Metformin in Nonalcoholic Steatohepatitis: A Randomized Controlled Trial

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ABSTRACT

BACKGROUND

Nonalcoholic steatohepatitis (NASH) is a common liver disease that can progress to cirrhosis or hepatocellular carcinoma. It is estimated that up to 3% of the Iranian population have this condition. Although the pathogenesis of NASH is incompletely understood, there is significant evidence pointing to the importance of insulin resistance. Metformin is an oral hypoglycemic agent known to improve insulin resistance. This study examines the effectiveness of metformin on biochemical and histological improvement among NASH patients in a randomized double-blind controlled trial.

METHODS

This study enrolled 33 biopsy-proven NASH patients. Other causes of liver disorders were excluded. Subjects were randomized to receive either metformin, 500 mg twice daily, or an identical-looking placebo. Overweight patients were also instructed to lose weight. Treatment continued for 6 months. Patients were regularly visited and liver enzyme levels recorded. Compliance and any adverse drug effects were recorded.

RESULTS

In the metformin group, the mean aspartate aminotransferase (AST) level dropped from 61.2 IU/L to 32.7 IU/L and the mean alanine aminotransferase (ALT) level dropped from 85.1 IU/L to 50.8 IU/L. The mean AST level in the placebo group dropped from 54.3 IU/L to 37.9 IU/L, whereas the mean ALT level dropped from 111.8 IU/L to 55.4 IU/L in the placebo group. The decrease in liver enzymes was significant in both groups, but the magnitude of decrease was not significantly different.

CONCLUSION

The improvement observed in liver enzyme levels is totally attributable to weight loss. Metformin had no significant effect on liver enzyme levels.

KEYWORDS:

Nonalcoholic Steatohepatitis; Metformin; Nonalcoholic Fatty Liver Disease.

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INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is an advanced stage in the spectrum of nonalcoholic fatty liver disease (NAFLD) first described in 1980.[1] The steep rise in the prevalence of NASH and its consequences on liver-related morbidity and mortally have made this condition an area of intense research. NAFLD is currently prevalent among 30% of the population in affluent countries and NASH is diagnosed in 10-25% of those affected by NAFLD.[2-4] It is estimated that in up to 22% of those affected by NASH, the condition leads to cirrhosis.[5, 6] Hepatocellular carcinoma (HCC) is the second most common consequence of NASH.[7] Although HCC and cirrhosis are not as common in NASH as in conditions such as hepatitis B or C, given the rising prevalence of NASH, this condition will be a main future concern in public health.

Several risk factors have been identified for NASH which include obesity, type 2 diabetes mellitus, hypertension and hyperlipidemia, all of which are components of metabolic syndrome.[8] Although weight loss is a definite treatment,[9-12] there is no universally accepted pharmacologic treatment for this condition. The importance of finding a pharmacologic treatment for NASH is better understood because not all individuals affected by NASH are overweight or have treatable risk factors. Moreover, weight loss is not achievable in many patients.

Several studies have been conducted to evaluate the effectiveness of various medications in treating the hepatic manifestations of NASH. Oxidative damage plays an important role in the pathogenesis of NASH.[13-18] Another mechanism that seemingly plays a central role in the pathogenesis of NASH is insulin resistance and disruption in insulin secretion.[19, 20] The results of a study performed by Pagano et al. have shown that insulin sensitivity is lower in NASH patients compared to controls and insulin secretion is higher, which signifies that insulin resistance may be pivotal in the pathogenesis of NASH.[21]

It is estimated that insulin resistance affects up to 45% of the general population.[22] The prevalence of insulin resistance and the availability and relative safety of insulin-sensitizing agents justifies the necessity of studying their effectiveness in treating NASH. Thiazolidinediones have been studied with some success,[23, 24] but results are still inconclusive. Biguanides (i.e., metformin) comprise another option. A study by Lin et al. has shown that metformin, but not caloric restriction, greatly reduced hepatomegaly and hepatic steatosis in mice with associated insulin resistance, but had no significant reduction in fasting serum glucose concentrations.[25]

However, the effectiveness of metformin in treating human cases of NASH and its mechanism of action are still controversial. Marchesini et al. have demonstrated significant improvement in biochemical manifestations of NASH in patients treated with metformin. In their study, histological improvements were not observed and it was not clear whether the improvements were achieved through the insulin-sensitizing action of metformin or through its effect on weight.[26] Lupi et al. have shown metformin's protective effect on human pancreatic B cells against fatty acids and its prevention of a disruption in insulin secretion.[27] Idilman et al. have conducted a randomized controlled trial and concluded that insulin sensitizers lead to improvements in metabolic, biochemical and histological abnormalities of NASH as a result of improved insulin sensitivity.[28] However, a pilot study by Loomba et al. has concluded that metformin lead to improvements in liver histology and ALT levels in 30% of patients with NASH, probably through its effects on weight loss, which was not related to its insulin sensitizing action or its effect on insulin secretion.[29] However, a few reviews question the effectiveness of metformin.[30, 31]

In the current study, we designed a randomized double-blind controlled trial to study the biochemical and histological effects of metformin

MATERIALS AND METHODS

Subjects with increased liver enzymes who referred to a university clinic (Shariati Hospital, Tehran) during 2003 and 2004 were evaluated for enrollment. Liver enzymes were considered to be increased if either alanine aminotransferase (ALT) or aspartate aminotransferase (AST) were more than 1.5 times the upper limit of normal on at least two occasions, at least three months apart. Patients who took medications known to elevate liver enzymes or experimental medications for NASH within the preceding three months were excluded. Viral and autoimmune hepatitis and other causes of liver disorders were excluded. Alcohol use was assessed by questioning the patient and at least two relatives. Patients taking more than 40 grams of alcohol per week were excluded. Other exclusion criteria were: diabetes mellitus, severe medical conditions, pregnancy or intent to become pregnant in the following six months, and not consenting to the study protocol. Liver biopsy was performed for all eligible patients and if results were suggestive of NASH, patients were recruited. NASH was confirmed if over 5% of hepatocytes contained fat droplets and any degree of necroinflammation or fibrosis was noticed.

Subjects were randomized to receive either metformin, 500 mg twice daily, or an identical-looking placebo. Randomization was performed by simple randomization using a computer-generated table. The randomization was implemented using sequentially numbered containers. Both patients and researchers were blinded to the true identity of medications. All patients with BMI greater than 25 kg/m^2 were instructed to lose weight. Treatment was continued for six months. Patients were regularly visited at monthly intervals and liver enzyme levels were recorded. Possible adverse drug effects, lipid profile, and weight changes, as well as compliance were also recorded. Poor compliance was defined as using less than 80% of the study medication. Another liver biopsy was performed after six months of treatment in subjects who agreed.

The liver histology was scored using a modification of the system developed by Brunt et al.[32] Brunt classifies fatty liver as mild, moderate or severe according to the degree of steatosis, hepatocyte ballooning, lobular inflammation and portal inflammation. This system can occasionally be confusing because the four variables may not always be in agreement. To overcome this problem, we modified Brunt's system by scoring the four variables separately. Each variable was graded from zero through three. The sum of these scores was considered as the total pathology grade or NASH activity index (NAI). Fibrosis was staged from zero through four (Table 1).

This modified Brunt system has been validated and proven to have good inter- and intra-observer agreement.[33]

The primary outcome of the study was decrease in liver enzymes. Secondary outcome variables included changes in histologic variables and adverse effects.

Assuming a 50% normalization of liver enzymes by metformin[26], a type one error of 5% and a type two error of 10%, we calculated the sample size to be 16 subjects for each group.

Per-protocol analysis was performed. The chi square test compared ordinal variables between the two treatment groups and the t-test compared continuous variables between groups. Paired-samples t-test compared quantitative variables before and after treatment and the Wilcoxon signed-ranks test compared changes in histology before and after treatment.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board and Ethics Committee of the Digestive Disease Research Center of Tehran University of Medical Sciences.

Table 1: Scoring of histology findings in the liver[33]

Steatosis	1: Mainly macrovesicular, up to 33% of the lobules
	2: 33%–66% commonly mixed steatosis
	3: 66% (panacinar): commonly mixed steatosis
Hepatocyte ballooning	0: None
	1: Occasional, zone 3
	2: Obvious, zone 3
	3: Moderate to marked, predominantly zone 3
Hepatocyte ballooning	0: None
	1: Occasional, zone 3
	2: Obvious, zone 3
	3: Moderate to marked, predominantly zone 3
Portal inflammation	0: None
	1: Mild, some portal areas
	2: Mild to moderate, most portal areas
	3: Moderate to severe, most portal areas
Fibrosis stage	0: None
	1: Zone 3 perivenular, perisinusoidal fibrosis
	2: Stage 1 changes + periportal fibrosis
	3: Bridging fibrosis
	4: Cirrhosis

RESULTS

Thirty-three subjects were included in the study of which 15 were treated by metformin and 18 received placebo. There were 26 male and 7 female subjects. Sex distribution was not significantly different between the metformin and placebo groups. None of the baseline characteristics significantly differed between the two groups (Table 2).

Five subjects withdrew from the study before the final assessment: 1 female and 3 male subjects from the placebo group and 1 male subject from the metformin group. None of the baseline clinical, biochemical, or histological characteristics of the 5 subjects who withdrew significantly differed from the subjects who completed the study.

In the metformin group, 3 subjects complained of diarrhea. Three subjects from the placebo group and 1 subject from the metformin group complained of nausea, with complaints of vomiting in 2 subjects from the placebo group. Abdominal distention was noted by 2 subjects from the metformin group and 1 subject from the metformin group complained of metallic taste. None of these complaints led to discontinuation of study drugs. Finally, among the 28 subjects who completed the study, 5 from the placebo group and 1 from the metformin group had poor compliance. The freguency of adverse effects was not significantly different between the placebo and metformin groups.

Among the 28 subjects who finished the study, 16 lost weight, 3 had no change in weight and the remaining 9 gained weight (Table 3). There was no difference between the two treatment groups. Overall, the BMI decreased (mean change: 0.54) with marginal significance ($p=0.049$), but the decrease within the treatment groups was significant. The decrease in BMI was not significantly different between genders.

Of study subjects, 13 in the metformin and 13 in the placebo group were overweight and received weight loss instructions. The decrease in BMI was not significantly different between overweight subjects who received weight loss instructions and normal weight subjects who received no instructions.

Liver enzymes (AST and ALT) showed significant decreases in both groups (Table 2). AST decreased significantly in both the metformin ($p< 0.001$) and placebo ($p=0.02$) groups, as did the level of ALT ($p<0.001$ for both groups). However, there was no significant difference in the magnitude of decrease in liver enzymes between the two treatment groups. Changes in other parameters were not significant; neither within each of the treatment groups nor between them, and neither within the entire group of subjects.

The coefficient of regressing change in AST on baseline AST ($p=0.006$) and the coefficient of regressing change in ALT on baseline ALT ($p<0.001$) were significantly positive, indicating that subjects with higher initial AST or ALT levels showed greater improvement. Biopsy was conducted on all 33 subjects before treatment and 8 subjects after treatment completion (6 subjects in the metformin and 2 in the placebo groups). Neither the grade of steatosis, fibrosis stage or NAI significantly differed between the two treatment groups, before or after treatment. NAI was significantly lower for

Table 2: Clinical and biochemical parameters before and after six months of treatment

Variable	Placebo (N=18)			Metformin (N=15)			*p* (metformin vs.. placebo)
	Before treatment	After treatment	*p*	Before treatment	After treatment	*p*	
Age, years (range)	41.5 (25-58)			43.5 (26-62)			0.55
Sex (M:F)	15:3			13:2			0.28
BMI (kg/m2)	27.1±2.9	26.8±2.9	0.40	28.6±3.5	27.8±3.5	0.08	0.28
Fasting blood sugar (mg/dL)	93.8±11.2	95.1±12.3	0.47	98.9±10.9	94.0±14.0	0.34	0.25
AST (IU/L; mean± SD)	54.3±26.9	37.9±26.5	0.02	61.2±25.6	32.7±12.3	0.001	0.19
ALT(IU/L; mean± SD)	111.8±68.1	55.4±35.1	0.001	85.1±27.8	50.8±22.9	0.001	0.14
Total cholesterol (mg/dL; mean±SD)	219.1±49.8	195.1±38.2	0.08	194.1±36.2	186.8±34.3	0.45	0.31
LDL (mg/dL; mean ±SD)	118.4±33.5	109.8±30.1	0.37	119.0±39.1	105.5±23.0	0.11	0.70
HDL (mg/dL; mean±SD)	43.4±14.9	42.2±8.5	0.72	42.5±12.3	46.5±6.2	0.08	0.22
Triglycerides (mg/dL; mean±SD)	208.3±80.1	189.7±140.8	0.58	193.5±84.8	159.9±43.6	0.09	0.70

BMI: body mass index; AST: aspartate aminotransferase;
ALT: alanine aminotransferase; LDL: low density lipoprotein;
HDL: high density lipoprotein

Table 3: Weight change among the two treatment groups.

Variables	Weight loss	No change	Weight gain	Total	*p*-value
Placebo	7 (50%)	2 (14%)	5 (36%)	14 (100%)	
Metformin	9 (64%)	1 (7%)	4 (29%)	14 (100%)	0.71
Total	16 (57%)	3 (11%)	9 (32%)	28 (100%)	

all 8 subjects after treatment (*p* = 0.048).

DISCUSSION

Weight loss and decrease in BMI were more prominent in the metformin group, but there was no statistically significant difference in the magnitude of decrease between both groups. The insignificant results in either of the groups might be due to inadequate sample size and the low power of the study.

ALT and AST levels decreased significantly in both treatment groups and in the entire group of subjects, but there was no significant difference in the magnitude of decrease between the two groups. These results again imply that unlike findings reported by Marchesini et al. and Idilman et al.[26, 28] metformin is probably not superior to placebo in decreasing liver enzymes. The observed significant decrease in liver enzymes within both groups may be entirely the result of weight loss.

The insignificant decrease on other biochemical determinants again shows that metformin has no particular effect on these risk factors.

In accord with findings of Marchesini et al.[26] and unlike the report by Idilman et al.,[28] neither steatosis, nor histology stage or grade significantly differed between the two groups, either before or after treatment. Moreover, no decline was observed in steatosis or histology stage post-treatment among the 8 subjects who underwent biopsies after treatment. However, NAI showed significant decreases in all 8 subjects, but the decline was not significant within the treatment groups. These numbers again indicate that insignificant results in either of the groups might be due to inadequate sample size and the low power of the study. With a larger sample size , we could probably detect significant histologic regression which would be attributed to weight loss.

Our results show that higher baseline levels of liver enzymes are associated with more prominent decreases in their levels after treatment. These results suggest the hypothesis that weight loss can be associated with improvements in biochemical profiles, especially in cases that baseline liver enzymes are higher.[9-12] However, since significant weight loss among subjects in our study was not achieved, we could not prove the same association between the improvement in biochemical profile and weight loss.

We were unable to show any associations between weight loss instructions or metformin with actual weight loss or biochemical improvements in NASH patients. The significant improvement in biochemical profile of NASH patients in our study can be solely attributed to weight loss, which is the only common predictor among the subjects. Although one might speculate that the observed improvement might be related to some other unmeasured variable that has changed in all our subjects during the study period, we find this unlikely. Metformin is demonstrated to be marginally associated with weight loss, but not biochemical improvement. However, weight loss and baseline insulin resistance, but not metformin, are marginally associated with biochemical improvements. The summary of these findings is in accord with results reported by Loomba et al.[29] and a few recent reviews,[22, 30, 31] which state that the association of metformin with biochemical improvement, whether significant or not, is probably mediated through weight loss and the apparent role of insulin resistance on the pathogenesis of NASH can probably be manipulated by weight loss, not by metformin.

However, as mentioned earlier, our insignificant results may be due to inadequate sample size and the low power of the study. It should be noted that our study was designed to detect a 50% improvement rate, so an improvement of less than 50% might not be detectable. Further studies with higher sample size, more compliant subjects and longer treatment duration are mandatory.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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