A Randomized Controlled Trial Comparing Efficacy of Pentoxifylline and Pioglitazone on Metabolic Factors and Liver Histology in Patients with Non-alcoholic Steatohepatitis

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Background and Aims: Non-alcoholic steatohepatitis (NASH) involves increased hepatic macrosteatosis due to increased insulin resistance and non-hepatic processes including oxidative stress, apoptosis, and increased pro-inflammatory cytokines. Present study compared the efficacy of pentoxifylline and pioglitazone therapy in improving the metabolic factors and liver histology in patients with NASH. **Methods:** Sixty consecutive biopsy proven NASH patients aged 18–70 years with ALT > 1.2 times the upper limit of normal were randomized to receive either pentoxifylline 1200 mg/day in three divided doses orally every day or pioglitazone (30 mg/day) daily for 6 months. All the patients were also instructed to reduce their calorie intake by 500 kcal/day as well as to perform modest exercise (brisk walking) regularly at least 5 days per week. Before and after treatment, liver function tests, serum insulin, C-peptide levels, TNF- α , adiponectin, leptin levels, HOMA-IR and hepatocyte injury and fibrosis scores on liver histology were assessed. **Results:** Both pentoxifylline and pioglitazone were effective in improving transaminases, insulin resistance (HOMA-IR) and adiponectin levels significantly. TNF- α levels improved with either of the drugs but did not achieve significant levels. Both the drugs improved the markers of acute liver injury. However, only steatosis improved significantly with either of the drugs. Patients treated with pioglitazone had significant improvement in lobular inflammation, portal inflammation and Bruns grade. Bruns grade improved significantly with pioglitazone as compared to pentoxifylline at the end of the therapy. **Conclusions:** Pioglitazone shows better improvement in both metabolic factors and liver histology in patients with NASH compared to pentoxifylline. (J CLIN EXP HEPATOL 2012;2:333–337)

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of disorders characterized by histological features of alcohol-induced liver disease occurring in individuals who do not consume significant amounts of alcohol. Usually, fatty liver does not progress to severe liver disease. However, approximately 20–30% of patients have histological signs of fibrosis and necroinflammation, suggesting the presence of non-alcoholic steatohepatitis (NASH).¹ It is this group of NASH patients, who are at higher risk of developing cirrhosis, terminal

liver failure, and hepatocellular carcinoma.¹ Approximately 15–20% of patients with non-alcoholic steatohepatitis develop cirrhosis over one or two decades.²

Adipocytokines and insulin resistance are the major driving force behind excessive fat accumulation in the liver and development of steatohepatitis and its progression.³ A number of adipocytokines have been linked to alterations in insulin sensitivity, including tumor necrosis factor- α (TNF α), adiponectin, leptin and resistin. These signaling molecules have been shown to be associated with suppressed hepatic insulin sensitivity, and it is thought that adipocytokines may contribute to the development of liver fibrosis.^{4,5} TNF α is overexpressed in adipose tissue in obesity and decreases with weight loss.⁶ This overexpression is elevated in patients with higher necroinflammation and with more advanced fibrosis stages in liver tissue of NASH patients.⁷ Pentoxifylline, due to its anti-TNF- α actions has been reported to have beneficial effects in biochemical, metabolic and histological parameters in patients with NASH.^{7,8}

Association of NASH with insulin resistance has provided the rationale for evaluation of medical therapies that increase insulin sensitivity.^{9–11} Pioglitazone, a thiazolidinediones (TZDs) class of antidiabetic drugs

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Abbreviations: NAFLD: nonalcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; TNF α : tumor necrosis factor- α ; TZDs: thiazolidinediones; ALT: alanine aminotransferase; HOMA: homeostatic model assessment; HOMA-IR: homeostasis model assessment insulin resistance index; WHR: waist hip ratio; AST: aspartate aminotransferase; FBS: fasting blood sugar; HDL: high density lipoprotein

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that increase insulin sensitivity, has shown promising results.^{12,13}

There has been no trial comparing the beneficial effect of pentoxifylline and pioglitazone in improving the biochemical, metabolic and histological profile in patients with NASH. We performed the study to compare the beneficial effects of pentoxifylline and pioglitazone therapy in improving the liver disease in patients with NASH and to assess if this improvement is associated with reduction of insulin resistance and improvement in cytokines profile.

PATIENTS AND METHODS

The study was performed between February 2006 and December 2009. The study protocol was approved by the institutional review board and informed written consent was obtained from all the patients.

Inclusion Criteria

Patients aged between 18 and 70 years; alanine aminotransferase (ALT) > 1.2 times the upper limit of normal on three occasions at least 1 month apart in the preceding 6 months; ultrasound showing diffusely echogenic liver suggestive of fatty infiltration of liver and liver biopsy showing steatosis of hepatocytes with necroinflammatory activity, ballooning hepatocytes &/or fibrosis were included for evaluation in the study.

Exclusion Criteria

The exclusion criteria were: alcohol intake of more than 20 g/week; evidence of viral or autoimmune hepatitis; primary biliary cirrhosis; biliary obstruction; Wilson's disease; hemochromatosis and decompensated cirrhosis. Drug ingestion of the following drugs, for a period of more than 4 weeks during past 6 weeks – amiodarone, methotrexate, perhexiline, glucocorticoids, estrogens, tamoxifen, nifedipine, diltiazem, tamoxifen. Pregnant women and patients requiring treatment with insulin for diabetes were also excluded from the study.

STUDY DESIGN

This study was an open label randomized controlled trial. All patients were instructed to reduce their calorie intake by 500 kcal/day as well as to perform modest exercise (brisk walking) regularly at least 5 days per week.

Information collected at the time of randomization included anthropometric data (height, weight, waist-hip ratio) and laboratory investigations namely hemogram, serum aminotransferases, total proteins, albumin, alkaline phosphatase, total bilirubin, hepatitis B serology (HBsAg, anti-HBe), anti-HCV or HCV-RNA by reverse transcription-polymerase chain reaction (RT-PCR), auto antibodies (antinuclear antibody, antismooth muscle anti-

body, anti mitochondrial antibody); iron profile (serum iron, transferrin saturation and ferritin), ceruloplasmin, fasting serum cholesterol, triglycerides, and glucose tolerance test.

Serum insulin and C-peptide levels were measured and quantitative assessment of insulin sensitivity was performed using the homeostasis model assessment insulin resistance index (HOMA-IR) = fasting serum insulin (μ IU/ml) X fasting serum glucose (mmol/l)/22.5[12]. TNF- α , adiponectin and leptin levels were estimated by ELISA method using standard kits on 50 μ L of plasma according to the manufacturer's recommendations (BioVendor Research, Modrice, Czech Republic).

All patients underwent a liver biopsy prior to randomization.

Patients were randomized to receive either pentoxifylline 1200 mg/day in three divided doses orally every day or pioglitazone (30 mg/day) daily for six months. Randomization was performed by computer program in blocks of 4. Patients were followed-up at monthly intervals for the initial 3 months and subsequently at 3-months interval. At every contact point, adverse events, concurrent medication and compliance with the study medication were assessed. Liver biochemistry was performed at 1-month interval for the initial 3 months and subsequently at 3-monthly intervals. Metabolic factors and liver biopsy were repeated at end of six months. Primary outcomes were reductions in hepatocyte injury and fibrosis scores on histology. Secondary outcomes were improvements in the biochemical and metabolic parameters in the pentoxifylline and pioglitazone groups.

The liver biopsy specimens were reviewed by one pathologist who was blinded to the drug administered as well as to whether the liver biopsy was performed at baseline or at the end of the study. Necroinflammatory grade and stage of fibrosis were assessed according to the method of Brunt et al.¹⁴

STATISTICAL ANALYSIS

Categorical data are expressed as frequencies (and percentages). Continuous variables are summarized as mean $\pm$ 1 standard deviation. Changes within each group over the study period were evaluated using the paired *t* test (for normally distributed data) or the Wilcoxon signed rank test (for nonparametric data). Comparisons between groups were made using the Mann-Whitney *U* test.

RESULTS

A total of 60 consecutive patients with NASH were enrolled in the study. One patient from the pioglitazone arm was lost to follow up after randomization. We report on the results of 29 patients in the pioglitazone arm and 30 patients in the pentoxifylline arm.

Baseline demographic, clinical, anthropometric data, biochemical and metabolic parameters were comparable in two groups (Table 1). Both pentoxifylline and pioglitazone were effective in improving transaminases, insulin resistance (HOMA-IR) and adiponectin levels significantly. TNF- α levels improved with either of the drugs but did not achieve significant levels. Leptin, on the contrary increased with either of the drugs inspite of improvement in liver histology after therapy (Table 2).

LIVER HISTOLOGY

Both the drugs improved the markers of acute liver injury. However, only steatosis improved significantly with either of the drugs. Patients treated with pioglitazone also had significant improvement in lobular inflammation, portal inflammation and Brunt's grade. However, when compared amongst the two groups, there was no significant difference in the improvement of various markers of liver injury except for Brunt's grade. Brunt's grade improved significantly with pioglitazone as compared to pentoxifylline at the end of the therapy (Table 3).

WITHDRAWALS AND SIDE EFFECTS

One patient was lost to follow up in pioglitazone group. None of the patients dropped out because of the side-effects of the drug. One patient reported pedal edema with pioglitazone but completed the study. Two patients reported heart-burn with pentoxifylline which re-

sponded with proton-pump inhibitors. None of the subjects developed heart failure or fractures during the study period.

DISCUSSION

NASH is now considered as a spectrum of NAFLD, characterized by histological features of alcohol-induced liver disease occurring in individuals who do not consume significant amounts of alcohol.

The "multi-hit" hypothesis of NASH has been proposed. The "first hit" is a hepatic process involving increased hepatic macrosteatosis due to increased insulin resistance. Proposed "second hits" are non-hepatic processes including oxidative stress, apoptosis, and increased pro-inflammatory cytokines. There are studies evaluated the efficacy of increasing the insulin sensitivity by pioglitazone a thiazolidinediones and drugs against pro inflammatory cytokine (TNF- α) like pentoxifylline.¹⁵

In present study hepatic steatosis improved significantly in both pentoxifylline and pioglitazone arms ($p=0.02$ & 0.005 respectively). Similar observation made by Satapathy et al⁷ in their study using pentoxifylline in 9 patients for 12 months. A 48 week trial of pioglitazone, 30 mg daily, in 18 patients by Promrat et al¹² showed significant improvement in steatosis ($P < 0.001$) and controlled trial with pioglitazone, Belfort et al¹³ in 55 patients showed significant improvement hepatic fat content (by 54%). Sanyal et al¹⁶ also reported significant improvement in hepatic steatosis with pioglitazone.

Table 1 Demographic, anthropometric and biochemical parameters in two groups before and after treatment.

Parameters	Pentoxifylline			Pioglitazone			P value (pio vs pentoxi)
	Baseline	Post-therapy	P value	Baseline	Post-therapy	P value	
Age (yr)	37.3 $\pm$ 7.2			40.4 $\pm$ 9.9			
Sex (M:F)	7:4			4:5			
Weight (kg)	66.6 $\pm$ 10.7	66.5 $\pm$ 9.1	0.81	71.1 $\pm$ 8.2	73.1 $\pm$ 9.1	0.31	
Body mass index (kg/m ²)	24.2 $\pm$ 2.7	24.2 $\pm$ 3.6	0.91	25.7 $\pm$ 3.58	26.2 $\pm$ 10.2	0.84	
Waist circumference (cm)	90.1 $\pm$ 7.1	90.1 $\pm$ 8.3	0.92	90.36 $\pm$ 7.85	90.8 $\pm$ 9.1	0.89	
WHR	0.94 $\pm$ 0.05			0.90 $\pm$ 0.08			
Bilirubin (mg/dl)	1.2 $\pm$ 0.6	0.99 $\pm$ 0.6	NS	1.1 $\pm$ 0.5	0.9 $\pm$ 0.5	NS	0.83
Total protein	7.5 $\pm$ 0.6	7.5 $\pm$ 0.63	0.69	7.9 $\pm$ 0.5	7.7 $\pm$ 0.5	0.11	0.34
Albumin	4.5 $\pm$ 0.4	4.4 $\pm$ 0.3	0.25	4.4 $\pm$ 0.2	4.5 $\pm$ 0.3	0.95	0.13
AST (IU/L)	63.25 $\pm$ 19.5	27.5 $\pm$ 9.7	0.002	66.5 $\pm$ 17.4	27.7 $\pm$ 9.1	0.003	0.97
ALT (IU/L)	86.6 $\pm$ 31.6	36.9 $\pm$ 19.6	0.002	106.3 $\pm$ 27.9	34.0 $\pm$ 16.1	0.003	0.65
FBS (mg/dl)	103.5 $\pm$ 28.8	97.6 $\pm$ 16.0	0.556	112.9 $\pm$ 47.2	98.0 $\pm$ 21.7	0.02	0.23
Total cholesterol	192.2 $\pm$ 18.0	190 $\pm$ 22.7	0.19	198.7 $\pm$ 39.9	195.8 $\pm$ 36.2	0.28	0.97
Serum triglyceride	178.2 $\pm$ 46.5	174.0 $\pm$ 37.8	0.36	234.9 $\pm$ 57.2	228.3 $\pm$ 45.6	0.24	0.97
HDL	46.0 $\pm$ 10.2	43.9 $\pm$ 9.4	0.06	38.5 $\pm$ 6.6	39.0 $\pm$ 6.6	0.80	0.08

WHR: waist hip ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, FBS: fasting blood sugar, HDL: high density lipoprotein.

Table 2 Metabolic parameters in two groups before and after treatment.

Parameters	Pentoxifylline			Pioglitazone			P value (pio vs pentoxi)
	Baseline	Post-therapy	P value	Baseline	Post-therapy	P value	
S. insulin (μ U/mL)	9.0 $\pm$ 4.6	6.8 $\pm$ 4.2	0.005	11.8 $\pm$ 3.7	8.5 $\pm$ 1.5	0.028	0.65
HOMA	2.4 $\pm$ 1.83	1.64 $\pm$ 1.2	0.006	3.29 $\pm$ 1.5	2.07 $\pm$ 0.61	0.016	0.28
C-peptide	2.9 $\pm$ 1.3	2.5 $\pm$ 1.3	0.09	3.3 $\pm$ 1.2	2.5 $\pm$ 0.5	0.13	0.34
TNF- α (pg/ml) (median, range)	12.1 (4–71.4)	8.7 (4–42)	0.286	13.3 (5.2–21.6)	11.7 (8.3–34.3)	0.285	0.26
Adiponectin (μ g/ml)	4.36 $\pm$ 1.87	7.18 $\pm$ 4.68	0.073	4.0 $\pm$ 1.87	7.17 $\pm$ 2.31	0.007	0.08
Leptin (ng/ml) (median, range)	4.48 (1.75–37.91)	23.57 (3.37–52.3)	0.007	13.47 (6.3–64.6)	14.38 (14.2–155.1)	0.007	0.76

HOMA: homeostatic model assessment, TNF- α : tumor necrosis factor alfa.

Other histological parameters like ballooning, lobular inflammation and portal inflammation were significantly improved with pioglitazone in contrast to pentoxifylline in the present study. But head to head comparison of both drugs did not reveal any significance. On contrary Satapathy et al⁷ showed a significant improvement in lobular inflammation on pentoxifylline therapy. Study from Belfort et al¹³ showed that administration of pioglitazone, as compared with placebo, was associated with improvement in ballooning necrosis ($P = 0.02$), and inflammation ($P = 0.008$) results similar to present study. Other study from Promrat et al¹² showed significant improvement in parenchymal inflammation and hepatocellular injury. Sanyal et al¹⁶ also found significant improvement in lobular inflammation with 86 weeks treatment with pioglitazone ($P = 0.004$).

There is no significant improvement in Brunt's grading and fibrosis in present study in pentoxifylline arm, but patients treated with pioglitazone had significant improvement in Brunt's grade ($P = 0.005$) though not in fibrosis ($P = 0.19$). When compared both arms showed significant improvement in Brunt's grade ($P = 0.04$) but not in fibrosis. The study by Satapathy et al showed significant improvement in both Brunt's grade and fibrosis with pentoxifylline therapy. Largest randomized controlled study on NASH patients with pioglitazone showed that there was no signif-

icant improvement in fibrosis like present study.¹³ Sanyal et al¹⁶ also reported the similar finding. There was no improvement in fibrosis with treatment with pioglitazone.

In our study, a reduction in transaminase levels occurred in all patients in both the study groups. All the previous studies with pentoxifylline (Adam et al,¹⁷ Satapathy et al⁸) and pioglitazone (Promrat et al,¹² Belfort et al,¹³ Sanyal et al¹⁶) showed reduction in transaminases similar to the present study. Reduction in transaminase levels was also seen in patients who had progression of disease in liver histology. Hence a reduction in transaminase level does not guarantee an improvement in liver histology. Other studies also suggest that advanced histologic changes in NAFLD can exist inspite of normal liver enzyme levels and changes in aminotransferase levels do not parallel the progression of fibrosis.¹³

We found significant reduction in fasting insulin level and improvement in homeostasis model assessment-IR in both the pioglitazone and pentoxifylline groups. C peptide did decrease with any of the drugs, though it did not reach significant levels suggesting that the drug acts by reducing insulin secretion. Serum insulin levels as well as C-peptide levels significantly decreased in patients treated with pioglitazone in their study by Promrat et al¹² Adiponectin levels increased significantly after treatment with pioglitazone ($P = 0.007$) as well as with pentoxifylline

Table 3 Liver histology in two groups before and after treatment.

Parameters	Pentoxifylline			Pioglitazone			P value (pio vs pentoxi)
	Baseline (n = 30)	Post-therapy (n = 24)	P value	Baseline (n = 30)	Post-therapy (n = 22)	P value	
Steatosis	2.08 $\pm$ 0.79	1.25 $\pm$ 0.86	0.02	2.18 $\pm$ 0.87	1.0 $\pm$ 0.6	0.005	0.60
Ballooning	1.33 $\pm$ 0.49	1.16 $\pm$ 0.71	0.414	1.45 $\pm$ 0.5	1.09 $\pm$ 0.7	0.15	0.52
Lobular inflammation	1.16 $\pm$ 0.57	0.75 $\pm$ 0.6	0.187	1.36 $\pm$ 0.50	0.45 $\pm$ 0.4	0.013	0.21
Portal inflammation	0.83 $\pm$ 0.38	0.75 $\pm$ 0.62	0.65	1.0 $\pm$ 1.0	0.36 $\pm$ 0.5	0.038	0.17
Brunt's grade	1.50 $\pm$ 0.67	1.16 $\pm$ 0.93	0.10	2.1 $\pm$ 0.83	0.9 $\pm$ 0.9	0.005	0.04
Fibrosis	0.83 $\pm$ 0.57	0.91 $\pm$ 0.71	0.70	1.36 $\pm$ 1.0	0.9 $\pm$ 0.9	0.19	0.26

($P = 0.073$). Adiponectin, which is secreted by visceral adipocytes and correlates strongly with insulin sensitivity¹⁸ may account for some of the beneficial effects of the pioglitazone as well as pentoxifylline in improving liver histology.

To conclude pioglitazone shows better improvement in both metabolic factors and liver histology in patients with NASH compared to pentoxifylline.

CONFLICTS OF INTEREST

All authors have none to declare.

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