# Beneficial Effects of Pentoxifylline on Hepatic Steatosis, Fibrosis and Necroinflammation in Patients With Non-alcoholic Steatohepatitis

**Sanjaya K. Satapathy; Puja Sakhuja; Veena Malhotra; Barjesh C. Sharma; Shiv K. Sarin**
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## Abstract and Introduction

### Abstract

**Background and Aim:** Inhibition of tumor necrosis factor (TNF)-α is a logical approach to manage patients with non-alcoholic steatohepatitis (NASH). Pentoxifylline reduces TNF-α and alanine aminotransferase (ALT) levels in patients with NASH. The aim of the present paper was to study if pentoxifylline can improve histological injury in patients with NASH.

**Methods:** Nine patients (mean age 31.6 ± 7.2 years) with histologically proven NASH and with persistently elevated ALT (>1.5 times) were given pentoxyfylline at a dosage of 400 mg t.i.d. for 12 months. Besides biochemical assessment, a repeat liver biopsy was performed and the degree of inflammation and fibrosis was compared.

**Results:** After 12 months of therapy a significant reduction in ALT (111 ± 53 IU/L *vs* 45 ± 19 IU/L, *P* = 0.003) and aspartate aminotransferase (AST) (61 ± 27 IU/L *vs* 33 ± 12 IU/L, *P* = 0.005) levels was observed. Steatosis and lobular inflammation each reduced in 55% and six (67%) patients down-staged on Brunt's staging (*P* = 0.009). Four out of six patients with baseline fibrosis had reduction in their fibrosis stage.

**Conclusions:** Long-term pentoxyfylline therapy effectively achieves sustained biochemical improvement. This correlates well with histological resolution of the disease.

### Introduction

Non-alcoholic steatohepatitis (NASH) is a form of chronic hepatitis with histological features of alcohol-induced liver disease that occurs in individuals who do not consume significant amounts of alcohol. [1] Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of clinicopathological syndromes with varying risks of cirrhosis. The probability of developing advanced hepatic

fibrosis is significantly greater in individuals with steatohepatitis than in those with simple steatosis. [2-4] Between 15 and 20% of patients with steatohepatitis develop cirrhosis over one or two decades. [2,4] Although lipid lowering agents like statins and fibrates, antioxidants like vitamin E, insulin sensitizing agents like metformin, and cytoprotective agents like ursodeoxycholic acid (UDCA) have shown some promise by reducing the levels of aminotransferases, and in a few studies improvement in liver histology; none of these drugs can be recommended as a targeted and specific therapy for NASH. Most of the studies in this area have been small, non-randomized and did not have any follow-up histological studies.

We have recently reported the beneficial effects of 6 months of pentoxifylline therapy in clinical, biochemical and metabolic parameters in patients with NASH. [5] The present study reports the observations on the same cohort of patients treated for 12 months. The special feature of this study is the histological evaluation after completion of the therapy.

## Methods

Patients with histological diagnosis of NASH were continued on oral pentoxifylline after completing a 6-month course of therapy which has been reported earlier. These patients continued the treatment for 12 months. From this cohort, patients who consented to a histological follow-up were included in the present study. In brief, the inclusion criteria in the initial study were: aged 18-70 years; alanine aminotransferase (ALT) > 1.5 times the upper limit of normal on three occasions tested at least 1 month apart in the preceding 6 months; ultrasound showing diffusely echogenic liver suggestive of fatty infiltration of liver; and liver histology diagnostic of NASH according to the criteria of Brunt *et al*.[6] The exclusion criteria were: alcohol abuse (>20 g/week); evidence of viral or autoimmune hepatitis; primary biliary cirrhosis; biliary obstruction; or Wilson's disease and hemochromatosis and decompensated cirrhosis. Patients taking medication known to cause steatosis, specifically amiodarone, methotrexate, perhexilene, glucocorticoids, estrogens, tamoxifen, nifedipine, diltiazem and chloroquine, were excluded. Patients with other comorbid conditions that could potentially elevate transaminases, such as congestive heart failure, valvular heart diseases, chronic obstructive pulmonary disease and pregnancy, were also excluded. It was also ensured that the patients abstained from any alcohol and drugs likely to cause hepatic steatosis during the therapy period.

A total of nine patients fulfilled the selection criteria and were enrolled (eight from an earlier study and one new entrant). Patients were administered pentoxifylline 1200 mg/day in three divided doses over a 1-year period. A detailed clinical assessment was carried out in every patient. Patients were followed-up at monthly intervals for the initial 3 months and subsequently at 3-month intervals for the duration of the treatment. At every contact point, adverse events, concurrent medication and compliance with the study medication were assessed. Liver biochemistry was performed at 1-month intervals. Patients were classified based on their body mass index (BMI, $kg/m^2$) according to the following Asia-Pacific cut-off values: normal, between 18.5 and 22.9 $kg/m^2$; overweight, between 23 and 24.9 $kg/m^2$; and obese, above ≥25 $kg/m^2$.[7]

Laboratory investigations included hemogram, serum aminotransferases, total proteins, albumin, alkaline phosphatase, gamma glutamyl transferase, total bilrubin, hepatitis B serology (HBsAg, anti-HBe), anti-HCV or undetectable HCV-RNA by reverse transcription-polymerase chain reaction (RT-PCR), auto-antibodies (antinuclear antibody, antismooth muscle antibody, anti mitochondrial antibody); iron profile (serum iron, transferrin saturation and ferritin), ceruloplasmin, fasting serum cholesterol, triglycerides, and glucose tolerance test. An abdominal ultrasound was performed to assess the liver parenchyma, biliary tree, vascular patency and presence of ascites or portal hypertension, and also to exclude the possibility of hepatocellular carcinoma. [8] All patients had compensated liver disease, serum creatinine < 1.5 mg/dL and thyroid-stimulating hormone within normal limits.

Ethical justification and approval was obtained from the institutional review board. Informed written consent was obtained from all the participants in this trial.

## Histological Evaluation

A liver biopsy was performed in every patient within 4 weeks of starting the therapy to confirm the diagnosis of NASH. A repeat liver biopsy was performed at the end of 12 months of therapy in seven patients and after 6 months in two patients. Necroinflammatory grade and stage of fibrosis were assessed according to the method of Brunt *et al*.[6] Steatosis was graded as: 1, 10-30% of hepatocytes affected; 2, 30-70%; or 3, >70% of hepatocytes affected. Hematoxylin and eosin stained sections were used to assess the necroinflammatory grades and the Masson's trichrome stain for grading the fibrosis. Two hepatopathologists (PS and VM), who were blinded to the timing of the biopsy and the clinical and laboratory parameters of the patients, independently studied the biopsy specimens. Histological response was defined as at least one grade reduction in Brunt's histological grade from the baseline.

## Statistical Analysis

Data are expressed as mean ± SD. Analysis of repeated measures (general linear model) was used for comparison of variables (AST and ALT) across different time-points during the treatment. Paired *t*-test was applied for pair-wise comparison of continuous variables. Spearman's correlation was used to assess the association between change in BMI, weight and change in aminotransferases. A two-tailed *P* -value of less than 0.05 was considered significant. All data were analyzed by statistical software SPSS 10.01 (SPSS, Chicago, IL, USA).

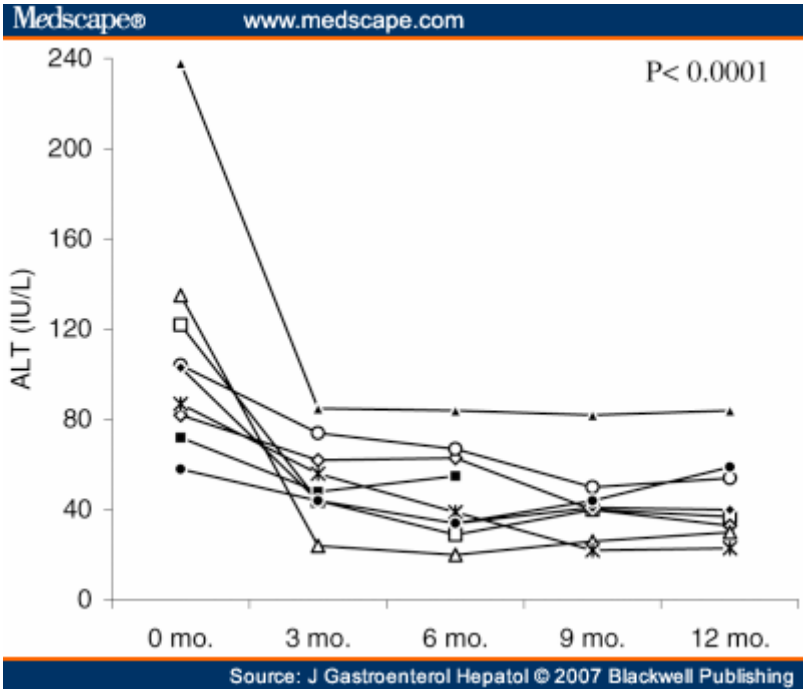
## Results

All the enrolled patients were male with a mean age of 31.6 ± 7.2 years (range 19-42 years) and a BMI of 26 ± 3.8 (range 22-34). Only one of the nine patients was lean, and four each were overweight and obese. Three patients had impaired glucose tolerance tests (GTT) at presentation. Hypertriglyceridemia (>160 mg/dL) and hypercholesterolemia (>200 mg/dL) were observed in five (mean serum triglyceride 201 ± 85 mg/dL) and three (mean serum cholesterol 162.6 ± 39.6 mg/dL) patients, respectively.

Insulin resistance (HOMA-IR, homeostasis model assessment of insulin resistance) was measured in only four of the included patients at baseline and was measured again at 12 months follow-up. Three of the four patients had a significant reduction in their HOMA-IR from baseline. Although one patient had a mild rise in HOMA-IR ( 1.96 to 2.51) at the end of 12 months of treatment, this patient still showed a significant reduction in transaminases and steatosis. However, he was noted to have one stage progression in fibrosis from a baseline of undetected fibrosis. No significant differences were noted from the baseline blood sugar level at 0, 1/2 h, 1 h, 1½ h, 2 h and 2½ h and repeat measurements after 12 months of pentoxifylline therapy ( *P* = 0.17, 0.92, 0.66, 0.79, 0.40, respectively). Of the three patients who had impaired glucose tolerance at presentation, one showed normal GTT after 12 months.

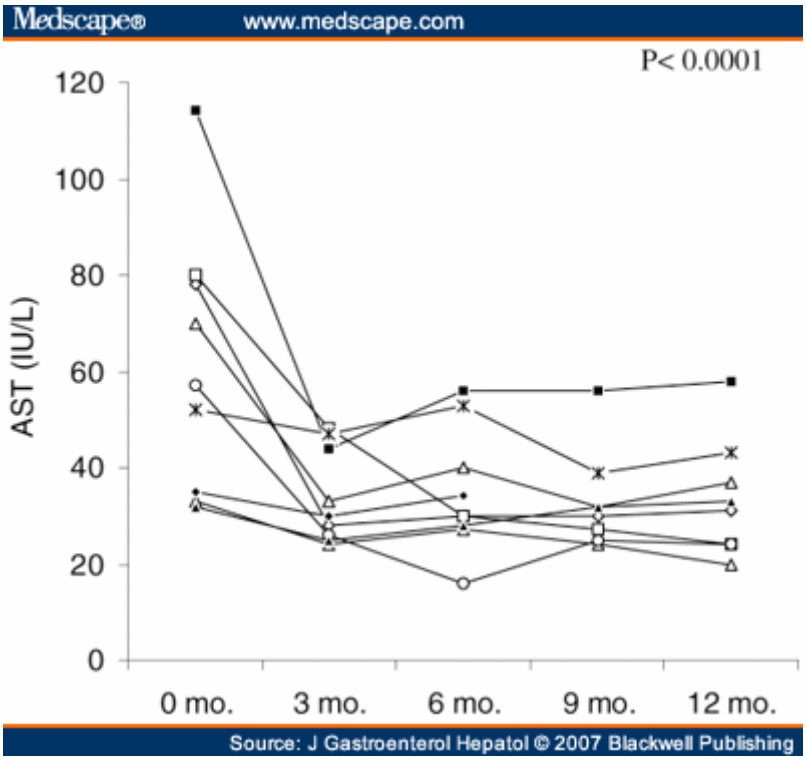
The ALT and AST values during therapy are shown in Table 1 . The mean pretreatment ALT for all the patients was 111 ± 53 IU/L. This substantially decreased at the end of therapy to 45 ± 19 IU/L (*P* = 0.003). Similarly, the mean pretreatment AST for all the patients was 61 ± 27 IU/L, which was significantly reduced to 33 ± 12 IU/L post-treatment ( *P* = 0.005). Statistical computation by the general linear model with repeated measures showed a significant drop in ALT (*P* < 0.0001) and AST (*P* < 0.0001) levels at the end of 12 months of treatment, respectively ( Table 2 , Figs 1,2). The ALT and AST were normal in five (55%) and six (67%) of the nine patients, respectively, after 12 months of therapy.

**Figure 1.**




Significant and sustained improvement in alanine aminotransferase (ALT) levels during 12 months of therapy with pentoxifylline in nine patients (specified with distinct symbols) with non-alcoholic steatohepatitis (NASH).

**Figure 2.**



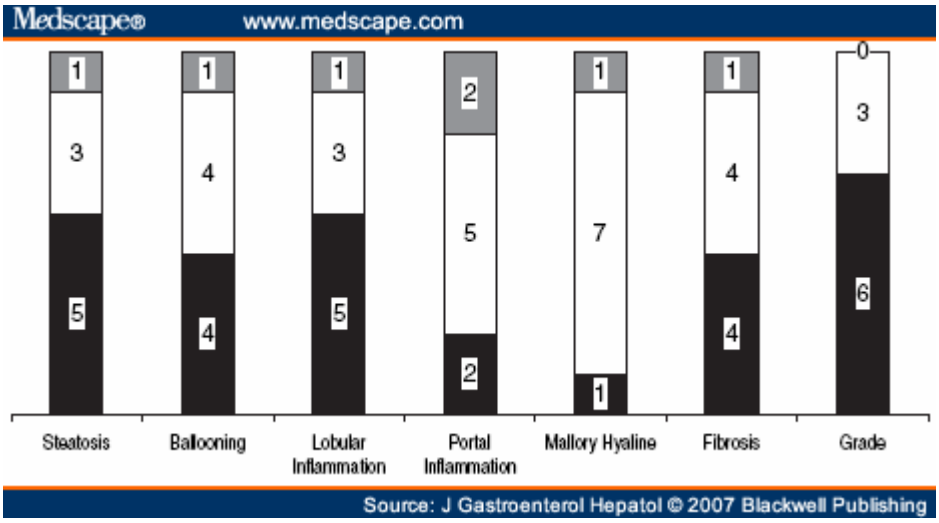

Significant and sustained improvement in aspartate aminotransferase (AST) levels during 12 months of therapy with pentoxifylline in nine patients (specified with distinct symbols) with non-alcoholic steatohepatitis (NASH).

No significant difference in BMI (26 ± 3.8 *vs* 25 ± 4.2 kg/m$^2$ , *P* = 0.098) or waist circumference (96.2 ± 8.7 *vs* 93.8 ± 10.2 cm, *P* = 0.246) was observed before and after treatment. There was no significant difference between the responders and non-responders in terms of age and BMI. However, when only weight was compared instead of BMI, a modest reduction in weight was observed following 12 months of treatment with pentoxifylline ( *P* = 0.022). We did not observe any correlation between improvement in ALT at 12 months and weight change (*r* = 0.07, *P* = 0.86), or change in the waist circumference (*r* = 0.4, *P* = 0.31).

Baseline liver biopsy was obtained in all patients and showed some degree of steatosis in all patients, moderate (grade II) in two, and severe (grade III) in four patients. Steatosis was predominantly centrilobular. All the patients had grade I to II lobular inflammation. Portal inflammation was mild in the majority of patients. Mallory hyaline bodies were present in a single case at presentation. Stage I fibrosis was present in three, stage II in one and stage III in two cases. After 12 months of therapy with pentoxifylline, significant overall improvement was noted in liver histology (Fig. 3). Sixty seven percent of patients had a decrease in overall grading based on Brunt's staging ( *P* = 0.009). Fifty-five percent of the patients had a reduction in their steatosis and lobular inflammation. Four out of the six patients with baseline fibrosis had a reduction in their fibrosis stage and another two patients remained stable on follow-up biopsy. Only one patient who did not have any evidence of fibrosis on follow-up showed stage I fibrosis. Three patients had one

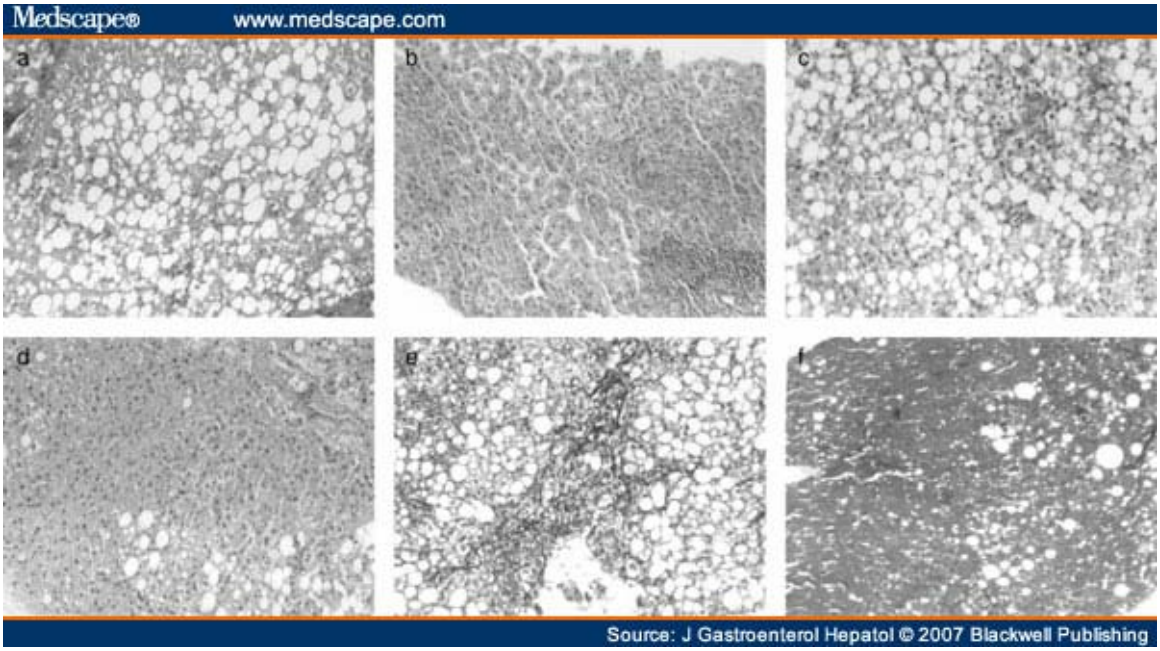
stage reduction in fibrosis and a single patient had a two stage reduction in fibrosis. Five of the six histological responders (defined as at least one grade reduction in Brunt's histological grade) were also biochemical responders with normalization of ALT. Histological improvement in necroinflammation and fibrosis is shown in two representative patients in Figure 4 (arbitrarily named, X and Y).

**Figure 3.**




Histological changes on paired liver biopsy after 12 months of pentoxifylline therapy. Out of the four patients showing no worsening of fibrosis stage, two had no fibrosis at baseline. (■), Decreased; (□), stabilized; (▨), worsened.

**Figure 4.**



Pre-therapy biopsy showing severe steatosis with mild lobular inflammation from patient X. (b) Post-therapy biopsy showing almost complete clearance of steatosis and no lobular inflammation, but some increase in portal inflammation (inset in b, arrow) in patient X. (c) Pre-therapy liver biopsy from patient Y showing severe steatosis, lobular inflammation and some ballooning. (d) Post therapy biopsy of patient Y showing significant reduction in steatosis, ballooning and lobular inflammation. (e) Pre therapy biopsy showing perisinusoidal fibrosis on Masson's trichrome stain in patient Y. (f) Significant reduction of fibrosis on Masson's trichrome stain in patient Y. *Photomicrograph a and b from patient X and photomicrograph c-f is from patient Y.

## Discussion

In the present study, we demonstrated significant histological improvement and normalization of aminotranferases at the end of 12 months of pentoxifylline treatment in patients with NASH.

NASH could progress to cirrhosis in up to 20-30% of patients.[2-4] No effective therapy for preventing disease progression or amelioration of this disease currently exists. The improvement in liver enzymes in the present study was associated with histological resolution of NASH. Sixty-seven percent of the patients in the present study had at least one grade improvement in Brunt's grading. Five of the six histological responders also had persistent normalization of their aminotransferases. Histological response was noted in steatosis, necroinflammation and fibrosis stage. Fibrosis could be down-staged in four out of six patients with baseline fibrosis. Most importantly, worsening of the fibrosis was observed in only one patient at the end of 12 months of treatment. Pentoxifylline thus could prove useful as an antifibrotic drug in NASH in addition to improving necroinflammation. However, in view of the small number of patients included in the study and because it was not a randomized controlled study, the possibility of sampling error in liver biopsy cannot be entirely excluded. Ratziu *et al*. recently found that histological lesions of NASH are unevenly distributed throughout the liver parenchyma, which might account for sampling error of liver leading to staging inaccuracies. [9] Although we agree to this theoretical possibility, the sustained biochemical improvement over the entire 12-month period and histological improvement in almost two-thirds of the patients is unlikely to be due to be a result of sampling error.

Pentoxyfylline has long been viewed as a potential antifibrotic agent. Its role as an antifibrotic drug has been shown in a yellow phosphorus-induced pig model of hepatic fibrosis, however, no improvement was found in bile duct ligated rat models. [10] However, in another study using a rat model of biliary fibrosis, pentoxifylline caused a dramatic decrease in profibrogenic cytokine transforming growth factor (TGF)-β, connective tissue growth factors and procollagen III peptide (PIIINP). [11] Its antifibrotic action is probably due to blockage of stellate cell activation *in vivo* independently of its inhibitory effects on phosphodiesterases by interfering with the oxidative stress cascade and the activation of NF-kappa B and c-myb. [12] No study has yet been conducted to ascertain its effect on liver fibrosis in humans. The present study gives us an opportunity to assess this important fact in NASH.

A steady decline in the AST and ALT levels within the first month of therapy was observed and this decline persisted throughout the treatment period. Because we had already shown a sustained improvement in ALT for up to 6 months with pentoxifylline therapy in our earlier published study, [5] this study further shows that this improvement can be carried on up to 12 months with long-term treatment. A recent study from the Mayo Clinic group has further reiterated these results with improvement in aminotransferases after 12 months of therapy. [13] In a recent animal study, pentoxifylline was found to achieve a significant reduction in serum ALT levels and hepatic inflammation in the methionine choline deficient (MCD) diet model of steatohepatitis. [14] The authors postulated that this improvement could be due to an increase in glutathione levels or a reduction in the TNF-α levels by pentoxifylline.

Although we did not find any significant change in BMI, a modest reduction in weight was observed. Because the patients included in the study were not prescribed any specific diet or enrolled in a weight-reducing program, we believe that the reduction in weight may be a long-term secondary effect of pentoxifylline. Adams *et al*. made a similar observation in their study of 18 patients with NASH after 12 months of therapy with pentoxifylline.[13] The fact that pentoxifylline improved steatosis from the liver itself proves that it has the potential to burn the adipose tissue in general, an effect that needs further evaluation.

An association with insulin resistance and NASH has been shown in various studies. [15-17] We have earlier reported a significant reduction in HOMA-IR in NASH patients after 6 months of therapy with pentoxifylline. [5] Three of the four patients in the present study showed improvement in their HOMA-IR after 12 months of therapy. The improvement in insulin resistance index could be due to the downregulation of TNF-α by pentoxifylline. In our earlier published paper, [5] we reported a significant reduction in TNF-α with pentoxifylline. The cytokine TNF-α is an important mediator of insulin resistance through its ability to influence the tyrosine kinase activity of the insulin receptor. [18] TNF-α is known to inhibit the propagation of insulin receptor initiated signals in hepatocytes. TNF-α promotes insulin resistance in ob/ob mice. [19] Thus modulation of insulin resistance by pentoxifyliine could be a potential mechanism for improvement in patients with NASH.

In conclusion, pentoxifylline leads to a significant reduction in the AST and ALT levels in patients with NASH. This improvement correlates with histological resolution of steatosis, inflammation and fibrosis stage. Randomized controlled trials with long-term pentoxyfylline treatment with histological follow-up are justified.

Table 1. Baseline Characteristics of the Study Patients

| Parameter | Observation [†] |
|---|---|
| Number of patients | 9 |
| Age (years) | 31.6 ± 7.2 (range 19-42 years)* |
| Sex (male : female) | 9:0 |
| Weight (kg) | 78.3 ± 11 (range 67-105) |
| Body mass index (kg/m$^2$ ) | 26 ± 3.8 (range 22-34) |
| AST (<40 IU/L) | 61 ± 27 (range 32-114) |
| ALT (<40 IU/L) | 111 ± 53 (range 58-238) |
| Serum cholesterol (<200 mg/dL) | 162.6 ± 39.6 (range 126-232) |
| S. triglyceride (<160 mg/dL) | 201 ± 85.6 (range 114-352) |
| HDL (>40 mg/dL) | 40 ± 3.3 (range 35-45) |
| Fasting blood sugar (70-90 mg/dL) | 86 ± 6.6 (range 77-98) |

[†]Expressed as mean ± SD unless specified. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein cholesterol.

Table 2. Influence of Pentoxifylline Therapy on the Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels

| | **Baseline data** | **Effects of therapy** | | | | |
|---|---|---|---|---|---|---|
| **Parameters** | **0 month (*n* = 9)** | **3 months (*n* = 9)** | **6 months (*n* = 9)** | **9 months [‡] (*n* = 8)** | **12 months [‡] (*n* = 8)** | ***P* -value** |
| AST (IU/L) | | | | | | |
| Mean | 61 ± 27 | 34 ± 10 | 35 ± 13 | 33 ± 10 | 33 ± 12 | <0.0001 [†] |
| Median | 57 | 30 | 30 | 31 | 32 | |
| Range | 32-114 | 24-48 | 16-56 | 24-56 | 20-58 | |
| ALT (IU/L) | | | | | | |
| Mean | 111 ± 53 | 53 ± 18 | 47 ± 21 | 43 ± 18 | 45 ± 19 | <0.0001 [†] |
| Median | 103 | 48 | 39 | 40 | 38 | |
| Range | 63-238 | 24-85 | 20-84 | 22-82 | 23-84 | |

[†]General linear model repeated measures were used to compute the changes in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) across time.
[‡]One patient withdrew from the therapy at 6 months.

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**Reprint Address**

Dr SK Sarin, Professor and Head, Department of Gastroenterology, Room No. 201, GB Pant Hospital, New Delhi-110002, India. Email: sksarin@nda.vsnl.net.in

**Sanjaya K. Satapathy**,* **Puja Sakhuja** ,† **Veena Malhotra**, † **Barjesh C. Sharma*** and **Shiv K. Sarin***

Departments of *Gastroenterology and †Pathology, GB Pant Hospital, New Delhi, India