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ORIGINAL ARTICLE

# A randomized controlled pilot study of Pentoxifylline in patients with non-alcoholic steatohepatitis (NASH)

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**Abstract**
*Purpose* Tumor necrosis factor-α (TNF-α) is implicated in non-alcoholic steatohepatitis (NASH). Pentoxifylline inhibits TNF-α. We wanted to evaluate the efficacy of Pentoxifylline on NASH patients.
*Methods* Patients with biopsy proven NASH and persistently elevated alanine aminotransferase (ALT) greater than 1.5 times the upper limit of normal were randomized to 3 months of treatment with a step 1 American Heart Association diet and daily exercise with Pentoxifylline or placebo. Liver function tests, serum lipids and TNF-α, Interleukin 6 (IL-6), and plasma hyaluronic acid were measured at baseline, at weeks 6 and 12. Categorical data were analyzed by Fisher's exact test while independent sample *t*-test and Mann–Whitney test were used for continuous data.
*Results* Eleven patients were randomized into the Pentoxifylline and nine to the placebo group. After 3 months of treatment body mass index (BMI), ALT and aspartate aminotransferase (AST) decreased significantly in both groups. There was no difference between the two groups in reduction of BMI ($P = 0.897$). There was significantly greater reduction in AST in the Pentoxifylline group ($P = 0.038$). There was a trend toward lower ALT level ($P = 0.065$) in the Pentoxifylline group. TNF-α and IL-6 decreased significantly in both groups after treatment, but there was no significant difference between the two groups.
*Conclusion* Three months of Pentoxifylline treatment in combination with diet and exercise results in significantly greater reduction in AST levels in patients with NASH as compared with controls.

**Keywords** Non-alcoholic steatohepatitis · Pentoxifylline · TNF alpha · Randomized controlled study

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## Background

Non-alcoholic fatty liver disease (NAFLD) comprises of a large spectrum of liver injury ranging from steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Natural history studies have shown that disease progression to fibrosis and cirrhosis only occurs in NASH [1]. The pathogenesis of NASH is complex with "first hit" being insulin resistance [2]. In addition it appears that a second hit is necessary for the induction of steatohepatitis and it is believed that the interaction of cytokines with oxidative stress has a pivotal role [3]. One cytokine, which is implicated in this process, is tumor necrosis factor-α (TNF-α). Elevated levels of TNF-α have been previously reported in patients with alcoholic hepatitis where TNF-α levels correlated with disease severity and also with

mortality [4]. In animal models of NASH, ob/ob mice show increased sensitivity to endotoxin-induced hepatotoxicity as well as increased TNF-α production [5]. Increased serum concentrations of TNF-α have also been reported in human subjects with NASH as opposed to controls [6]. TNF-α has also been shown to interfere with insulin signaling resulting in steatosis and may have a pro-inflammatory effect in NASH [7]. Another cytokine implicated in the pathogenesis of NASH is Interleukin 6 (IL-6). IL-6 was found to be increased in patients with NAFLD as compared to age, sex, and ethnicity-matched controls [8]. In another pilot study, it was found that IL-6 was significantly elevated in NASH patients and decreased after treatment with lifestyle modification and Vitamin E for 3 months [9]. In the same study, plasma hyaluronic acid (HA), which is used frequently as a marker of fibrosis, was found to be significantly elevated at baseline in NASH patients compared with controls. HA was found to be a reliable marker for severe fibrosis (Stages 3–4) in 79 patients with NASH with AUC of 0.9 (95% CI: 0.83–0.97) [10].

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 [11, 12]. In a randomized controlled study of Pentoxifylline versus placebo in 101 patients with severe acute alcoholic hepatitis, 4 weeks of Pentoxifylline significantly improved short-term survival [13]. Therefore, Pentoxifylline may be an effective treatment for NASH through inhibition of TNF-α. There are currently two cohort studies demonstrating improvements in liver aminotransferase levels in NASH patients who were treated with Pentoxifylline [14, 15].

The purpose of our study is to evaluate the effects of Pentoxifylline on patients with NASH in the context of a randomized controlled pilot study. In order to provide the proof of principle that Pentoxifylline is effective in the treatment of NASH, we studied the effect of Pentoxifylline plus a calorie-restricted diet as compared to placebo plus a calorie-restricted diet on liver enzymes and HA (as surrogate indicator of hepatic fibrosis). We also wanted to determine if the therapeutic effect of Pentoxifylline in NASH patients was mediated through TNF-α and IL-6.

## Materials and methods

### Subjects

From January 2005 to September 2005, we recruited patients who were between 21 and 65 years of age from our Gastroenterology/Hepatology clinic. After other causes of liver disease were ruled out, those patients who had persistently abnormal alanine aminotransferase (ALT) (greater than 1.5 times the upper limit of normal and repeated at least twice over 6 months), imaging (ultrasound or CAT scan) showing fatty infiltration and who had histological evidence of NASH were enrolled in the study. The minimal criteria for a diagnosis of NASH included the presence of lobular inflammation and either ballooning of cells or perisinusoidal or pericellular fibrosis in Zone 3 of the hepatic acinar [16]. We received Institutional Review Board approval for our research protocols before any patients were enrolled. Each biopsy was read and scored by a single pathologist according to the Brunt criteria [17]. Patients were excluded if they had decompensated liver disease as evidenced by a Bilirubin of ≥35 μmol/l, serum Albumin of (35 g/l or an INR greater than or equal to 1.7; or overt ascites and or gastrointestinal bleeding documented on upper GI endoscopy; ongoing total parenteral nutrition; jejunal-ileal bypass; human immunodeficiency virus infection; alcohol intake of more than 30 g a week in past 6 months or a history of alcohol dependence; pregnancy or lactation; hypersensitivity to methylxanthines (e.g., caffeine, theophylline, theobromine); concomitant use of ketorolac; recent retinal/cerebral hemorrhage; acute myocardial infarction or severe cardiac arrhythmias and impaired renal function.

### Study design

There were 20 patients who fulfilled the criteria and were treated for 3 months with a low-calorie diet (1,200 kcal for women, 1,500 kcal for men) and daily exercise. The exercise consists of a walk or jog for 30 min daily. The patients were randomized to receive either Pentoxifylline 400 mg thrice daily or placebo thrice daily for 12 weeks. Compliance to diet and exercise was determined at by checking patients' entries in a daily diary. Compliance to medication was determined at each visit by counting of tablets by the research pharmacists. The randomization process was concealed and consisted of computerized randomization with varying block. Both investigators and patients were unaware of group allocation as the trial medication was issued by the research pharmacy. The study was conducted over 12 weeks with a 2-week run in where a dietician would instruct the family and patient how to follow the diet and instructions for daily exercise. They had dietary follow-up by telephone weekly and in person every fortnightly. The outcome measures for this study included the measurement of biochemical and cytokine profiles before and after treatment.

Blood was obtained at entry, weeks 8 and 12 during fasting for

1. Liver panel: ALT, aspartate aminotransferase (AST), total bilirubin, albumin, alkaline phosphatase, and total protein.
2. Lipid panel: total cholesterol/low-density lipoprotein/high-density lipoprotein/triglyceride.
3. Cytokines: TNF-α and IL-6.
4. HA.

### Biochemical assays

(1) Liver and lipid panel was determined by the hospital clinical laboratory.
(2) Cytokine and HA analysis.

Plasma TNF-α and IL-6 levels were determined by using highly sensitive enzyme-linked immunoassay (Research and Diagnostic systems, Minneapolis).

HA was determined by a commercially available kit (Corgenix).

## Statistics

The categorical data were presented by proportion, whereas continuous data were presented using mean ± SD. Categorical data were analyzed by Fisher's exact test. Continuous data were first tested for normality of their distribution by Shapiro–Wilk test. Changes in continuous measures between baseline and post-treatment levels were tested by means of the paired *t*-test, while the Wilcoxon Signed Rank test was used for non-parameteric data. The differences in the changes between the groups were tested by the independent sample *t*-test, while the Mann–Whitney test was used for non-normally distributed data.

## Results

### Baseline characteristics

All the 20 patients completed the study. The mean age of our patients was 47.4 years. There were 11 patients randomized to the Pentoxifylline arm and 9 to the placebo arm.

Both groups did not differ significantly with regard to age, body mass index (BMI), gender, presence of diabetes mellitus or hypercholesterolemia, ALT and AST, fasting glucose, and lipid profile at baseline. The baseline characteristics are shown in Table 1. Baseline liver biopsy was obtained in all patients. Biopsy showed that all patients had steatosis which was moderate in 55% (Grade 2) and severe in 10%. Most patients (95%) had mild to moderate lobular inflammation (Grades 1–2) and 75% had mild fibrosis (Grade 1) (Table 2). There were no adverse events in the placebo group, and one patient in the Pentoxifylline arm reported an episode of sore throat and running nose suggestive of an upper respiratory tract infection.

### Response to treatment

After 3 months of treatment, BMI decreased significantly in both the Pentoxifylline group ($P = 0.001$) as well as the placebo group ($P = 0.02$) compared to baseline (Table 3). There was however no significant difference between the groups in the reduction of BMI ($P = 1$). AST decreased significantly in both the Pentoxifylline ($P = 0.000$) and the placebo ($P = 0.018$) groups as compared with baseline (Table 3). The reduction in the AST was significantly greater in the Pentoxifylline group as compared with the placebo patients ($P = 0.0380$) (Fig. 1). ALT also decreased significantly in the Pentoxifylline group

**Table 1** Baseline characteristics

| Variable | Pentoxifylline ($n = 11$) | Placebo ($n = 9$) | *P*-value |
|---|---|---|---|
| Age (years) | 47.00 (8.39) | 47.89 (14.05) | 0.863 |
| Gender (male) | 7 | 6 | 1.000 |
| Gender (female) | 4 | 3 | |
| BMI (baseline) | 26.67 (3.43) | 29.91 (5.35) | 0.118 |
| Diabetes | 2 | 0 | 0.479 |
| No diabetes | 9 | 9 | |
| Hypercholesterolemia | 3 | 2 | 1.000 |
| No hypercholesterolemia | 8 | 7 | |
| ALT (baseline) (IU/l) | 108.45 (32.57) | 108.33 (23.32) | 0.993 |
| AST (baseline) (IU/l) | 61.55 (16.95) | 68.89 (17.45) | 0.354 |
| TC (baseline) (mmol/l) | 5.24 (0.98) | 5.13 (0.92) | 0.807 |
| LDL (baseline) (mmol/l) | 3.25 (0.73) | 3.38 (0.66) | 0.706 |
| TG (baseline) (mmol/l) | 2.05 (0.63) | 1.61 (0.61) | 0.136 |
| Fasting glucose (mmol/l)[a] | 6.4 (7.5) | 5.9 (9.5) | 0.659 |

Data shown as mean (SD)

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglycerides

[a] Data shown as median (range)

**Table 2** Hepatic histological scores of study patients according to Brunt's classification [17]

| Grading | Steatosis | Inflammation | Fibrosis |
|---|---|---|---|
| 0 | | 1 | 1 |
| 1 | 7 | 12 | 15 |
| 2 | 11 | 7 | 1 |
| 3 | 2 | | 3 |
| 4 | | | |
| Total number of patients | 20 | 20 | 20 |

($P = 0.000$) and the placebo group ($P = 0.008$) (Table 3). There was a trend toward a greater reduction in ALT level after treatment in the Pentoxifylline group versus placebo ($P = 0.065$) (Fig. 2). In addition, there were signficantly more patients who had AST normalization in the Pentoxifylline arm compared to the placebo group ($P = 0.026$). However there was no difference in both groups with regard to ALT normalization ($P = 0.16$).

Out of the 20 patients, there was one patient each from the Pentoxifylline and placebo groups who did not have TNF-α and IL-6 samples available for analysis.

After treatment, TNF-α and IL-6 decreased significantly in both Pentoxifylline and placebo groups but there was no significant difference in the reduction of TNF-α ($P = 0.27$) and IL-6 ($P = 0.78$) between the two groups (Table 4). There was also no significant difference in the reduction of HA between the two groups at the end of treatment.

**Table 3** Comparison of outcomes in both groups after treatment

| Variable | Pentoxifylline before treatment | Pentoxifylline after treatment | *P*-value | Placebo before treatment | Placebo after treatment | *P*-value | *P*-value (Pentoxifylline versus placebo) |
|---|---|---|---|---|---|---|---|
| Weight (loss/kg) | | 3.20 (2.14) | | | 2.98 (3.23) | | 0.856 |
| Body mass index | 26.67 (3.43) | 25.49 (3.07) | 0.001 | 29.91 (5.35) | 28.78 (5.39) | 0.020 | 1 |
| ALT (IU/l) | 108.45 (32.57) | 50.73 (15.71) | 0.000 | 108.33 (23.32) | 75.44 (34.70) | 0.008 | 0.065 |
| AST (IU/l) | 61.55 (16.95) | 33.18 (6.87) | 0.000 | 68.89 (17.45) | 49.33 (19.20) | 0.018 | 0.038 |
| TC (mmol/l) | 5.24 (0.98) | 5.58 (1.16) | 0.270 | 5.13 (0.92) | 4.98 (0.68) | 0.598 | 0.188 |
| LDL (mmol/l) | 3.25 (0.73) | 3.57 (1.05) | 0.258 | 3.38 (0.66) | 3.37 (0.73) | 0.963 | 0.630 |
| HDL (mmol/l) | 1.06 (0.23) | 1.42 (0.61) | 0.101 | 1.05 (0.15) | 1.14 (0.35) | 0.252 | 0.247 |
| TG (mmol/l) | 2.05 (0.63) | 1.65 (0.64) | 0.064 | 1.61 (0.61) | 1.45 (0.50) | 0.403 | 0.455 |

Data shown as mean (SD)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglycerides

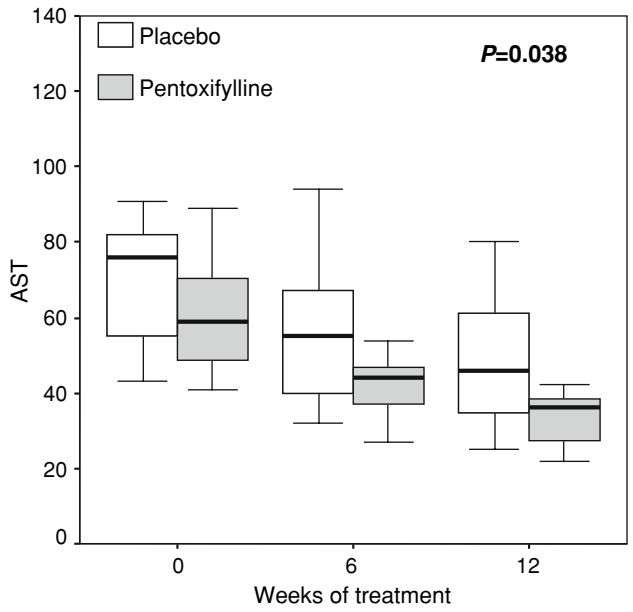


**Fig. 1** Comparison of AST levels before and after treatment

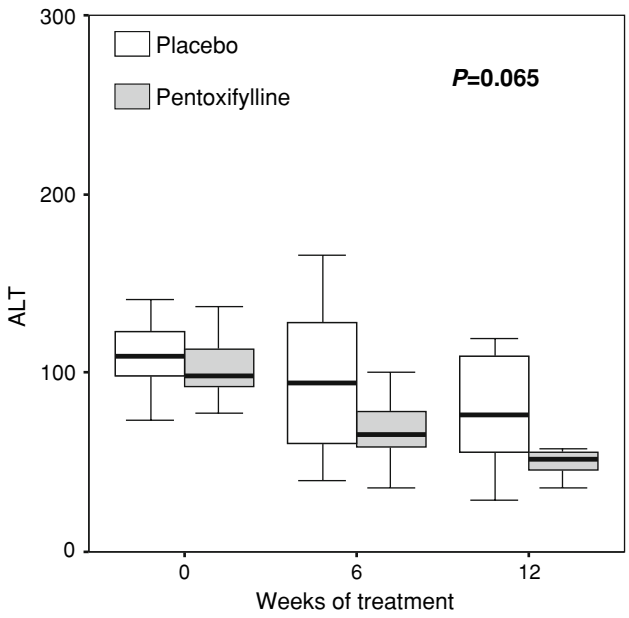


**Fig. 2** Comparison of ALT levels before and after treatment

**Table 4** Comparison of decrease in cytokines at the end of treatment

| Variable | Pentoxifylline ($n = 10$) | Placebo ($n = 8$) | $P$-value |
|---|---|---|---|
| Decrease from weeks 0 to 14 | | | |
| TNF-α (pg/ml) | 10.86 (15.68) | 31.35 (54.54) | 0.272 |
| IL-6 (pg/ml) | 7.47 (6.81) | 8.26 (4.36) | 0.780 |
| HA (ng/ml) | 7.32 (22.6) | 18.85 (17.55) | 0.254 |

Data shown in mean (SD)

TNF-α, tumor necrosis factor alpha; HA, hyaluronic acid; IL, interleukin

## Discussion

The pathogenesis of NASH is not clearly elucidated. There are few randomized controls trials or successful treatments for this condition. A recent placebo-controlled trial of Pioglitazone in subjects with NASH and impaired glucose tolerance or type 2 diabetes showed metabolic and histological improvement in those treated with Pioglitazone [18]. In another double-blind randomized placebo controlled trial of Orlistat in patients with NAFLD, Orlistat resulted in the improvement of serum ALT levels and steatosis beyond its effect on weight reduction [19]. This was attributed to decreased insulin resistance resulting in decreased-free fatty acid flux into the liver.

Our study is the first randomized controlled trial demonstrating that Pentoxifylline improves liver aminotransferase levels in patients with NASH. We have shown that in the Pentoxifylline group, AST was significantly reduced at the end of 3-months treatment compared to the placebo group. Furthermore there was significantly greater AST normalization in the Pentoxifylline group compared to the placebo group. ALT levels also showed a trend toward greater improvement in the Pentoxifylline group. However possibly due to the small numbers in our study, this did not reach statistical significance. In addition, most of our study patients had mild inflammation and fibrosis. This may explain why there was no significant difference between the Pentoxifylline and the placebo groups with regards to ALT, TNF-α, and IL-6 and also why there was a greater improvement in AST as compared to ALT. The beneficial effect of Pentoxifylline liver transaminases in patients with NASH has been previously demonstrated by two cohort studies. However there was a significant drop-out rate of nearly 50% in one study due to a higher dose of Pentoxifylline used in the study (1,600 mg/day) [14]. In contrast our patients tolerated Pentoxifylline at a dose of 1,200 mg/day. In addition to its effect on liver aminotransferase levels, a recent study also reported significant improvements in steatosis, lobular inflammation, and fibrosis in nine patients with NASH who received Pentoxifylline for 1 year [20].

Our study suggests that TNF-α and IL-6 may be implicated in the pathogenesis of NASH. We have demonstrated that there is a significant reduction of TNF-α and IL-6 in all study patients after treatment as compared with baseline levels. The decrease in the placebo group occurred probably as a result of the significant loss of weight at the end of 3 months that decreased insulin resistance. There was no significant difference however in the reduction of TNF-α and IL-6 between the Pentoxifylline and the control groups. This is may be due to the short duration of treatment of 3 months and the mild inflammation and fibrosis score in most of our patients. Similarly, although HA decreased significantly in each group at the end of treatment, there was no significant difference between the two groups. Other noteworthy features of our study were that the patients in both groups were similar at baseline and tolerated the treatment with no adverse events and none was lost to follow-up. Compliance to study medication was good and patients had benefit of a 2-week run in to obtain dietary counseling and advice on exercise which helped them adhere to the weight reduction program.

The limitations of our study include the short period of treatment of 3 months and the small numbers of patients investigated. Secondly we did not demonstrate histological improvement after treatment with Pentoxifylline. This may be important as improvement in liver aminotransferase levels may not be sufficient or reliable by itself as a marker for treatment response. However Satapathy et al. [20] showed that in five out of six histological responders to Pentoxifylline, there was also biochemical response in normalization of ALT. Furthermore we used HA as a surrogate marker for fibrosis in our study but there was no significant difference in the reduction of HA probably due to the short period of treatment.

In conclusion, we have achieved the main objective of our study which is to provide proof of principle that Pentoxifylline had a beneficial effect on liver aminotransferase levels in patients with NASH. Therefore a larger clinical trial is needed to determine the efficacy of Pentoxifylline in patients with NASH where liver histology is a primary outcome measure in addition to liver aminotransferase levels.

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