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ORIGINAL ARTICLE

Comparative clinical study between the effect of fenofibrate alone and its combination with pentoxifylline on biochemical parameters and liver stiffness in patients with non-alcoholic fatty liver disease

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Abstract
Background Non-alcoholic fatty liver disease is a common health problem associated with increased liver and vascular specific complications.
Aim The purpose of this study was to assess and compare the effect of fenofibrate alone or in combination with pentoxifylline on the measured biochemical parameters, inflammatory pathway and liver stiffness in patients with non-alcoholic fatty liver disease.
Methods The study design was randomized controlled trial. From July 2013 to June 2014, we recruited 90 non-alcoholic fatty liver patients from the Internal Medicine Department at Tanta University Hospital, Egypt. They were classified randomly into two groups to receive fenofibrate 300 mg daily or fenofibrate 300 mg daily plus pentoxifylline 1200 mg/day in three divided doses for 24 weeks. Fasting blood sample was obtained before and 24 weeks after treatment for biochemical analysis of liver and lipid panels, tumor necrosis factor-alpha, hyaluronic acid, transforming growth factor beta 1, fasting plasma insulin and fasting glucose. Liver stiffness measurement was carried out using fibro-scan. Data were statistically analyzed by paired and unpaired Student's *t* test.
Results The data obtained suggests that adding pentoxifylline to fenofibrate does not provide a beneficial effect on lipid panel, but has a beneficial effect on indirect biochemical markers of hepatic fibrosis, a direct marker linked to matrix deposition (hyaluronic acid), a cytokine/growth factor linked to liver fibrosis (transforming growth factor beta 1), the inflammatory pathway, insulin resistance and liver stiffness as compared to fenofibrate alone.
Conclusion The combination pentoxifylline plus fenofibrate may represent a new therapeutic strategy for non-alcoholic fatty liver disease as it resulted in more beneficial effects on direct and indirect markers of liver fibrosis, liver stiffness, insulin resistance and inflammatory pathway implicated in NAFLD.

Keywords Non-alcoholic fatty liver · Fenofibrate · Pentoxifylline · TNF-α · HA · TGF-β1 · Livers stiffness

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common health problem associated with increased liver and vascular specific complications [1]. NAFLD comprises a large spectrum of liver injury ranging from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [2]. NAFLD is typically asymptomatic but non-specific complaints including fatigue, malaise and right upper quadrant discomfort may be present [3]. Elevation of aminotransferase activities, especially of alanine aminotransferase (ALT) and γ-glutamyltranspeptidase (GGT) are markers of hepatocellular damage [4]. From a pathophysiological viewpoint, NAFLD is associated with imbalanced influx versus removal of triglycerides (TG) in the liver [5]. Increased body weight considerably increases the risk of this abnormality [6]. Dyslipidemia, predominantly hypertriglyceridemia, and insulin resistance play a key role in

The limitation of our study includes the relatively small number of patients investigated and hence this work needs further extension.

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pathogenesis of NAFLD [7]. Also, in NAFLD the liver overproduces various atherogenic factors, including inflammatory cytokines [8]. Among the pro-inflammatory molecules, TNF-α has been proposed to be the key link between obesity and insulin resistance [9]. To date, an established treatment of NAFLD is gradual weight loss [10]. Interest is increasing regarding the effect of lipid-lowering drugs such as fenofibrate on NAFLD and alteration of the inflammatory process implicated in NAFLD using the anti-TNF-alpha agent pentoxifylline [11, 12].

Fibrates are first-line drugs for reducing TG levels, their hypolipidemic action is attributed to activation of the peroxisome proliferator-activated receptors (PPAR), particularly PPARα that control the transcription of genes regulating lipid and glucose metabolism [13]. These receptors may also modulate hepatic lipid homeostasis, inflammation and fibrosis, by directing the proliferative and inflammatory response of specific cell types [13]. Furthermore, fenofibrate may improve insulin sensitivity by limiting lipid accumulation in several tissues, including the liver and muscles [14]. This can also be attributed to increased adiponectin together with reduced expression and plasma levels of several other adipokines, including tumor necrosis factor-α (TNF-α), leptin, resistin and plasminogen activator inhibitor (PAI)-1 [14, 15]. Considering its anti-inflammatory properties, hypolipidemic and insulin-sensitizing actions it could be assumed that fenofibrate is useful for the prevention and management of NAFLD [16].

Pentoxifylline is a non-selective phosphodiesterase inhibitor reported to decrease TNF-α gene transcription as well as affecting multiple steps in the cytokine/chemokine pathway by direct or indirect inhibition of TNF-α [12]. The cytokine TNF-α is an important mediator of insulin resistance through its ability to influence the tyrosine kinase activity of the insulin receptor [17]. Thus, modulation of insulin resistance by pentoxifylline could be a potential mechanism for improvement in patients with NAFLD [18]. Data are inconclusive regarding the effect of fenofibrate on NAFLD in the clinical setting [16, 19, 20]. In addition the available clinical studies include small sample size [16, 20] and the use of fenofibrate in combination with other strategies [19, 20]. Furthermore, there are few small size trials about the implication of pentoxifylline in NAFLD [18, 21], and to our knowledge, no other trial compares the effect of fenofibrate and fenofibrate-pentoxifylline combination on NAFLD patients.

In this context, we aimed to compare the outcome of fenofibrate and fenofibrate plus pentoxifylline combination as a therapeutic strategy on NAFLD patients. We aimed to investigate the effect of adding pentoxifylline as anti-TNF-α to anti-hyperlipidemic agent fenofibrate on indirect biochemical markers of hepatic fibrosis (ALT, AST, GGT), direct markers linked to matrix deposition (hyaluronic acid), cytokine/growth factor linked to liver fibrosis (TGF-β1), the inflammatory pathway implicated in NAFLD (TNF-α), insulin resistance and liver stiffness.

Patients and methods

From July 2013 to June 2014, we recruited patients from the Internal Medicine Department at Tanta University Hospital, Egypt. The inclusion criteria were: patients who had persistently abnormal aminotransferase levels in two separate occasions over the past six months. NAFLD was assumed in patients with moderately elevated aminotransferase activities (<3× the upper limit of normal) together with relevant ultrasonographic findings, after excluding other causes of abnormal liver function tests [22]. In addition, patients were diagnosed with NAFLD according to the practice guidelines of the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association 2012; there is evidence of hepatic steatosis either by imaging or by histology; and there is no cause for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders [23]. The ultrasonographic criteria used to diagnose fatty liver disease included the presence of increased liver echogenicity (bright), stronger echoes in the hepatic parenchyma than in the renal parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins [24]. The ultrasonograph (Sonoline Sienna, Mitsubishi P 91, Siemens) used during the current study to diagnose NAFLD.

The exclusion criteria were: alcohol abuse (alcohol intake of more than 20 g/week), evidence of viral or autoimmune hepatitis, Wilson's disease, hemochromatosis and decompensated liver disease, hypersensitivity to methylxanthines, and impaired renal function. Patients taking medication known to cause steatosis were excluded. Patients with other comorbid conditions that could potentially elevate transaminases, such as congestive heart failure and pregnancy, were also excluded. Patients with previous episodes of retinal/cerebral hemorrhage and lactating mothers were also excluded.

Study design

A total number of 90 patients who fulfilled the selection criteria were enrolled in the study. All individuals included in the study were submitted to: full medical history, demography (age, diet supplement), measurement of weight and height. Height and weight were measured using Detecto scale (Detecto Company, 203 East Daugherty Street, USA). Body mass index (BMI) was also calculated which is defined as the weight in kilograms divided by the square

of the height in meters, i.e. BMI = [weight (kg)]/height^2 (m)]. The study design was a parallel randomized study where the enrolled patients were classified randomly into two groups: group I, 45 patients assigned to receive fenofibrate 300 mg daily after principal meal for 24 weeks (Lipanthyl®; Minipharm Egypt under license of laboratories Fournier France); and group II, 45 patients assigned to receive fenofibrate 300 mg daily plus pentoxifylline (Trental®; Sanofi Aventis) 1200 mg/day in three divided doses after meals for 24 weeks.

Patients were followed-up at monthly intervals for assessment of compliance to the study medication. Adverse events, tolerability to studied medication, and concurrent medication were also assessed in order to ensure that the patients abstained from any alcohol and drugs likely to cause hepatic steatosis during the therapy period. Blood samples were obtained before treatment and at the end of the study. Fasting blood samples were taken from all patients in the morning after the patients had fasted overnight for 12 h. Plasma and sera were separated from blood samples for biochemical analyses.

Biochemical assays

Liver panel

Alanin-aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) were measured by colorimetric method (Biodiagnostic, Dokki, Giza, Egypt).

Lipid panel

Fresh sera are used for determination of sera lipid profiles including TG, total cholesterol and high-density lipoprotein cholesterol (HDL-C) which were measured colorimetrically (enzymatic colorimetric method) using commercial kits (Biodiagnostic, Dokki, Giza, Egypt). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula [25] where LDL = [TC − HDL − (TG/5)] provided that TG level is less than 400 mg/dl [25].

Assay of TNF-α, hyaluronic acid (HA) and transforming growth factor beta 1 (TGF-β1)

Quantitative detection of human plasma TNF-α was done using Enzyme-Linked Immunosorbent Assay kit (TNF-α Platinum ELISA; e Bioscience). Sensitive Sandwich Enzyme-Linked Immunosorbent Assay kit was used for the quantitative determination of hyaluronic acid (TECOmedical AG). Enzyme-Linked Immunosorbent Assay kit was used for quantitative measurement of human TGF-β1 (RayBiotech, Inc.).

Blood glucose and insulin

Fasting blood glucose (FBG) levels were assayed using glucose oxidase method. Fasting plasma insulin (FPI) was assayed using Enzyme-Linked Immunosorbent Assay kits (Diagnostic Automation/ Cortez Diagnostics, Inc). We estimated insulin resistance (IR) using the HOMA-IR index or homeostasis model assessment of insulin resistance [26] which is defined as fasting insulin (μIU/ml) times fasting glucose (mmol/l) divided by 22.5 or divided by 405 if FBG is expressed in mass units (mg/dl).

Liver stiffness measurement (LSM)

LSM was performed using transient elastography (Fibroscan, Echosens, Paris). This procedure has been well described previously [27]. The LSM was performed by a single experienced and well trained operator. At least ten valid measurements were obtained for each patient. Results were included in the final analysis only if the following three criteria were met: at least ten valid measurements, success rate >60 % (success rate represented by the ratio of the number of valid measurements and total shots and which has to be at least >60 %) and the interquartile range (IQR)-to-liver stiffness ratio was ≤0.30 (IQR shows the variability between the 10 valid determinations and which is shown to not exceed 30 % of the mean, i.e final results). The median values of the validated measurements for each patient were representative of the liver stiffness and expressed in units of kilopascals (kPa).

Statistical analysis

Data were statistically analysed by paired Student's *t* test to compare between the results before (baseline) and after treatment within the same groups, and unpaired Student's *t* test to compare between means of the different groups using SPSS statistical package version 22.0 (IBM corporation software group, USA). All results were expressed as mean ± SD. The level of significance was set at <0.05.

Results

All 90 patients completed the study, although seven patients (15.6 %) in group I and nine patients (20 %) in group II complained of tolerable abdominal discomfort. Those patients preferred to complete their therapy and were reinstructed to take their medications after meals with plenty of water. No one showed hypersensitivity reaction to medication or complained of abnormal events. The laboratory parameters in patients treated with fenofibrate (group I) and fenofibrate plus pentoxifylline (group II)

Table 1 Demographic data, TNF-α, liver and lipid panels and HOMA-IR in patients treated with fenofibrate (group I) and fenofibrate plus Pentoxifylline (group II) before and 24 weeks after treatment

Laboratory parameters	Group I (Fenofibrate) $n = 45$		Group II (Fenofibrate plus pentoxifylline) $n = 45$	
	Before treatment	24 weeks after treatment	Before treatment	24 weeks after treatment
Age (years)	43.78 ± 6.30		45.34 ± 5.34	
Gender (male/female)	30/15		32/13	
BMI (kg/m^2)	27.26 ± 1.06	26.73 ± 1.43	27.14 ± 1.27	26.54 ± 1.26
TNF-α (pg/ml)	5.39 ± 1.04	3.60[a] ± 0.86	5.67 ± 1.24	2.32[a,b] ± 0.55
AST (IU/l)	108.44 ± 9.08	58.94[a] ± 7.88	112.96 ± 9.6	42.81[a,b] ± 8.83
ALT (IU/l)	114.56 ± 8.50	57.34[a] ± 7.81	117.80 ± 9.90	46.40[a,b] ± 9.02
GGT (IU/l)	107.84 ± 7.60	61.22[a] ± 7.44	109.34 ± 7.75	50.03[a,b] ± 6.27
Cholesterol (mg/dl)	248 ± 26.62	159.06[a] ± 20.22	240 ± 26.34	160.94[a] ± 21.98
HDL-C (mg/dl)	39.90 ± 4.03	47.22[a] ± 3.63	38.90 ± 3.10	46.19[a] ± 3.89
Triglycerides (mg/dl)	249.80 ± 33.42	176.22[a] ± 27.74	255.60 ± 36.34	169.69[a] ± 24.92
LDL-C (mg/dl)	158 ± 25.85	76.93[a] ± 19.88	150 ± 29.11	80.88[a] ± 22.40
Insulin (μIU/ml)	19.56 ± 3.29	14.22[a] ± 3.11	20.06 ± 4.05	9.88[a,b] ± 3.02
Blood glucose (mg/dl)	111.31 ± 6.17	96.62[a] ± 6.85	109.78 ± 5.66	87.28[a,b] ± 6.51
HOMA-IR	5.38 ± 0.98	3.37[a] ± 0.72	5.43 ± 1.08	2.14[a,b] ± 0.61

Data are represented as mean ± SD. No significant difference between group I and group II before treatment ($p > 0.05$)

BMI body mass index, *TNF-α* tumor necrosis factor-α, *AST* aspartate aminotransferase, *ALT* alanin-aminotransferase, *GGT* gama-glutamyl transpeptidase, *HDL-C* high-density lipoprotein, *LDL-C* low-density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance

[a] Data obtained after treatment showed significant difference versus data obtained before treatment within group I or group II ($p < 0.05$)

[b] Data obtained 24 weeks after treatment in group II showed significant difference versus data obtained 24 weeks after treatment in group I

before and 24 weeks after treatment are shown in Tables 1, 2 and 3. As shown from these tables, within the group, either group I or II, there was significant difference ($p < 0.05$) in all the measured parameters (except for BMI) between data obtained before initiation of any treatment (baseline) and those obtained 24 weeks after treatment. There was significant decrease of TNF-α, liver panel (ALT, AST, GGT), direct marker linked to matrix deposition (HA), cytokine linked to liver fibrosis (TGF-β1), total cholesterol, TG, LDL-C, FPI, FBG, HOMA-IR and liver stiffness. In contrast, there was a significant increase in HDL-C in both groups following treatment. However, there was non-significant decrease in BMI after treatment as compared to baseline values within both groups ($p > 0.05$).

The comparison of the two groups (fenofibrate and fenofibrate plus pentoxifylline) before and 24 weeks after treatment revealed that both groups (groups I and II) did not differ significantly ($p > 0.05$) before initiation of treatment (baseline data) with regard to age, body mass index (BMI), measured biochemical parameters and liver stiffness. Six months after treatment, there was a significant difference between the two groups in all the measured parameters except for BMI and lipid panel ($p > 0.05$). At the end of the study, group II (fenofibrate plus pentoxifylline) showed significantly lower levels of TNF-α, plasma insulin, FBG, HOMA-IR, liver panel (AST, ALT and GGT), direct marker linked to matrix deposition (HA) and cytokine linked to liver fibrosis (TGF-β1). Furthermore, group II (fenofibrate plus pentoxifylline) showed significant improvement in liver stiffness as compared to group I (fenofibrate alone).

Discussion

NAFLD is a cluster of liver disorders associated with hepatic lipid accumulation (steatosis) in the absence of viral hepatitis or alcohol abuse [5]. Our study demonstrates that fenofibrate and fenofibrate plus pentoxifylline significantly improve lipid profile as compared to baseline data. Fenofibrate hypolipidemic action is attributed to activation of the PPAR, particularly PPARα [28]. Also, this result matches with previously reported findings demonstrating treatment with fenofibrate 200 mg/day for 48 weeks was associated with significant improvement of the serum lipid profile [16]. Furthermore, a non-statistically significant difference was detected between both groups at the end of the study regarding lipid panel. The later result is matched with previously reported findings which demonstrated that pentoxifylline did not influence serum lipids [29, 30].

Table 2 Hayalourinic acid (HA) and transforming growth factor beta 1 (TGF-β1) in patients treated with fenofibrate (group I) and fenofibrate plus pentoxifylline (group II) before and 24 weeks after treatment

Laboratory parameters	Group I (Fenofibrate) $n = 45$		Group II (Fenofibrate plus pentoxifylline) $n = 45$	
	Before treatment	24 weeks after treatment	Before treatment	24 weeks after treatment
HA (ng/ml)	91.51 ± 29.84	57.01[a] ± 19.35	86.89 ± 28.28	42.06[a,b] ± 13.84
TGF-β1 (ng/ml)	15.23 ± 5.12	8.14[a] ± 3.20	14.34 ± 4.86	5.75[a,b] ± 2.13

Data are represented as mean ± SD. No significant difference between group I and group II before treatment ($p > 0.05$)

HA hyaluronic acid, *TGF-β1* transforming growth factor beta 1

[a] Data obtained after treatment showed significant difference versus data obtained before treatment within group I or group II ($p < 0.05$)

[b] Data obtained 24 weeks after treatment in group II showed significant difference versus data obtained 24 weeks after treatment in group I

Table 3 Liver stiffness measurement (LSM) in patients treated with fenofibrate (group I) and fenofibrate plus pentoxifylline (group II) before and 24 weeks after treatment

Laboratory parameters	Group I (Fenofibrate) $n = 45$		Group II (Fenofibrate plus pentoxifylline) $n = 45$	
	Before treatment	24 weeks after treatment	Before treatment	24 weeks after treatment
LS (kPa)	12.95 ± 5.11	9.82[a] ± 3.71	12.61 ± 5.03	7.47[a,b] ± 3.01
IQR	2.70 ± 1.74	2.54 ± 1.19	2.83 ± 1.51	1.86 ± 0.87
IQR/LS ratio	0.21 ± 0.07	0.26 ± 0.05	0.22 ± 0.07	0.25 ± 0.04
Success rate	84.44 ± 11.91	88.22 ± 9.17	86.81 ± 8.82	86.00 ± 10.37

Data are represented as mean ± SD. No significant difference between group I and group II before treatment ($p > 0.05$)

LS liver stiffness, *IQR* interquartile range

[a] Data obtained after treatment showed significant difference versus data obtained before treatment within group I or group II ($p < 0.05$)

[b] Data obtained 24 weeks after treatment in group II showed significant difference versus data obtained 24 weeks after treatment in group I

In NAFLD the liver overproduces various atherogenic factors, including inflammatory cytokines [8] such as TNF-α, which correlates with disease severity [31]. At the end of the study, patients on fenofibrate and fenofibrate plus pentoxifylline showed significant reduction in TNF-α level as compared to their baseline data. It has been demonstrated that TNF-α hepatic expression and plasma levels can be decreased by PPARα activation and by fenofibrate [11, 32]. In addition, our study showed that the pentoxifylline plus fenofibrate treated group showed significantly lower TNF-α level than that detected with the fenofibrate treated group. Pentoxifylline is reported to decrease TNF-α gene transcription as well as affecting multiple steps in the cytokine/chemokine pathway by direct or indirect inhibition of TNF-α [33–35]. It seems that pentoxifylline decreases lipopolysaccharide-stimulated TNF-α production that in turn triggers the production of additional cytokines that collectively recruit inflammatory cells, with subsequent destruction of hepatocytes and induction of fibrogenesis [36, 37]. Our finding is in agreement with the finding of Lee et al. [21] who demonstrated significant reduction of TNF-α in all studied NASH patients after 12 weeks treatment with pentoxifylline 400 mg thrice daily as compared with baseline level [21]. The aforementioned information justifies our results concerning a more beneficial effect reported with the pentoxifylline plus fenofibrate treated group as compared to the fenofibrate treated group.

Insulin resistance plays a key role in the pathogenesis of NAFLD [7, 38] which could be attributed to enhanced hepatic fatty acid flux and uptake [7, 38]. Insulin resistance results in hyperinsulinemia and increased circulating levels of free fatty acids, which enter hepatocyte cytoplasm to create TG [7, 38]. At the end of the study, patients on fenofibrate and fenofibrate plus pentoxifylline showed significant reduction in plasma insulin level and significant decline in HOMA-IR as compared to baseline data. In addition, pentoxifylline plus fenofibrate treatment showed a significantly better effect on insulin sensitivity than that detected with the fenofibrate treated group. Fenofibrate may improve insulin sensitivity by limiting lipid accumulation in several tissues, including the liver and muscles [14, 39, 40] and by reducing expression and plasma levels of several adipokines including TNF-α [14, 15]. This result

is in accordance with previously reported findings which demonstrated that fenofibrate treatment was associated with significant improvement of insulin sensitivity [16]. On the other hand, pentoxifylline is reported to decrease TNF-α gene transcription [33]. It was reported that a significant improvement in insulin resistance index in NASH patients was detected after six months treatment with pentoxifylline due to the down regulation of TNF-α [18]. The later finding justifies the better effect on HOMA-IR reported with the pentoxifylline plus fenofibrate group as compared to the fenofibrate treated group.

NAFLD is typically asymptomatic; however, elevation of aminotransferase activities, especially of ALT, AST and GGT could be used as markers of hepatocellular damage [4]. Twenty-four weeks after therapeutic intervention, fenofibrate and fenofibrate plus pentoxifylline significantly reduced ALT, AST and GGT levels as compared to baseline data. The results obtained with the fenofibrate treated group is in accordance with a previously reported study which showed significant reduction in GGT activities and a decrease in AST and ALT levels in NAFLD patients treated with 200 mg daily of fenofibrate for 48 weeks [16]. In addition, the pentoxifylline plus fenofibrate group showed significantly lower levels of such parameters than that detected in the fenofibrate treated group which may be attributed to pentoxifylline. This improvement could be attributed to an increase in glutathione levels and a reduction in the TNF-α level by pentoxifylline [41] since pentoxifylline could decrease lipopolysaccharide-stimulated TNF-α production [37]. Our results are in accordance with Georgescu and Georgescu [29] who demonstrated that treatment of NASH patients with pentoxifylline 400 mg twice daily determined a fast and significant decrease of ALT and GGT after ten weeks of treatment and this effect remained stable during the whole period of his study (37 weeks). Furthermore, the beneficial effect of pentoxifylline on liver transaminases in patients with NASH has been previously demonstrated by other studies [21, 35, 42]. The later findings give logical justification for more significant improvement in liver enzymes in patients on pentoxifylline plus fenofibrate as compared to those on fenofibrate alone.

TGF-β is a cytokine/growth factor with immunosuppressive, anti-inflammatory, and pro-fibrotic properties [43]. In the liver, TGF-β1 is the most abundant isoform, and it is secreted by immune cells, stellate cells and epithelial cells [44]. TGF-β1 plays a critical role in liver apoptosis, fibrogenesis [45] and collagen production [46] during chronic liver injury. Stärkel et al. [47] showed that the up-regulation of TGF-β1 is an early molecular step in the progressive fibrotic steatohepatitis [47]. Das and Balakrishnan [48] reported a significant elevation in TGF-β1 levels in NAFLD patients [48]. In the present study, TGF-β1 level was significantly reduced in NAFLD patients 24 weeks after treatment as compared to baseline values. The reason behind the decrease in TGF-β levels 24 weeks after treatment may be related to the anti-inflammatory effects of both pentoxifylline and fenofibrate and their beneficial effect on TNF-alpha. The production of TNF-alpha is one of the primary events in liver injury triggering the production of various cytokines [33] that recruit inflammatory cells which in their turn could damage hepatocytes and induce fibrogenesis. Interestingly, TNF-α and growth factors activation of receptor tyrosine kinases can convert a cytostatic TGF-beta signal to collagen-producing character in activated hepatic stellate cells under the influence of inflammatory microenvironments [49]. The notion that fenofibrate may reduce TGF-β1 expression [50, 51] may give logical justification for decreased TGF-β1 level noted in the fenofibrate treated group. Furthermore, the anti-inflammatory effect of pentoxifylline and its ability to down-regulate TGF-β1 and to decrease hepatic mRNA expression of TGF-β1 [52, 53] together with the beneficial effect of fenofibrate on TGF-β1 expression [50, 51] may explain the lower TGF-β1 level detected in the fenofibrate plus pentoxifylline treated group than that detected in the fenofibrate treated group.

Hyaluronic acid (HA) is a glycosaminoglycan that is produced by hepatic stellate cells [54] and represents a component of the ECM. HA has a great value in detecting liver fibrosis [55]. We reported a significant decrease in HA levels in both groups 24 weeks after treatment as compared to baseline data. The decrease in HA level that was reported in the fenofibrate treated group may be related to antifibrotic, anti-inflammatory and anti-oxidant effects of fenofibrate since it was postulated that the degree of liver fibrosis in mice induced by CCl4 was reduced through up-regulation of PPARα, inhibition of the inflammatory response and enhancement of SOD anti-oxidant activity [51]. Xie et al. [51] also reported lower level of hepatic HA content with treatment of fenofibrate due to antifibrotic effect of fenofibrate [51], a finding that could justify lower serum level of HA obtained with 24 weeks treatment with fenofibrate during our study. Furthermore, we reported a more decrease in HA level in the fenofibrate plus pentoxifylline treated group as compared to the fenofibrate treated group, a result which seems to be related to antifibrotic effect of fenofibrate [51], in addition to the beneficial effect of pentoxifylline on HA level [21, 56]. It was postulated that administration of pentoxifylline caused significant decline in HA levels which may be an index and surrogate indicator reflecting improvement of hepatic fibrosis [21, 56]. This beneficial effect of pentoxifylline on hyaluronic acid, which is produced by hepatic stellate cells [54], may be attributed to the antifibrogenic effect of pentoxifylline on activated hepatic stellate cells [57, 58].

Interestingly, fenofibrate improves the liver microvascular environment and oxygen metabolism [59], decreases hepatic lipid accumulation [13], modulates insulin resistance [14], activates expression of genes involved in fatty acid turnover and decreases hepatic lipid peroxidation through anti-oxidant mechanisms that include an increase in catalase activity by up-regulating its mRNA levels [11, 60]. On the other hand, pentoxifylline may have hepatoprotective effects [41]. Pentoxifylline increases hepatic glutathione levels and reduces the production of oxygen radicals [61]. Additionally, pentoxifylline is well known to improve tissue oxygenation via its vasodilator effect and to restore hepatocellular function [62, 63]. Therefore, these aforementioned findings could provide a number of auxiliary explanatory mechanisms for improvement of NAFLD and for decreasing both HA and TGF-β1 levels in NAFLD patients including patients with steatosis with or without inflammation and fibrosis.

Although liver biopsy remains the gold standard for assessing liver fibrosis, patients may be unwilling to undergo a biopsy procedure and clinicians may be reluctant to advocate it because of the potential adverse effects associated with this invasive procedure [64]. Liver stiffness measurement (LSM) has become one of the most reliable alternative non-invasive methods to biopsy in assessing liver fibrosis, grading liver fibrosis [65], evaluating prognosis and predicting long-term treatment response [66]. The results of the present study revealed that the liver stiffness was decreased 24 weeks after treatment with fenofibrate alone and with treatment by fenofibrate plus pentoxifylline. The result obtained in the fenofibrate treated group seems to be attributed to the antifibrotic effect of fenofibrate [51, 67] which may be related to up-regulation of PPARα, inhibition of the inflammatory response, enhancement of SOD anti-oxidant activity and enhanced catalase expression [51, 67]. The pentoxifylline plus fenofibrate treated group showed more improvement in liver stiffness than that detected in the fenofibrate treated group, a result which seems to be attributed to the beneficial effect of pentoxifylline. It seems that pentoxifylline decreases lipopolysaccharide-stimulated TNF-α production, improves hepatic histology and diminishes both steatosis and necroinflammation [37] with subsequent improvement in liver stiffness. Zein et al. [68] evaluated the effects of pentoxifylline in biopsy-proven NASH patients and found that pentoxifylline improved inflammation, fibrosis score and histological features of NASH patients [68]. The aforementioned information may justify our results concerning liver stiffness. The overall results indicate that the combination of fenofibrate plus pentoxifylline results in more improvement in liver fibrosis than fenofibrate alone as indicated by more improvement in indirect markers of hepatic fibrosis (ALT, AST, GGT), a direct marker linked to matrix deposition (HA) and cytokine/growth factor linked to liver fibrosis (TGF-β1) together with more improvement in liver stiffness. This favorable effect of fenofibrate plus pentoxifylline on liver fibrosis may be attributed to the anti-inflammatory effect of both drugs since both drugs can reduce the expression and down-regulate TNF-α [14, 15, 18], whereas TNF-α activation of receptor tyrosine kinases can convert a cytostatic TGF-beta signal to collagen-producing character in activated hepatic stellate cells under the influence of inflammatory microenvironments [49]. Furthermore, the decrease in liver fibrosis provoked by fenofibrate plus pentoxifylline may be related to the antifibrogenic effect of both drugs on activated hepatic stellate cells, the major cells involved in hepatic fibrosis [51, 57, 58]. PPARα activation by fenofibrate may reverse fibrosis by reducing the expression of fibrotic markers and the number of stellate cells [69]. Also, pentoxifylline attenuates liver fibrosis by inhibition of hepatic stellate cells proliferation [70] and by blockage of stellate cells activation [71].

At the end of the study, our results demonstrate that fenofibrate or fenofibrate plus pentoxifylline reduce BMI non-significantly as compared to baseline data since the patients included in our study were not prescribed any specific diet or enrolled in an exercise program.

Conclusion

These important and promising overall results showed an improvement in indirect biochemical markers of hepatic fibrosis (ALT, AST, GGT) and a direct marker linked to matrix deposition (HA) and cytokine/growth factor linked to liver fibrosis (TGF-β1) together with improvement of liver stiffness, which give the suggestion that fenofibrate improves liver histology. The combination pentoxifylline plus fenofibrate may represent a new therapeutic strategy for NAFLD as it resulted in more beneficial effects on liver markers of fibrosis, liver stiffness, insulin resistance and the inflammatory pathway implicated in NAFLD. However, a large scale clinical trial seems necessary.

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Compliance with ethical requirements and Conflict of interest The study was approved by the national research ethics committee (Tanta University ethical committee) and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. An informed written consent was obtained from all individuals included in the study. Sahar Mohamed El-Haggar and Tarek Mohamed Mostafa declare that they have no conflicts of interest.

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