

# Metformin is effective in achieving biochemical response in patients with nonalcoholic fatty liver disease (NAFLD) not responding to lifestyle interventions\*

Ajay Duseja;<sup>1</sup> Ashim Das;<sup>2</sup> Radha Krishan Dhiman;<sup>1</sup> Yogesh Kumar Chawla;<sup>1</sup> Kiran K. Thumburu;<sup>1</sup> Sanjay Bhadada;<sup>3</sup> Anil Bhansali<sup>3</sup>

## Abstract

**Background:** Insulin resistance plays an important role in the pathogenesis of NAFLD. Pharmacological treatment of patients with NAFLD is still evolving. Insulin sensitizing drugs like metformin may be effective in these patients. Twenty five adult patients with NAFLD who did not achieve normalization of alanine transaminases (ALT) after 6 months of lifestyle interventions and UDCA were treated with metformin 500mg tid for 6 months. Insulin resistance was determined by HOMA- IR. Liver function tests were done monthly and patients were defined having no response, partial response or complete biochemical response depending on the change in ALT. Results were compared with 25 patients with NAFLD from the

same cohort treated only with lifestyle interventions (disease controls). **Results:** Thirteen (52%) patients had class III (n = 5) or class IV (n = 8) disease amounting to histological NASH. Of these 13 patients none had severe inflammation and none had stage 4 fibrosis (cirrhosis). All 25 patients with NAFLD had insulin resistance in comparison to healthy controls. In comparison to disease controls (127.5 ± 41.8 vs. 118 ± 21.6 p = NS), all patients treated with metformin had partial biochemical response (mean ALT 122.2 ± 26.8 vs 74.3 ± 4.2 p < 0.01) and 14 (56%) of them achieved complete normalization of ALT. **Conclusions:** Metformin is effective to achieve biochemical response in patients with NAFLD who do not respond to lifestyle interventions and UDCA.

**Key words:** Nonalcoholic steatohepatitis, NASH, insulin resistance, diabetes mellitus, metabolic syndrome, UDCA, metformin,

\* This work was presented at the Asian Pacific Digestive Week at Cebu City, Philippines in November 2006 (*J Gastroenterol Hepatol* 2006; 21 (suppl 6): A457).

<sup>1</sup> Departments of Hepatology.

<sup>2</sup> Histopathology.

<sup>3</sup> Endocrinology.

Postgraduate Institute of Medical Education & Research, Chandigarh, India

## Abbreviations

Nonalcoholic fatty liver disease - NAFLD, nonalcoholic steatohepatitis - NASH, insulin resistance -IR, ursodeoxycholic acid - UDCA, alanine transaminase - ALT, hepatitis B surface antigen -HBsAg, anti hepatitis C virus antibodies - anti HCV, anti-nuclear antibody - ANA, anti-smooth muscle antibody - ASMA, anti-liver kidney microsomal antibody - LKM, anti-mitochondrial antibody - AMA, body mass index -BMI, world health organization - WHO, fasting plasma glucose - FPG, high density lipoprotein -HDL, low density lipoprotein - LDL, triglycerides - TG, tumor necrosis factor alpha - TNF- $\alpha$ , homeostasis model assessment for insulin resistance - HOMA- IR.

Address for correspondence:

Dr. Ajay Duseja MD, DM, MNAMS, FACG  
Department of Hepatology  
PGIMER Chandigarh, India  
Phone-91-172-2756336 Fax-91-172-2744401  
E-mail: ajayduseja@yahoo.co.in

Manuscript received and accepted: 10 and 17 October 2007

## Introduction

Nonalcoholic fatty liver disease (NAFLD) includes patients with simple steatosis and nonalcoholic steatohepatitis (NASH), which can even progress to cirrhosis and hepatocellular carcinoma.<sup>1,2</sup> Pharmacological treatment of patients with NAFLD is still evolving and since insulin resistance (IR) plays a key role in the pathogenesis of nonalcoholic fatty liver disease (NAFLD), insulin sensitizing drugs like metformin may have role in the treatment of patients with NAFLD.<sup>3-9</sup>

Objective of this study was to determine the role of metformin in achieving biochemical response in patients with nonalcoholic fatty liver disease who did not respond to lifestyle interventions and ursodeoxycholic acid (UDCA).

## Patients and methods

### Patients

In a prospective analysis (April 2001 – March 2007), 25 patients with NAFLD who did not have complete biochemical response after 6 months of lifestyle interventions and

UDCA were included in the study after an informed consent. The project had the approval of institute's ethical committee. Life style interventions included moderate sustained exercise like brisk walking, jogging, swimming, cycling etc at least for 30-45 minutes per day, low fat and low calorie diet and slow weight reduction (10% of base line in 6 months, not more than 1.6 Kg/week) in those with overweight and obesity. UDCA was given in a dosage of 300 mg twice daily. Inclusion criteria were adult (> 16 yrs.) nonalcoholic individuals (total abstinence or intake less than 20 g/day, confirmed by two family members) with raised serum alanine aminotransferase (ALT) ( $> 1.5 \times \text{ULN}$  x at least 6 months), ultrasound showing hyperechoic liver or other features of steatosis, negative viral markers (HBsAg, anti HCV - 3rd generation), negative autoimmune markers [anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-liver kidney microsomal antibody (LKM), anti-mitochondrial antibody (AMA)], normal serum ceruloplasmin & absent Keyser Fleisher rings on slit lamp examination, normal iron parameters and a liver biopsy consistent with NAFLD. Pregnant females and patients with diabetes mellitus were excluded from the study.

### Controls

Twenty five patients with NAFLD from the same cohort with comparable age, gender, BMI, waist, baseline ALT, insulin resistance and metabolic syndrome were included as disease controls. These patients were also non responder to lifestyle interventions and UDCA and were continued on only lifestyle interventions without metformin

### Anthropometry

All patients underwent a detailed physical examination including anthropometry. Overweight (BMI  $\geq 23$  but  $< 25 \text{ kg/m}^2$ ), obesity (BMI  $\geq 25 \text{ kg/m}^2$ ) and abnormal waist circumference [ $> 90 \text{ cm}$  (males),  $> 80 \text{ cm}$  (females)] were defined as per the Asia Pacific criteria.<sup>10,11</sup>

### Biochemistry

Diabetes mellitus was defined as per the WHO criteria with fasting plasma glucose (FPG)  $\geq 126 \text{ mg/dL}$  or plasma glucose  $\geq 200 \text{ mg/dL}$  in a symptomatic patient or a 2-hour plasma glucose on glucose tolerance test  $\geq 200 \text{ mg/dL}$ <sup>12</sup> and patients qualifying these criteria were excluded from the study. Lipid profile was taken as abnormal when serum cholesterol was  $> 200 \text{ mg/dL}$ , serum high-density lipoprotein (HDL)  $< 40 \text{ mg/dL}$  in males &  $< 50 \text{ mg/dL}$  in females, serum low-density lipoprotein (LDL)  $> 130 \text{ mg/dL}$  and serum triglycerides were  $> 150 \text{ mg/dL}$ .<sup>13</sup>

In addition to measurement of fasting serum insulin levels (radioimmunoassay), fasting C-peptide (ELISA) and serum TNF- $\alpha$  (ELISA) levels were also determined in these patients.

### Metabolic syndrome

Metabolic syndrome was defined by the presence of at least  $\geq 3$  out of five modified adult treatment panel III criteria including modified abnormal waist as per the Asia Pacific criteria, FPG  $> 110 \text{ mg/dL}$ , hypertension (blood pressure  $\geq 130/85 \text{ mmHg}$  or on anti-hypertensive drugs), serum triglycerides  $> 150 \text{ mg/dL}$ , and serum high density lipoprotein (HDL)  $< 40 \text{ mg/dL}$  (males) &  $< 50 \text{ mg/dL}$  (females).<sup>13</sup>

### Histopathology

Histologically patients were classified into four classes as per Matteoni et al (Class - 1 = Simple steatosis, Class - 2 = Steatosis + lobular inflammation, Class - 3 = + Ballooned hepatocytes, Class - 4 = + Mallory hyaline or fibrosis) and those patients with class 3 or 4 were defined as having NASH.<sup>14</sup> Further patients with NASH were graded and staged according to Brunt et al.<sup>15</sup>

### Insulin resistance

Insulin resistance was determined by homeostasis model assessment for insulin resistance (HOMA-IR) calculated as the product of fasting insulin ( $\mu\text{U/L}$ ) and fasting plasma glucose (mmol/L) divided by 22.5. An absolute value of HOMA-IR  $> 1.64$  was taken as abnormal.<sup>3,16</sup>

### Treatment and follow up

Patients were treated with tablet metformin 500 mg/tid for 6 months in addition to the life style interventions. Disease controls were continued on only lifestyle interventions without metformin. Liver function tests were done monthly and patients were defined as having no response, partial response or complete biochemical response depending on the change in ALT.

### Statistical analysis

All data is expressed in mean  $\pm$  SD until otherwise specified. Students't' test, chi square test and Mann-Whitney U test were used to determine the differences between different groups. A p value of  $< 0.05$  was taken as significant.

## Results

### Patients

There were 25 patients (males 17 mean age  $36.4 \pm 9.2$  years). Other clinical and biochemical details including metabolic syndrome are shown in *Table I*.

**Histopathology**

Details of the histopathology are shown in Table II. Thirteen (52%) patients had class III (n = 5) or class IV (n = 8) disease amounting to histological NASH. Of these 13 patients none had severe inflammation and none had stage 4 fibrosis (cirrhosis).

**Insulin resistance**

Patients with NAFLD had significantly higher HOMA-IR in comparison to the historical healthy controls. (Mean HOMA – IR = 7.6 ± 2.9 vs 1.4 ± 1.2) (p = < 0.01).

**Treatment**

Though BMI did not change significantly in both the groups, in comparison to disease controls (127.5 ± 41.8 vs 118 ± 21.6 p = NS), all patients treated with metformin

had partial biochemical response (mean ALT 122.2 ± 26.8 vs 74.3 ± 4.2 p < 0.01) and 14 (56%) of them achieved complete normalization of ALT and continue to do so on follow up for 3 months (Figure 1). No patient experienced any side effect and there were no dropouts.

**Discussion**

Insulin resistance, which is responsible for increased lipolysis from adipose tissue and hepatic steatosis, is very common in patients with NAFLD.<sup>3,17</sup> Increased fatty acid oxidation, oxidative stress and cytokines may lead on to steatohepatitis and its consequences in some of these patients.<sup>18</sup> All our patients with NAFLD had IR as demonstrated by significantly higher HOMA-IR in comparison to healthy controls. High C-peptide to insulin ratio (Table I) in our patients with NAFLD is indicative of primary insulin resistance in these patients rather than the hyperinsulinemia occurring due to decreased hepatic extraction of insulin due to any liver disease.<sup>1</sup>

First line of treatment in patients with NAFLD is always weight reduction by life style interventions and control of other risk factors. Pharmacological treatment for NAFLD is still evolving and there is no single agent, which is effective in achieving the complete response.<sup>19</sup> Earlier studies did show UDCA to be an effective mode of treatment in these patients<sup>20</sup> but a recent randomized placebo controlled trial showed that UDCA is no better than placebo in the treatment of patients with NASH.<sup>21</sup> The options in patients like ours who do not respond to above measures are limited. Since insulin resistance is the major pathogenetic mechanism, insulin-sensitizing drugs have a role in the treatment.<sup>22-24</sup> Thiazolidinediones group of drugs including troglitazone, rosiglitazone and

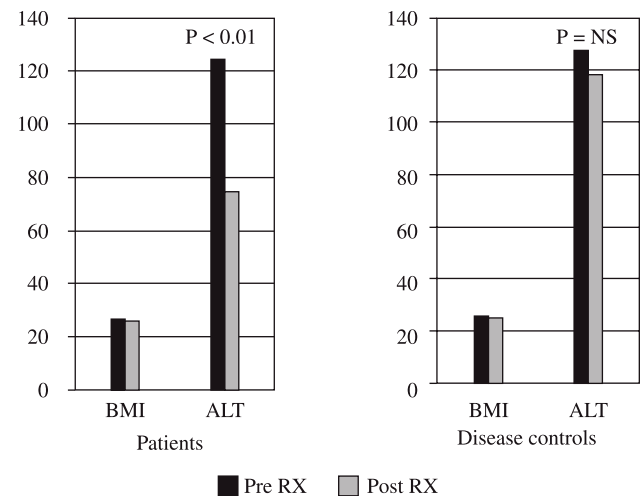
**Table I.** Baseline characteristics of 25 patients with NAFLD.

Parameter	
Males: Females	17 : 8
Mean age (yrs.)	36.4 ± 9.2
Mean BMI (kg/m <sup>2</sup> )	26.8 ± 3.6
Mean waist (cm)	96.8 ± 10.0
Mean ALT (IU/L)	122.2 ± 26.8
C- peptide (pmol/L)	422 ± 1255
Mean insulin (pmol/L)	212.1 ± 164.3
Mean TNF-α (pg/mL)	26.7 ± 17.5
Mean Cholesterol (mg/dL)	192 ± 46
Mean HDL (mg/dL)	37 ± 9.5 mg/dL
Mean LDL (mg/dL)	112 ± 38.5 mg/dL
Mean TG (mg/dL)	202 ± 98.4 mg/dL
Hypertension	2(8%)
Metabolic syndrome	13 (52%)

BMI – Body mass index  
 ALT – Alanine aminotransferase  
 TNF – Tumor necrosis factor  
 HDL – High-density lipoprotein  
 LDL – Low-density lipoprotein  
 TG – Triglycerides

**Table II.** Liver histology in 25 patients with NAFLD.

Class I-	2 (8%)
Class II-	10 (40%)
Class III-	5 (20%)
Class IV-	8 (32%)
NASH (class III+ IV) on histology (n =13)	
Grade- 1	6 (46%)
2	7 (54%)
3	0
Stage- 0	3 (23%)
1	6 (46%)
2	2 (15%)
3	2 (15%)
4	0



**Figure 1.** Shows significant change in ALT in the patients who got metformin (patients) in comparison to those continued on only lifestyle modifications (disease controls). There was no change in BMI in both groups.

pioglitazone have been used in the treatment of NAFLD. Troglitazone has been withdrawn from the market due to its potential hepatotoxicity, which has been described even with rosiglitazone and pioglitazone.<sup>22-24</sup>

Metformin through its anti- TNF and insulin sensitizing actions is an effective drug for achieving biochemical response in patients with NAFLD, and data on its use both experimental<sup>25</sup> and in patients<sup>4-9</sup> with NAFLD is evolving. Most of the studies on metformin are open label studies.<sup>4,8</sup> One randomized trial using metformin found it to be better than vitamin E and lifestyle interventions in improving the liver biochemistry and histological activity including steatosis, inflammation and fibrosis and none of the patients had any side effects.<sup>9</sup> In another study 36 patients with NASH were randomized to lipid and calorie-restricted dietary treatment alone and metformin 850 mg b.d. plus dietary treatment for 6 months. The mean serum alanine/aspartate aminotransferase, insulin and C-peptide levels decreased and the index of insulin resistance improved significantly from baseline in the group given metformin, significantly greater than those in the group given dietary treatment alone. Though not significant, more patients in the metformin group showed improvement in the necro-inflammatory activity, compared with the group given dietary treatment alone.<sup>7</sup>

Though we did not evaluate them for histological improvement, our patients had significant biochemical response in comparison to patients continued only on lifestyle interventions with normalization of ALT in 14 (56%) of them. We also found it to be safe with no patient experiencing any side effect and there were no dropouts.

Our study has certain drawbacks. Number of patients is small and results may change with large number of patients. We evaluated patients only for biochemical response and since ALT may not have good correlation with histology in patients with NAFLD, improvement in ALT in our patients may not signify histological response.

In conclusion, our study shows that insulin resistance is common in patients with NAFLD.

Metformin is an alternative option to achieve biochemical response in patients with NAFLD who do not respond to lifestyle interventions and UDCA. Results need to be reproduced in larger number of patients in a randomized placebo controlled trial with histological data.

## References

- Falck-Ytter Y, Younossi ZM, Marchesini G, Mc Cullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001; 21: 17-26.
- Duseja A, Nanda M, Das A, Das R, Bhansali A, Chawla Y. Prevalence of obesity, diabetes mellitus and hyperlipidemia in patients with cryptogenic liver cirrhosis. *Trop Gastroenterol* 2004; 25: 15-17.
- Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; 35: 373-9.
- Chavez-Tapia NC, Barrientos-Gutierrez T, Tellez-Avila FI, Sanchez-Avila F, Montano-Reyes MA, Uribe M. Insulin sensitizers in treatment of nonalcoholic fatty liver disease: Systematic review. *World J Gastroenterol* 2006 28; 12: 7826-31.
- Duseja A, Murlidharan R, Bhansali A, Sharma S, Das A, Das R, Chawla Y. Assessment of insulin resistance and effect of metformin in nonalcoholic steatohepatitis – a preliminary report. *Indian J Gastroenterol* 2004; 23: 12-5.
- Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in nonalcoholic steatohepatitis. *Lancet* 2001; 358: 893-4.
- Uygun A, Kadayifci A, Isik AT, Ozqurtas T, Deveci S, Tuzun A, Yesilova Z, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2004; 19: 537-44.
- Nair S, Diehl AM, Wiseman M, Farr GH Jr, Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther* 2004; 20: 23-8.
- Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; 100: 1082-90.
- Steering Committee of the Western Pacific region of the World Health Organization. *The International Association for the study of Obesity and the International Study Task Force*. The Asia-Pacific perspective; redefining obesity and its treatment, 8-56. Australia: Health Communications; 2000.
- Dhiman RK, Duseja A, Chawla Y. Asians need different criteria for defining overweight and obesity. *Arch Intern Med* 2005; 165: 1069-1070.
- World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications – Part 1: *Diagnosis and classification of diabetes mellitus*, 31-3. Geneva: World Health Organisation; 1999.
- Expert panel on detection evaluation and treatment of high cholesterol in adults. Executive summary of the third report of the national Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment panel III). *JAMA* 2001; 285: 2486-97.
- Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Non-alcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; 116: 1413-19.
- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander – Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; 94: 2467-74.
- Duseja A, Das A, Das R, Dhiman RK, Chawla Y, Bhansali A, Kalra N. Clinicopathological profile of Indian patients with non-alcoholic fatty liver disease is different from that in the west. *Dig Dis Sci* 2007; 52: 2368-74.
- Duseja A, Das A, Dhiman RK, Chawla YK, Das R, Bhadada S, Sialy R, et al. Indian patients with nonalcoholic fatty liver disease presenting with raised transaminases are different at presentation. *World J Gastroenterol* 2007; 13: 649-50.
- Day C, James O: Steatohepatitis: a tale of two «hits»? (Editorial). *Gastroenterology* 1998; 114: 842-5.
- Tilg H, Kaser A. Treatment strategies in nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol* 2005; 2: 148-55.
- Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, et al. Ursodeoxycholic acid or clofibrate in the treatment of nonalcoholic induced steatohepatitis: A pilot study. *Hepatology* 1996; 23: 1464-67.
- Lindor KD, Kowdley KV, Heathcote EJ, Harrison ME, Jorgensen R, Angulo P, Lymp JF, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 2004; 39: 770-8.

22. Caldwell SH, Hespeneide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL. A pilot study of a thiazolidinedione, Troglitazone in nonalcoholic steatohepatitis. *Am J Gastroenterol* 2001; 96: 519-25.
23. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR -  $\gamma$  Ligand rosiglitazone. *Hepatology* 2003; 38: 1008-17.
24. Sanyal AJ, Mofrad PS, Contos MJ, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, et al. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2004; 2: 1107-15.
25. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin deficient mice. *Nat Med* 2000; 6: 998-1003.