomly assigned to the three treatment groups. Due to the COVID-19 pandemic, 6 patients dropped out and 2 other patients did not complete the study. A total of 94 subjects completed the 3-month study period (Figure 1).

The mean age of the included participants was 44.5 ± 10.5 years. The majority of subjects (80%) were overweight (BMI ≥25 <30 kg/m^2). Gender distribution was 52 males and 42 females contributing to 55.3% and 44.7% of the participant population, respectively (Table I). As revealed from ANOVA test, and illustrated in Table I, there was no statistically significant difference between the three groups at baseline regarding demographics, anthropometric characteristics, CBC findings, liver function test, lipid profile, IL6, CCL2/MCP-1 and clinical symptoms.

After 3 months, there was a statistically significant ($p < 0.05$) reduction in serum levels of the inflammatory cytokine (IL6) and chemokine CCL2/MCP-1 in the three treatment groups compared to baseline values (Table I and Figure 2). Specifically, IL6 level was reduced by 57%, 47%, and 39% after treatment with Vit. E, UDCA, and PTX, respectively (Figure 2A) and CCL2/MCP-1 level was reduced by 55%, 37%, and 20% in the Vit. E, UDCA, and PTX groups, respectively

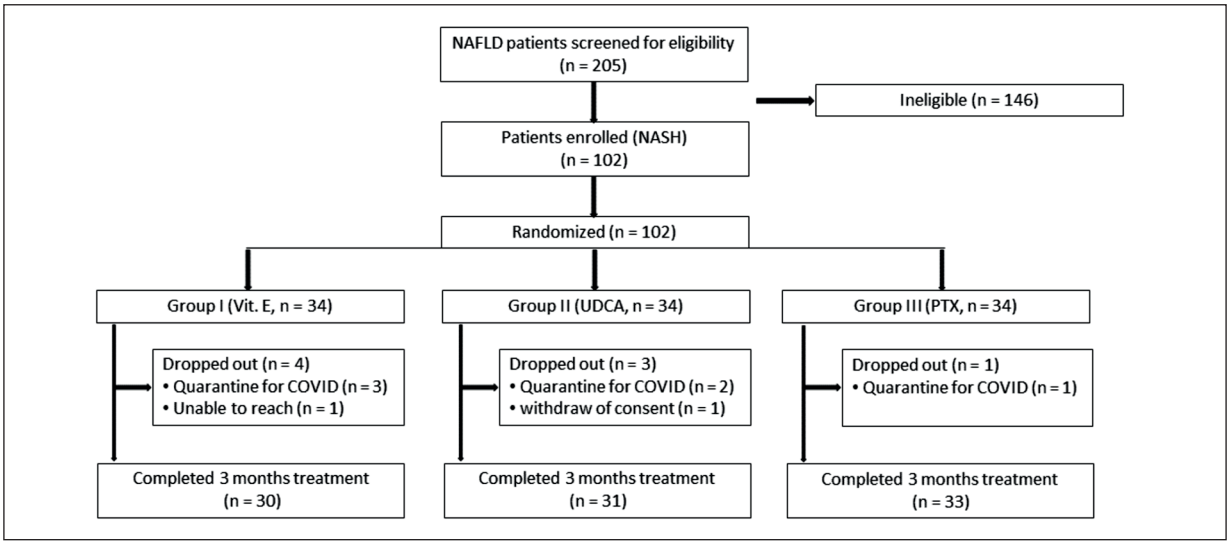

Figure 1. Patient enrollment flow diagram. NAFLD; Nonalcoholic fatty liver disease, NASH; Nonalcoholic steatohepatitis, Vit. E; Vitamin E, UDCA; Ursodeoxycholic acid, PTX; Pentoxifylline.

(Figure 2B). Similarly, patients in the three treatment groups showed a significant reduction in the liver enzymes AST and ALT (Table I and Figure 2). After 3-month treatment with Vit. E, UDCA, and PTX, serum level of AST was reduced by 43%, 35%, and 19%, respectively (Figure 2C). Likewise, serum level of ALT was significantly reduced by 50%, 41%, and 17% in the Vit. E, UDCA, and PTX groups, respectively (Figure 2D). Although these biochemical markers showed significant reduction in the three treatment arms, the changes produced by Vit. E supplementation were greater and statistically significant compared to the other two treatments. However, changes in IL6, CCL2/MCP-1, AST, and ALT were comparable between the UDCA and PTX groups ($p > 0.05$, for between groups). The other tested parameters (CBC, lipid profile, bilirubin, and albumin) did not change significantly in any of the treatment groups compared to baseline (Table I).

At baseline, abdominal pain and malaise were the most common complaints reported by > 50% of the study subjects. After treatment, Vit. E resulted in significant reduction in abdominal pain (80%) and malaise (73%) incidence compared to baseline ($p < 0.05$). Although UDCA and PTX resulted in symptomatic relief, none of these changes were statistically-significant (Table I).

All study subjects tolerated the treatment intervention, and the reported drug related side effects were mild and rare (Table II). The most commonly reported adverse effects were gastrointestinal and were reported in 21% of the patients and occurred more frequent in the Vit. E and PTX arms compared to the UDCA arm. However, the differences in frequency of adverse effects were not statically significant among groups.

A positive and significant correlation was observed between the changes in ALT and changes in the inflammatory cytokine (IL6) and chemokine CCL2/MCP-1 with a Pearson correlation factor of 0.51 and 0.49, respectively in the Vit. E-treated group (Table III). A similar correlation (R = 0.56) was observed after treatment with UDCA, but only with CCL2/MCP-1. On the contrary, no significant correlation was detected between the changes in liver enzymes and either of the tested inflammatory markers in the PTX-treated group.

Discussion

The pathogenesis of NASH and mechanisms responsible for liver injury are still incompletely understood[25]. Up till now, there are no Food and Drug Administration–approved pharmacologic agent for NASH[26,27]. Currently, promising treatments recommended by American Association for the Study of Liver Diseases (AASLD) in patients with NASH include vitamin E, pentoxifylline and Ursodeoxycholic acid (UDCA). To our knowledge, this study was the first to compare the efficacy and toxicity of these drugs in Egyptian populations with NASH and explore

Table I. Characteristics of study subjects at baseline and at end of study period.

	All patients (n = 94) Baseline	Group I (Vit. E) (n = 30)		Group II (UDCA) (n = 31)		Group III (PTX) (n = 33)	
		Baseline	3 months	Baseline	3 months	Baseline	3 months
Lab values							
Weight (kg)	93.6 ± 12.0	93.8 ± 9.3		90.7 ± 15		96.3 ± 10	
BMI (kg/m^2)	27.5 ± 2.5	28.1 ± 2.2		26.8 ± 2.1		27.0 ± 3.1	
Age (year)	44.5 ± 10.5	44.8 ± 9.7		43.4 ± 11		45.2 ± 11	
Gender (number)							
Male	52	16		17		19	
Female	42	14		14		14	
Hemoglobin (g/dl)	13.1 ± 1.2	12.8± 1.3		13.5 ± 1.4		13.2 ±1.1	
RBCs (10^6/dl)	5.1 ± 0.76	5.3 ± 0.8		5.2 ± 0.5		4.9 ± 0.9	
WBCs (10^3/dl)	6.9 ± 1.7	7.3 ± 1.5		7.0 ± 1.7		6.6 ±1.8	
Platelet (10^3/dl)	262 ± 53	269 ± 36		265 ± 50		252 ± 66	
IL6 (pg/ml)	122.7 ± 16	123.4 ± 15	52.6 ± 6.9*	118 ± 20	62.8 ± 9.5*	126 ± 9.9	76.4 ± 6.2*
CCL2/MCP-1 (pg/ml)	282.8 ± 62	289 ± 75	131 ± 43*	293 ± 52	185 ± 52*	268 ± 58	215 ± 48*
AST (U/L)	97.4 ± 25	99.5 ±15	56.8 ± 17*	99 ± 33	64 ± 18*	94.1 ± 24	76.3 ± 29*
ALT (U/L)	109 ± 43	97.6 ± 33	48.5 ± 10*	121 ± 44	71.8 ± 25*	108 ± 32	89.7 ± 43*
TC (mg/dl)	259 ± 30.5	269 ± 39	255 ± 40	251 ± 23	238 ± 30	259 ± 26	246 ± 26
Triglyceride (mg/dl)	199 ± 32.5	197.6 ± 26	184 ± 35	200 ± 42.2	195 ± 46	199 ± 28	192 ± 30
LDL (mg/dl)	183.8 ± 42	195 ± 51.1	190 ± 49	180 ± 36	172 ± 34	177 ± 37	171 ± 34
HDL (mg/dl)	53.3 ± 7.4	53.7 ± 5.1	55 ± 5.6	54.9 ± 7	56 ± 7.7	51.3 ± 9.1	52.8 ± 7.2
D. Bilirubin (mg/dl)	0.2 ± 0.06	0.24 ± 0.04	0.22 ± 0.04	0.22 ± 0.13	0.19 ± 0.07	0.23 ± 0.03	0.20 ± 0.06
T. Bilirubin (mg/dl)	0.81 ± 0.1	0.85 ± 0.07	0.81 ± 0.10	0.84 ± 0.08	0.81 ± 0.11	0.87 ± 0.13	0.80 ± 0.13
Albumin (g/dl)	3.8 ± 0.5	3.63 ± 0.3	3.75 ± 0.4	3.71 ± 0.3	3.89 ± 0.6	3.66 ± 0.3	3.93 ± 0.6
Clinical symptoms							
Abdominal pain	54 (57%)	10 (33%)	2 (6.5%)$^{\#}$	15 (48.5%)	8 (26%)	12 (36%)	7 (21%)
Malaise	52 (55%)	11 (37%)	3 (10%)$^{\#}$	17 (55%)	9 (29%)	13 (39%)	8 (24%)

*Significant change (paired *t*-test $p < 0.05$), $^{\#}$Significant change (Chi square test, $p < 0.05$). BMI: Body mass index; RBC: red blood cell; WBC: white blood cell; IL6: Interleukin 6; CCL2/MCP-1: Monocyte chemoattractant protein- 1; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; LDL: Low density lipoprotein; HDL: High density lipoprotein; TC: total cholesterol; TG: triglyceride; T. Bilirubin: total bilirubin; D. Bilirubin: direct bilirubin; (Vit. E): vitamin E; (UDCA): Ursodeoxycholic acid; (PTX): pentoxifylline.

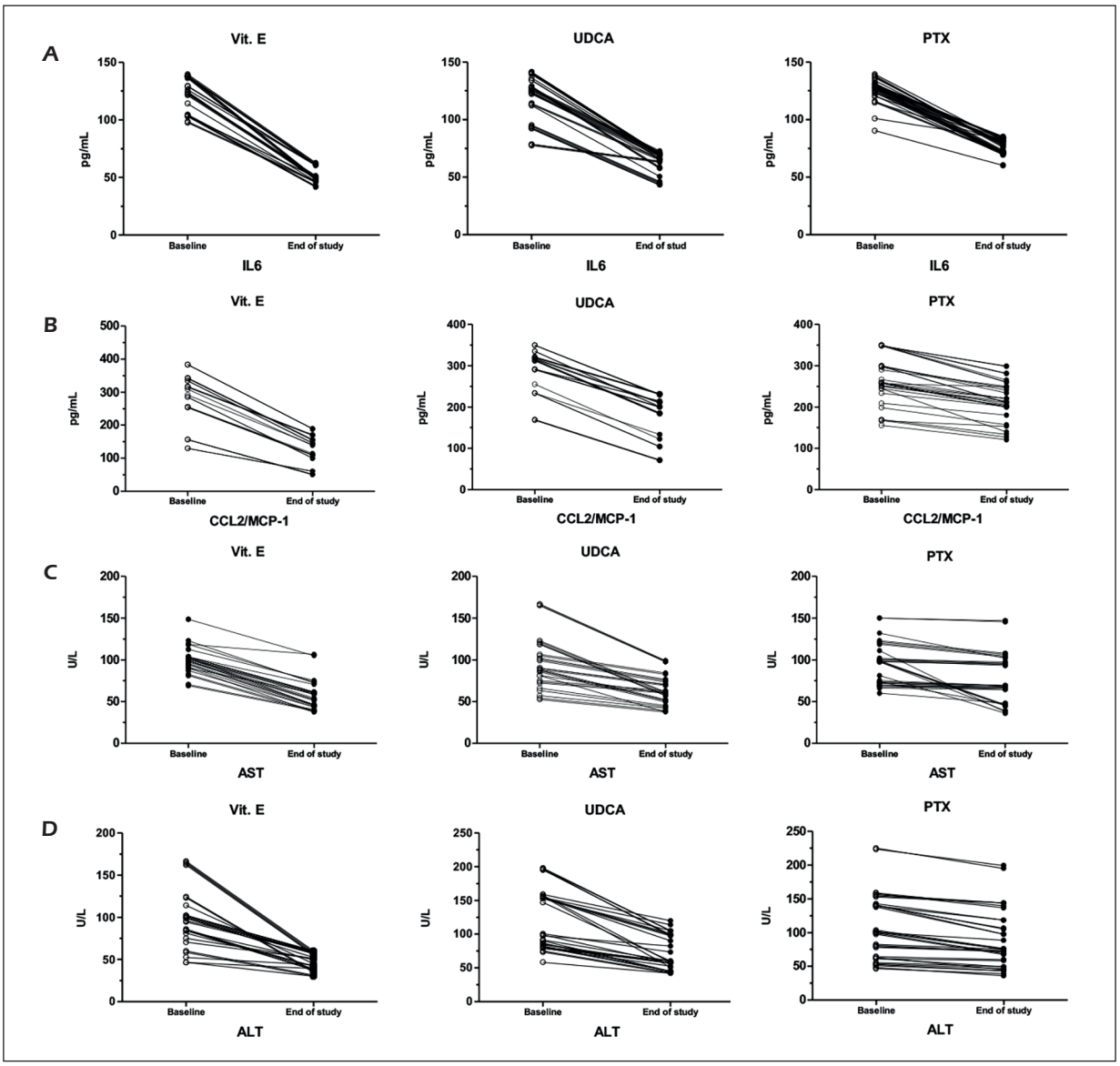

Figure 2. The Change in the serum IL6 **(A)**, Monocyte Chemo-attractant protein-1 CCL2/MCP-1 **(B)**, Aspartate aminotransferase (AST) **(C)**, and Alanine aminotransferase (ALT) **(D)**, levels after 3-month treatment course with vitamin E (Vit. E), Ursodeoxycholic acid (UDCA), and pentoxifylline (PTX) compared to baseline.

their possible roles on inflammatory cytokine and chemokine "IL6 and CCL2/MCP-1".

The current and most prevailing understanding of the pathogenesis of NAFLD and NASH is

Table II. Reported adverse drug effects.

Side Effect	Vit. E (n = 30)	UDCA (n = 31)	PTX (n = 33)	Total (n = 94)
Nausea	2 (6.5%)	0 (0%)	3 (9%)	5 (5.5%)
Vomiting	0 (0%)	1 (3%)	2 (6%)	3 (3.2%)
Diarrhea	3 (10%)	1 (3%)	2 (6%)	6 (6.4%)
Headache	2 (6.5%)	0 (0%)	1 (3%)	3 (3.2%)
Bloating	2 (6.5%)	1 (3%)	3 (9%)	6 (6.4%)

Data presented as n (%). Vit. E: vitamin E; UDCA: Ursodeoxycholic acid; PTX: pentoxifylline.

Table III. Pearson correlation coefficient (R) between the change in liver function tests and the change in the inflammatory cytokines and chemokines after treatment course in the three groups.

		IL6	CCL2/MCP-1
Vit. E	ALT	0.51*	0.49*
	AST	0.15	0.21
UDCA	ALT	-0.14	0.56*
	AST	0.17	0.35
PTX	ALT	-0.05	0.14
	AST	-0.21	-0.07

Data presented as n (%). Vit. E: vitamin E; UDCA: Ursodeoxycholic acid; PTX: pentoxifylline.

based on the "2-hit" hypothesis which involves initial metabolic disturbance causing steatosis followed by subsequent oxidative stress, lipid peroxidation, and production of inflammatory cytokines[28-31]. In the present study, patients with NASH had significantly higher serum levels of ALT, AST in addition to prominent rise in serum levels of IL6 and CCL2/MCP-1 which confirmed the potential role of inflammation in the pathogenesis and progression of NASH. The results of this study show a significant reduction in serum levels of liver transferases, serum cytokine and chemokine in patients with NASH after 3-month treatment with Vit. E, UDCA and PTX, indicating the beneficial effect of these drugs in decreasing liver injury and disease progression and potential therapeutic role in clinical practice. These findings are supported by earlier studies that demonstrated the antioxidant and anti-inflammatory effects of these drugs. Yakaryilmaz et al[32] reported that treatment with oral Vit. E for 24 weeks resulted in suppression of oxidative stress, reduction of insulin resistance, and peroxisome proliferator-activated receptor-alpha (PPAR-alpha) expression in NASH. Similarly, several studies[23,33-35] have demonstrated the efficacy of UDCA and PTX in NAFLD and NASH patients by the inhibition of apoptosis of hepatocytes[33], and pro-inflammatory cytokines including TNF-α[23,34,35]. In addition, our study demonstrated that these drugs were safe at the administered doses and well tolerated similar to non-significant side effects indicated from previous report[36].

Compared to the UDCA and PTX groups, ALT and AST were significantly reduced after vitamin E administration with a greater tendency to ALT normalization. Similarly, IL6 and CCL2/MCP-1 were significantly reduced after vitamin E intake compared to the UDCA and PTX groups. These pronounced activities of vitamin E on ALT, CCL2/MCP-1, IL6, and AST indicated that oxidative stress played the main role in the pathogenesis of NASH. In addition, Vit. E had a significant reduction in clinical symptoms compared to UDCA and PTX indicating a better improvement in clinical symptoms of NASH population and confirming the powerful role of antioxidants like vitamin E in improving the quality of life of NASH populations. The efficacy of Vit. E in NASH has been previously reported by pilot and clinical studies. Recent meta-analyses[37,38] studies documented that vitamin E supplementation improved the levels of amino transaminase in NASH patients supporting the therapeutic potential of vitamin E. Similarly, Sanyal et al[39] showed that Vitamin E was effective in NAFLD and non-cirrhotic nondiabetic NASH patients by AASLD. Compared to Pioglitazone, vitamin E demonstrated a superior activity, with 40% improvement in NASH patients[40]. In contrast, Bugianesi et al[41], reported that Vit. E addition to lifestyle modifications (exercise and low-fat diet) had no role on NASH people and it was less effective than metformin.

From clinical aspects, these results indicated that vitamin E may have a greater potential for the management of NASH in clinical practice than PTX or UDCA and Vit. E may be the drug of choice for NASH treatment in recent guidelines, but further large clinical trials may be required.

Our study also demonstrated the efficacy of PTX in improving liver transaminases as well as inflammatory markers in NASH patients. Findings from previous studies[42,43] agree with our results. For example, Lee et al[44] reported a significant decrease of ALT and AST as well as serum levels of TNF-α and IL6 in patients with NASH after 3 months of treatment with PTX. However, a systematic review on the treatment with PTX in patients with NALFD/NASH including six studies, concluded that PTX reduces liver transaminases and improve liver histological score without affecting cytokines[45].

Although our study demonstrated a significant improvement in liver enzymes and inflammatory markers, there is a huge discrepancy in the literature in regard to the efficacy of UDCA in NASH individuals. Recent human and animal studies suggested that UDCA improved liver enzymes and steatosis in humans and animal model of hepatic injury[33,46,47]. Moreover, a randomized

controlled study showed a beneficial effect of UDCA on ALT without histological improvement. Furthermore, Dufour et al[48] have reported a positive effect and steatosis regression after UDCA treatment in combination with vitamin E for 2 years. Ratziu et al[49] demonstrated that high dose (28-35 mg/kg/day) UDCA for 12 months was safe, well-tolerated, and produced a significant and sustained reduction of ALT levels and fibrosis markers in patients with NASH. On the contrary, larger randomized studies reported no effect of UDCA in NASH patients. For example, a single large multicenter RCT concluded no significant changes in liver enzymes, the degree of steatosis, necroinflammation, or fibrosis after 2 years of treatment with UDCA at daily doses of 13-15 mg/kg[50].

Our study demonstrated for the first time a significant correlation between reduction of ALT and inflammatory markers in the Vitamin E group. Such findings may indicate the essential role of controlling oxidative stress in reducing hepatic inflammation. These results were consistent with those of Chu et al[51], who found that individuals with NAFLD exhibit dysregulated cytokine metabolism at baseline and a substantial drop in IL6 levels after implementing lifestyle adjustments and supplementation with Vit. E and this drop of IL-6 was significantly correlates with ALT[52-54].

From clinical point of view, this correlation between IL6 and CCL2/MCP-1 and ALT may indicate that serum IL6 or CCL2/MCP-1 with or without ALT can be used for treatment evaluation of Vit. E in NASH population. In addition, IL6 and CCL2/MCP-1 may have a role in NASH diagnosis and evaluation of disease progression but further studies are required in the future.

Despite the novel findings of our study, it has a few limitations. We only tested the effect of the drugs under investigation for 3 months. Although several similar studies have implemented this study duration, and even shorter, we believe that a longer treatment duration might be necessary to establish effects. Moreover, we did not demonstrate histological improvement by the end of the study period. It is not unlikely that improvement in liver transaminases or inflammatory markers to be insufficient as surrogate markers of treatment response. Further larger clinical studies, with histological examination of NASH patients for a longer period of time (up to 1 year) may be recommended in the future. In addition, Combination of Vit. E with either PTX or UDCA may be considered for further investigation of possible synergistic activities. Similarly, other inflammatory mediators like Interferon, C-reactive protein (CRP), Interleukin 10 (IL10), Interleukin 1 (IL1), and Tumor Necrosis Factor alpha (TNF-α) should be investigated for possible role in treatment evaluation and disease progression in NASH population in the future.

Conclusions

This study indicated that monotherapy with Vit. E, PTX, or UDCA for 3 months had a beneficial role in NASH population through inhibition of ALT, AST, IL6, and CCL2/MCP-1. In addition, we demonstrated that Vit. E had a more powerful effects on liver aminotransferases, cytokine, chemokine and clinical symptoms than PTX and UDCA with expectation of a better clinical outcomes in practical setting. Moreover, IL6 and CCL2/MCP-1 may be used with or without ALT for treatment evaluation of NASH people. These findings will aid in the development of innovative NASH treatment strategies in Egypt improving the quality of clinical care.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethical Approval

The study protocol was approved by the Ethics Committee of Kafrelsheikh University in accordance with the Declaration of Helsinki and its amendments.

Informed Consent

All subjects provided written informed consent before participation.

Authors' Contribution

Conceptualization: FE, AF and KA; methodology: AF, AEA, MH, AAA and KA; validation: FE, AF, AAA and KA; formal analysis: FE, AF, AEA and AAA; investiga-

tion: FE, AF, MH, and KA; resources: FE, MH, and KA; data curation: FE, AF, KA, AEA, and AAA; writing – original draft preparation: AF, KA and FE; writing – review and editing, FE, AF, KA, AEA, and AAA; supervision, FE, KA; project administration: KA, AF, and FE; All authors have read and agreed to the published version of the manuscript.

Consent for Publication

Patients expressed no objection for the publication of the results.

Availability of Data and Material (Data Transparency)

The datasets generated during and/or analyzed during the current study they are not publicly available due to confidentiality reasons but are available from the corresponding author on reasonable request. Trial registration: ClinicalTrials.gov NCT04977661, retrospectively registered on 071421.

Authors' Declaration

They have made a substantial contribution to research design, the acquisition, analysis and/or interpretation of data; they have drafted the paper or revised it critically; they have given approval of the submitted and final versions, and they don't have any conflict of interest.

References

1) Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, Wai-Sun Wong V, Yilmaz Y, George J, Fan J, Vos MB. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2019; 69: 2672-2682.
2) Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology 1999; 29: 62-67.
3) Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. 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 2012; 55: 2005-2023.
4) Sberna AL, Bouillet B, Rouland A, Brindisi MC, Nguyen A, Mouillot T, Duvillard L, Denimal D, Loffroy R, Vergès B, Hillon P, Petit JM. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) clinical practice recommendations for the management of non-alcoholic fatty liver disease: evaluation of their application in people with Type 2 diabetes. Diabet Med 2018; 35: 368-375.
5) Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010; 52: 1836-1846.
6) Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998; 114: 842-845.
7) Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. Hepatology 1998; 27: 128-133.
8) Hill DB, Awad JA. Increased urinary F2-isoprostane excretion in alcoholic liver disease. Free Radic Biol Med 1999; 26: 656-660.
9) Haukeland JW, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, Torjesen PA, Birkeland K, Bjøro K, Aukrust P. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. J Hepatol 2006; 44: 1167-1174.
10) Debbabi M, Nury T, Zarrouk A, Mekahli N, Bezine M, Sghaier R, Grégoire S, Martine L, Durand P, Camus E, Vejux A, Jabrane A, Bretillon L, Prost M, Moreau T, Ammou SB, Hammami M, Lizard G. Protective Effects of α-Tocopherol, γ-Tocopherol and Oleic Acid, Three Compounds of Olive Oils, and No Effect of Trolox, on 7-Ketocholesterol-Induced Mitochondrial and Peroxisomal Dysfunction in Microglial BV-2 Cells. Int J Mol Sci 2016; 17: 1973
11) Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 2014; 72: 76-90.
12) Hasegawa T, Yoneda M, Nakamura K, Makino I, Terano A. Plasma transforming growth factor-beta1 level and efficacy of alpha-tocopherol in patients with non-alcoholic steatohepatitis: a pilot study. Aliment Pharmacol Ther 2001; 15: 1667-1672.
13) Oseini AM, Sanyal AJ. Therapies in non-alcoholic steatohepatitis (NASH). Liver Int 2017; 37 (Suppl. 1): 97-103.
14) Banini BA, Sanyal AJ. Current and future pharmacologic treatment of nonalcoholic steatohepatitis. Curr Opin Gastroenterol 2017; 33: 134-141.
15) Rimbach G, Moehring J, Huebbe P, Lodge JK. Gene-regulatory activity of alpha-tocopherol. Molecules 2010; 15: 1746-1761.
16) Ahsan H, Ahad A, Iqbal J, Siddiqui WA. Pharmacological potential of tocotrienols: a review. Nutr Metab (Lond) 2014; 11: 52.
17) Ortiz GG, Pacheco-Moisés FP, Gómez-Rodríguez VM, González-Renovato ED, Torres-Sánchez ED, Ramírez-Anguiano AC. Fish oil, melatonin and vitamin E attenuates midbrain cyclooxygenase-2 activity and oxidative stress after the administration of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine. Metab Brain Dis 2013; 28: 705-709.
18) Ishizaki K, Imada T, Tsurufuji M. Hepatoprotective bile acid 'ursodeoxycholic acid (UDCA)' Property

and difference as bile acids. Hepatol Res 2005; 33: 174-177.

19) Kotb MA. Molecular mechanisms of ursodeoxycholic acid toxicity & side effects: ursodeoxycholic acid freezes regeneration & induces hibernation mode. Int J Mol Sci 2012; 13: 8882-8914.

20) Hepgül G, Tanrikulu S, Unalp HR, Akguner T, Erbil Y, Olgaç V, Ademoğlu E. Preventive effect of pentoxifylline on acute radiation damage via antioxidant and anti-inflammatory pathways. Dig Dis Sci 2010; 55: 617-625.

21) Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. Surgery 1991; 110: 192-198.

22) Duman DG, Ozdemir F, Birben E, Keskin O, Ekşioğlu-Demiralp E, Celikel C, Kalayci O, Kalayci C. Effects of pentoxifylline on TNF-alpha production by peripheral blood mononuclear cells in patients with nonalcoholic steatohepatitis. Dig Dis Sci 2007; 52: 2520-2524.

23) Younossi ZM, Loomba R, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE, Friedman SL, Abdelmalek MF, Harrison SA, Sanyal AJ, Lavine JE, Mathurin P, Charlton MR, Chalasani NP, Anstee QM, Kowdley KV, George J, Goodman ZD, Lindor K. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2018; 68: 361-371.

24) Rickham PP. Human experimentation. Code of ethics of the world medical association. Declaration of Helsinki. Br Med J 1964; 2: 177.

25) Carter-Kent C, Zein NN, Feldstein AE. Cytokines in the pathogenesis of fatty liver and disease progression to steatohepatitis: implications for treatment. Am J Gastroenterol 2008; 103: 1036-1042.

26) Blond E, Disse E, Cuerq C, Drai J, Valette PJ, Laville M, Thivolet C, Simon C, Caussy C. EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease in severely obese people: do they lead to over-referral? Diabetologia 2017; 60: 1218-1222.

27) Oseini AM, Cole BK, Issa D, Feaver RE, Sanyal AJ. Translating scientific discovery: the need for preclinical models of nonalcoholic steatohepatitis. Hepatol Int 2018; 12: 6-16.

28) Albano E, Mottaran E, Vidali M, Reale E, Saksena S, Occhino G, Burt AD, Day CP. Immune response towards lipid peroxidation products as a predictor of progression of non-alcoholic fatty liver disease to advanced fibrosis. Gut 2005; 54: 987-993.

29) Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Wakasa K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. J Hepatol 2002; 37: 56-62.

30) Begriche K, Igoudjil A, Pessayre D, Fromenty B. Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. Mitochondrion 2006; 6: 1-28.

31) Albano E, Mottaran E, Occhino G, Reale E, Vidali M. Review article: role of oxidative stress in the progression of non-alcoholic steatosis. Aliment Pharmacol Ther 2005; 22: 71-73.

32) Yakaryilmaz F, Guliter S, Savas B, Erdem O, Ersoy R, Erden E, Akyol G, Bozkaya H, Ozenirler S. Effects of vitamin E treatment on peroxisome proliferator-activated receptor-alpha expression and insulin resistance in patients with non-alcoholic steatohepatitis: results of a pilot study. Intern Med J 2007; 37: 229-235.

33) Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996; 23: 1464-1467.

34) Koppe SW, Sahai A, Malladi P, Whitington PF, Green RM. Pentoxifylline attenuates steatohepatitis induced by the methionine choline deficient diet. J Hepatol 2004; 41: 592-598.

35) Balibrea JL, Arias-Díaz J, García C, Vara E. Effect of pentoxifylline and somatostatin on tumour necrosis factor production by human pulmonary macrophages. Circ Shock 1994; 43: 51-56.

36) Zein CO, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, McCullough AJ. Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. Hepatology 2011; 54: 1610-1619.

37) Xu R, Tao A, Zhang S, Deng Y, Chen G. Association between vitamin E and non-alcoholic steatohepatitis: a meta-analysis. Int J Clin Exp Med 2015; 8: 3924-3934.

38) Sato K, Gosho M, Yamamoto T, Kobayashi Y, Ishii N, Ohashi T, Nakade Y, Ito K, Fukuzawa Y, Yoneda M. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Nutrition 2015; 31: 923-930.

39) Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, Shiffman ML, Clore J, Mills AS. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2004; 2: 1107-1115.

40) Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 2011; 140: 124-131.

41) Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 2005; 100: 1082-1090.

42) Adams LA, Zein CO, Angulo P, Lindor KD. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. Am J Gastroenterol 2004; 99: 2365-2368.

43) Satapathy SK, Garg S, Chauhan R, Sakhuja P, Malhotra V, Sharma BC, Sarin SK. Beneficial effects of tumor necrosis factor-alpha inhibition by pentoxifylline on clinical, biochemical, and metabolic parameters of patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004; 99: 1946-1952.

44) Lee YM, Sutedja DS, Wai CT, Dan YY, Aung MO, Zhou L, Cheng CL, Wee A, Lim SG. A randomized controlled pilot study of Pentoxifylline in patients with non-alcoholic steatohepatitis (NASH). Hepatol Int 2008; 2: 196-201.

45) Li W, Zheng L, Sheng C, Cheng X, Qing L, Qu S. Systematic review on the treatment of pentoxifylline in patients with non-alcoholic fatty liver disease. Lipids Health Dis 2011; 10: 1-6.

46) Pathil A, Mueller J, Warth A, Chamulitrat W, Stremmel W. Ursodeoxycholyl lysophosphatidylethanolamide improves steatosis and inflammation in murine models of nonalcoholic fatty liver disease. Hepatology 2012; 55: 1369-1378.

47) Buko VU, Kuzmitskaya-Nikolaeva IA, Naruta EE, Lukivskaya OY, Kirko SN, Tauschel HD. Ursodeoxycholic acid dose-dependently improves liver injury in rats fed a methionine-and choline-deficient diet. Hepatol Res 2011; 41: 647-659.

48) Dufour JF, Oneta CM, Gonvers JJ, Bihl F, Cerny A, Cereda JM, Zala JF, Helbling B, Steuerwald M, Zimmermann A. Randomized placebo-controlled trial of ursodeoxycholic acid with vitamin e in nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2006; 4: 1537-1543.

49) Ratziu V, De Ledinghen V, Oberti F, Mathurin P, Wartelle-Bladou C, Renou C, Sogni P, Maynard M, Larrey D, Serfaty L, Bonnefont-Rousselot D, Bastard JP, Rivière M, Spénard J. A randomized controlled trial of high-dose ursodesoxycholic acid for nonalcoholic steatohepatitis. J Hepatol 2011; 54: 1011-1019.

50) Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, Burgart L, Colin P. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology 2004; 39: 770-778.

51) Chu CJ, Lu RH, Wang SS, Chang FY, Lin SY, Yang CY, Lin HC, Chang CY, Wu MY, Lee SD. Plasma levels of interleukin-6 and interleukin-8 in Chinese patients with non-alcoholic fatty liver disease. Hepatogastroenterology 2007; 54: 2045-2048.

52) Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol 2008; 103: 1372-1379.

53) Hill DB, Devalaraja R, Joshi-Barve S, Barve S, McClain CJ. Antioxidants attenuate nuclear factor-kappa B activation and tumor necrosis factor-alpha production in alcoholic hepatitis patient monocytes and rat Kupffer cells, in vitro. Clin Biochem 1999; 32: 563-570.

54) Lee KS, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. J Clin Invest 1995; 96: 2461-2468.