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Original Article

Efficacy of N-Acetylcysteine on Liver Function and Metabolic Profiles in Patients with Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A Double-Blind, Randomized Controlled Trial

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Abstract

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD), previously referred to as non-alcoholic fatty liver disease (NAFLD), is a common liver disorder associated with metabolic abnormalities. This study aimed to evaluate the effectiveness of N-acetylcysteine (NAC) in improving liver function and metabolic profile in patients with MASLD.

Methods: In this randomized controlled trial (RCT), 69 patients with MASLD were randomly assigned to either the NAC group (600 mg, administered three times daily, n = 34) or the placebo group (n = 35) for eight weeks. The severity of hepatic steatosis, liver enzymes, and metabolic profile were measured at baseline and the final trial. Data were analyzed using SPSS.

Findings: Following eight weeks of NAC administration in patients with MASLD, no significant changes were observed compared to the placebo in hepatic steatosis grade ($P = 0.215$), serum aspartate aminotransferase (AST) ($P = 0.21$), alanine transaminase (ALT) ($P = 0.28$), malondialdehyde (MDA) ($P = 0.79$), total antioxidant capacity (TAC) ($P = 0.56$), triglycerides ($P = 0.15$), total cholesterol ($P = 0.28$), low-density lipoprotein cholesterol ($P = 0.32$), and high-density lipoprotein cholesterol ($P = 0.16$). However, NAC administration resulted in significant reductions in fasting blood glucose (FBG) ($P = 0.01$), fasting insulin levels ($P < 0.001$), homeostatic model assessment for insulin resistance (HOMA-IR) ($P < 0.001$), and C-reactive protein (CRP) ($P < 0.001$), along with a significant increase in total glutathione levels ($P = 0.003$), compared to the placebo group.

Conclusion: NAC administration in patients with MASLD does not significantly impact hepatic steatosis, liver enzymes, or lipid profiles; however, it improves oxidative, glycemic, and inflammatory markers. Therefore, NAC may be a beneficial adjunct therapy for managing metabolic parameters and reducing inflammation and oxidative stress in MASLD patients.

Trial Registration: The trial was registered with the Iranian Registry of Clinical Trials (IRCT20201220049772N1) on February 20, 2021.

Keywords: N-acetylcysteine, Fatty liver disease, Hepatic steatosis, Metabolic dysfunction, Metabolic profile, Clinical trial

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD), is defined as fat accumulation in more than 5% of liver cells when other risk factors for fat accumulation, such as alcohol or drugs, have been ruled out.[1] MASLD may lead to a wide range of disorders, ranging from hepatic steatosis to inflammation beyond hepatic steatosis, which is known as non-alcoholic steatohepatitis (NASH),[2] chronic liver disease with various degrees of fibrosis and cirrhosis,[2] to hepatocellular carcinoma.[3] The global prevalence of MASLD is increasing over

time. A recent meta-analysis has reported an incidence of 46.9 cases per 1,000 person-years, suggesting that approximately 32% of the worldwide adult population is affected.[4] In Iran, a meta-analysis estimated the national prevalence of NAFLD to be 33% (95% CI: 27 – 37%), underscoring its significance as a growing public health concern.[5]

Considering the association between the development of MASLD and the increasing prevalence of metabolic syndrome with obesity and type 2 diabetes in recent decades,[6] there is a need for effective interventions and treatment strategies to manage them. Currently, there are limited drug options for the management of MASLD, and the primary focus of treatment has been weight loss and improving insulin resistance through lifestyle modification, such as avoiding alcohol and losing at least 5% of total body weight. Diet, nutrition, and exercise should be followed in conjunction with the usual anti-obesity measures.[7,8] However, for many patients, lifestyle changes can be challenging and require adjunctive drug therapy.

Recent studies have found that administering antioxidant drugs, in conjunction with lifestyle modifications, may help alleviate the symptoms of both MASLD and metabolic syndrome.[9] N-acetylcysteine (NAC), approved by the US Food and Drug Administration (FDA) since 1963 as a standard treatment for acetaminophen overdose and as a mucolytic, has also been used as an adjunct for decades [10,11]. The antioxidant and anti-inflammatory properties of NAC make it a promising treatment option for disorders involving oxidative stress.[10,11]

Previous studies have suggested that NAC could improve liver function in various liver disorders.[12-14] However, the specific effects of NAC on MASLD, particularly in terms of liver enzyme levels, lipid profiles, and comprehensive metabolic markers, have yet to be thoroughly investigated. Previous studies have provided some insights, but often need a more rigorous design and an extensive scope to draw definitive conclusions. This gap highlights the need for a well-designed randomized controlled trial (RCT) to assess NAC's efficacy in MASLD patients, addressing the critical need for more effective pharmacological interventions in managing this increasingly prevalent liver disorder.

This study aimed to evaluate the effects of NAC on serum liver enzyme levels and metabolic profiles in MASLD patients through an RCT. The research uses a comprehensive set of biomarkers to determine if NAC can be a viable pharmacological option for managing MASLD. The study aimed to provide evidence on the therapeutic potential of NAC, address the current lack of effective treatments, and offer insights into its benefits and limitations.

Materials and Methods

Trial Design and Settings

This study was a parallel-group, double-blind, placebo-controlled RCT phase III conducted in 2021 at the Gastroenterology clinic of Shahid Beheshti Hospital in Kashan, Iran. It adhered to CONSORT guidelines and aimed to determine the therapeutic effect of NAC on serum liver enzyme levels and metabolic profiles in patients with MASLD.

Eligibility Criteria

Participants were eligible for inclusion in the study if they were adults aged 18 to 75 years and had a confirmed diagnosis of metabolic dysfunction-associated steatotic liver disease (MASLD) with elevated liver enzyme levels indicative of liver inflammation. Additional criteria included having a stable weight, defined as no significant changes (± 5%) in the last three months, and an HbA1c level of less than 9% for those with diabetes, to ensure adequate diabetes management. Participants needed to be capable of providing informed consent and, for women of childbearing potential, to have a negative pregnancy test and agree to use effective contraception throughout the study. All participants were required to demonstrate willingness to adhere to the study protocol, attend follow-up visits, and abstain from alcohol consumption during the study period.

Diagnosis of MASLD was confirmed through a combination of imaging techniques, including ultrasound, Magnetic Resonance Imaging (MRI), and Fibroscan, which assessed liver fat accumulation and liver stiffness, alongside liver biopsy, which established the presence of liver fat of ≥ 5% to indicate steatosis; this comprehensive approach ensured accurate identification of the disease, as imaging alone can sometimes underestimate the extent of liver fat, while biopsy provides definitive histological evidence necessary for a reliable diagnosis.

Participants were excluded from the study if they met any of the following criteria: a history of alcohol consumption; the presence of coexisting liver diseases, including viral hepatitis, autoimmune hepatitis, or hemochromatosis; a body mass index (BMI) outside the range of 18.5 to 40 kg/m^2; prior or current use of hepatotoxic medications or substances; severe comorbid conditions such as advanced cardiovascular disease, chronic kidney disease, or uncontrolled diabetes; active acute infections; a history of liver transplantation or major liver surgery; pregnant or breastfeeding status; participation in another clinical trial within the past three months; known allergies or contraindications to the investigational drug or its components; and mental health conditions that could impair the ability to provide informed consent or adhere to the study protocol.

Randomization and Blinding

Participants were randomly assigned to either the

intervention or control groups in a 1:1 ratio using a computer-generated randomization sequence. Both participants and investigators were blinded to group assignments. Tablets were coded as 1 and 2 to maintain blinding.

Intervention

Research indicates that NAC is generally safe and well-tolerated at doses between 600 mg and 3000 mg per day [15]. In this study, the intervention group received NAC, administered as three 600 mg tablets daily for eight weeks. The control group received placebo tablets that matched the NAC in color, shape, size, and other physical characteristics, following the same administration protocol as the intervention group.

Education and Follow-Up

Participants were instructed to follow a balanced diet, focusing on whole foods and healthy fats while limiting processed foods, sugars, and saturated fats. They were also asked to maintain their usual physical activity levels. Compliance was tracked through weekly calls and in-person visits, during which empty medication packages were checked. Non-compliant participants were excluded.

Outcome Measures

The measured outcomes included changes in hepatic steatosis severity, liver enzyme levels (aspartate transaminase [AST] and alanine transaminase [ALT]), serum lipid profile (triglycerides [TG], total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]), glycemic markers (fasting blood glucose [FBG], fasting insulin levels, and insulin resistance assessed via the homeostatic model assessment for insulin resistance [HOMA-IR]), oxidative stress markers (glutathione, malondialdehyde [MDA], and total antioxidant capacity [TAC]), and the inflammatory marker C-reactive protein [CRP].

Anthropometric indicators, including weight, height, and BMI, were assessed at baseline and after eight weeks. Weight was measured in a fasting state, without shoes, and with minimal clothing. Height was measured using a tape measure. BMI was calculated by dividing a person's weight in kilograms by the square of their height in meters.

Biochemical Evaluation

Biochemical evaluations included determining lipid profiles, FBG, insulin resistance (HOMA-Index), CRP, glutathione, and MDA levels. These were assessed at baseline and the end of the 8-week intervention period.

Ethical Considerations

This study adhered to the ethical principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments, with approval from the Ethics Committee of Kashan University of Medical Sciences (Ethical code: IR.KAUMS.MEDNT.REC.1399.150). Informed consent was obtained from all participants, and confidentiality was strictly maintained throughout the study. The study was conducted under the supervision of a gastroenterology specialist, ensuring proper oversight during interviews and interventions. Data management protocols ensured participant privacy, and all medical services were provided at no charge. Participants had the right to withdraw at any time. No severe side effects were reported following NAC consumption. The study is registered in the Iranian Registry of Clinical Trials (IRCT20201220049772N1), ensuring transparency and adherence to international research standards.

Statistical Analysis

Data were analyzed using SPSS version 16, with a comprehensive statistical analysis plan. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Quantitative data were expressed as mean and standard deviation (SD) to summarize the central tendency and dispersion of the data. Considering the anticipated effect size and variability, the sample size was calculated to ensure sufficient power to detect a statistically significant difference between the drug and placebo groups. Missing data were handled using appropriate imputation techniques to maintain the integrity of the dataset and minimize bias. An interim analysis was conducted to evaluate the preliminary effects and ensure the study's validity and ethical compliance. ANCOVA was employed to determine the impact of the NAC compared to the placebo on quantitative parameters during the 8-week intervention, adjusting for confounding variables such as age, baseline values of the variable, and BMI at the start of the intervention. A p-value of 0.05 was considered statistically significant.

Results

Study Characteristics

In this RCT, the efficacy of NAC on serum liver enzyme levels and metabolic profiles in patients with MASLD was evaluated over eight weeks. Initially, 74 patients with MASLD who met the inclusion criteria were selected and randomly assigned into two groups, with 37 patients in each group. One group received NAC, while the other group received a placebo. During the trial and follow-up, five patients were excluded due to loss to follow-up and nonadherence to the medication regimen, including two participants from the placebo group and three from the intervention group. Ultimately, 69 participants completed the study, consisting of 34 individuals in the NAC (case) group and 35 in the placebo (control) group, who were included in the final analysis (Figure 1). It is worth noting that patient compliance with the medication was high, with more than 90% of the tablets being consumed during the study.

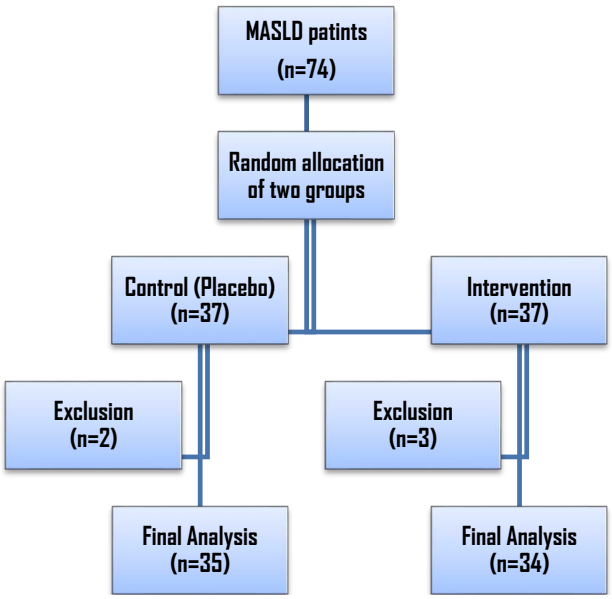

Figure 1. Flowchart of patient allocation and analysis

Patients' Characteristics

Table 1 presents the demographic characteristics of the patients. Gender distribution was not significantly different between the groups, with males comprising 51.4% of the placebo group and 68.8% of the NAC group ($P=0.38$). Females comprised 48.6% of the placebo group and 38.2% of the NAC group ($P=0.38$).

The participants' ages were similar between the groups, with a mean age of 53.12 ± 5.5 years in the placebo group and 55.12 ± 8.6 years in the NAC group ($P=0.45$). Height was also comparable, with the placebo group averaging 161.7 ± 2.6 cm and the NAC group averaging 161.8 ± 8.3 cm ($P=0.80$).

Weight and BMI measurements were consistent across both groups at baseline and the end of the study. The placebo group had a baseline weight of 73.1 ± 1 kg and an end weight of 73.9 ± 0.9 kg, while the NAC group had a baseline weight of 72.12 ± 9.7 kg and an end weight of 72.12 ± 4.6 kg. The *P* values for weight differences at baseline and the end were 0.97 and 0.83, respectively. Similarly, BMI at baseline was 27.3 ± 8.8 kg/m² for the placebo group and 28.3 ± 1.2 kg/m² for the NAC group (p = 0.76), with end values of 27.3 ± 6.8 kg/m² and 28.3 ± 0.1 kg/m², respectively ($P=0.60$).

Overall, there were no significant differences in gender distribution, age, height, weight, or BMI between the placebo and NAC groups, indicating that the groups were well-matched in terms of these baseline characteristics.

Severity of Hepatic Steatosis

Both the NAC and placebo groups showed improvements in hepatic steatosis over the 8-week trial. In the NAC group, the proportion of Grade II declined from 26.5% to 12%, and that of Grade 0 increased from 0% to 32%. In the placebo group, the prevalence of Grade II hepatic steatosis decreased from 35% to 23%, while that of Grade 0 increased from 0% to 23%. Within-group improvements were statistically significant ($P<0.001$), but the between-group difference was not significant ($P=0.215$). While NAC may have led to greater improvements, its effect was not significantly different from that of the placebo group (Table 2).

Table 1. Demographic characteristics of patients with MALSD (n = 69)

		NAC (n = 34)	Placebo (n = 35)	*P*-value
Gender	Male	21 (68.8%)	18 (51.4%)	0.38
	Female	13 (38.2%)	17 (48.6%)	0.38
Age (year)		55.12 ± 8.6	53.12 ± 5.5	0.45
Height (cm)		161.8 ± 8.3	161.7 ± 2.6	0.80
Weight (kg)	Baseline	72.12 ± 9.7	73.10 ± 1.0	0.97
	Final	72.12 ± 4.6	73.9 ± 0.9	0.83
BMI (kg/m²)	Baseline	28.3 ± 1.2	27.3 ± 8.8	0.76
	Final	28.3 ± 0.1	27.3 ± 6.8	0.60

Standard differences ± SD; BMI: Body mass index.

Liver Enzymes

Statistical analysis revealed a decreasing trend in the liver enzyme levels of both groups, as indicated by AST and ALT. In the placebo group, AST levels decreased from 51.3 ± 13.0 to 48.0 ± 11.1 mg/dL ($P=0.21$), and ALT levels decreased from 54.5 ± 13.9 to 53.6 ± 12.8 mg/dL ($P=0.28$). In the NAC group, AST levels decreased from 42.4 ± 10.2 to 40.1 ± 9.3 mg/dL ($P=0.21$), and ALT levels decreased from 60.1 ± 19.5 to 54.8 ± 14.4 mg/dL ($P=0.28$) (Table 3).

Glycemic Indices

Statistical analysis revealed significant improvements in glycemic indices in the NAC group. FBG levels decreased from 105.7 ± 11.9 to 98.7 ± 10.8 mg/dL in the NAC group ($P=0.01$), while the placebo group showed an increase from 99.0 ± 9.9 to 101.7 ± 12.4 mg/dL. Fasting insulin levels significantly decreased in the NAC group from 18.8 ± 4.8 to 13.3 ± 6.4 µIU/mL ($P<0.001$), whereas the placebo group showed no significant change. Additionally, insulin resistance, as measured by HOMA-IR, decreased from 4.9 ± 1.4 to 3.2 ± 1.7 in the NAC group ($P<0.001$), whereas it remained relatively unchanged in the placebo group (Table 3).

Lipid Profile

Statistical analysis showed that changes in serum lipid profile parameters were not statistically significant. TG levels decreased from 182.2 ± 59.8 to 162.6 ± 41.9 mg/dL in the NAC group ($P=0.15$) and from 183.2 ± 63.0 to 176.0 ± 57.3 mg/dL in the placebo group. TC, LDL-C, and HDL-C levels showed similar non-significant trends in both groups (Table 3).

Table 2. Changes in the grade of hepatic steatosis in the NAC and placebo groups at baseline and final (0 vs. 8th week) in patients with MALSD

Groups		Grade 0	Grade I	Grade II	*P* value [1]	*P* value [2]
Placebo (n = 35)	Baseline	0	23 (65%)	12 (35%)	<0.001*	0.215
	Final	8 (23%)	19 (54%)	8 (23%)		
NAC (n = 34)	Baseline	0	25 (73.5%)	9 (26.5%)	<0.001*	
	Final	11 (32%)	19 (56%)	4 (12%)		

P value[1]: Statistical analysis by Wilcoxon signed-rank test; *P* value[2]: Mann–Whitney U test.

Table 3. Changes in the laboratory metabolic indices in the NAC and placebo groups at baseline and final (0 vs. 8th week) in patients with MALSD

Laboratory panels	Markers	Placebo group (n = 35)		NAC group (n = 34)		*P* value[1]
		Baseline	Final	Baseline	Final	
Liver enzymes	AST (mg/dL)	51.3 ± 13.0	48.0 ± 11.1	42.4 ± 10.2	40.1 ± 9.3	0.21
	ALT (mg/dL)	54.5 ± 13.9	53.6 ± 12.8	60.1 ± 19.5	54.8 ± 14.4	0.28
Glycemic Indices	FBG (mg/dL)	99.0 ± 9.9	101.7 ± 12.4	105.7 ± 11.9	98.7 ± 10.8	**0.01***
	FIL (μIU/ml)	18.0 ± 7.4	17.3 ± 7.7	18.8 ± 4.8	13.3 ± 6.4	**<0.001***
	HOMA-IR	4.4 ± 1.8	4.3 ± 1.9	4.9 ± 1.4	3.2 ± 1.7	**<0.001***
Lipid profile	TG (mg/dL)	183.2 ± 63.0	176.0 ± 57.3	182.2 ± 59.8	162.6 ± 41.9	0.15
	TC (mg/dL)	174.6 ± 40.5	170.4 ± 50.4	162.8 ± 28.5	153.2 ± 24.4	0.28
	LDL (mg/dL)	94.0 ± 42.3	89.1 ± 51.1	84.3 ± 31.2	74.6 ± 23.6	0.32
	HDL (mg/dL)	43.9 ± 6.5	46.1 ± 5.8	41.9 ± 6.1	46.0 ± 5.9	0.16
Oxidative stress	MDA (mmol/L)	2.7 ± 0.9	2.4 ± 0.8	2.5 ± 0.4	2.3 ± 0.4	0.79
	Total Glutathione (mmol/L)	560.4 ± 164.9	545.1 ± 176.1	552.1 ± 44.8	641.9 ± 145.2	**0.003***
	TAC (mmol/L)	1251.7 ± 299.9	1182 ± 282.1	1138.7 ± 192.8	1135.1 ± 169.3	0.56
Inflammatory marker	CRP (mg/L)	7.2 ± 1.1	7.6 ± 0.9	7.1 ± 1.0	6.3 ± 1.1	**<0.001***

AST: Aspartate Aminotransferase; ALT: Alanine Transaminase; FBG: Fasting blood glucose; FIL: Fasting insulin levels; TG: Triglycerides; TC: Total Cholesterol; LDL: Low-density Lipoprotein; HDL: High-density Lipoprotein; MDA: Malondialdehyde; TAC: Total Antioxidant Capacity; CRP: C-reactive protein.
Data are expressed as mean ± standard deviation.
[1]Analyzed by ANCOVA (Analysis of Covariance) test.

Oxidative Stress and Inflammatory Marker

Statistical analysis showed a significant increase in total glutathione levels in the NAC group, rising from 552.1 ± 44.8 to 641.9 ± 145.2 mmol/L ($P = 0.003$). In contrast, the placebo group showed a decrease from 560.4 ± 164.9 to 545.1 ± 176.1 mmol/L. Serum MDA levels and TAC showed no significant changes in either group. CRP, an inflammatory marker, showed a significant decrease in the NAC group from 7.1 ± 1.0 to 6.3 ± 1.1 mg/L ($P < 0.001$), while the placebo group showed an increase from 7.2 ± 1.1 to 7.6 ± 0.9 mg/L (Table 3).

Discussion

Aim and Main Findings

This study aimed to evaluate the efficacy of NAC administration on liver function and metabolic profiles in patients with MASLD. The findings suggest that an 8-week administration of NAC in patients with MASLD did not lead to significant changes in the severity of steatosis, liver enzymes (AST, ALT), or lipid profiles (TG, TC, LDL-C, HDL-C), compared to placebo. However, improvements were observed in glycemic indices (FBG, insulin, HOMA-IR) and CRP levels, indicating potential benefits in glucose metabolism and inflammation. Additionally, while total glutathione levels increased significantly, reductions in MDA and TAC were observed but did not reach statistical significance, though they approached a meaningful threshold. These results highlight NAC's potential role in modulating oxidative stress and inflammation in MASLD, warranting further investigation with larger sample sizes and more extended intervention periods.

Potential Mechanism

NAC primarily exerts its metabolic effects through its role as a precursor to glutathione, a crucial antioxidant that mitigates oxidative stress, a key contributor to metabolic dysfunction.[16] By replenishing glutathione levels and scavenging reactive oxygen species (ROS), NAC helps protect pancreatic β-cells, enhances insulin signaling, and reduces endoplasmic reticulum stress, leading to improved glycemic control and insulin sensitivity.[17,18] Additionally, NAC inhibits the activation of nuclear factor-kappa B (NF-κB), a regulator of pro-inflammatory cytokines, thereby reducing systemic inflammation and CRP levels.[19,20] These effects reduce oxidative and inflammatory stress, thereby preventing further metabolic deterioration; however, they do not directly alter lipid metabolism, which explains why NAC does not impact lipid profiles.

NAC also reduces lipid peroxidation and enhances cellular resilience by replenishing intracellular glutathione, which neutralizes ROS and reactive nitrogen species (RNS) that initiate lipid peroxidation.[21,22] This antioxidant action prevents the oxidation of polyunsaturated fatty acids in cellular membranes, resulting in a reduction in MDA, a marker of lipid peroxidation.[22,23] However, despite its ability to reduce oxidative stress, NAC does not directly affect hepatic lipid accumulation or lipogenesis pathways, such as fatty acid synthesis or triglyceride formation. Therefore, while NAC reduces oxidative damage and lipid peroxidation, it does not influence liver fat deposition or liver function markers, highlighting its protective role against oxidative damage rather than modulating lipid metabolism.

NAC on Liver Function

The study's main findings suggest that NAC administration led to significant improvements in hepatic steatosis in patients with MALSD over eight weeks. While both the NAC and placebo groups showed reductions in hepatic steatosis severity, the NAC group exhibited a greater improvement. However, the difference between the two groups was not statistically significant.

This study suggested that NAC administration improved hepatic steatosis in MALSD patients over eight weeks, with greater reductions in severity compared to the placebo group. However, the between-group difference was not statistically significant. These findings align with a systematic review and meta-analysis by Nikbaf-Shandiz et al,[13] which also reported no significant impact of NAC on liver enzymes across eight RCTs. However, preclinical studies have demonstrated more promising effects of NAC, including improved systemic and hepatic lipid metabolism, reduced inflammation-induced liver damage, and enhanced glucose tolerance in NAFLD-induced mice, likely due to its role in restoring hepatic glutathione and glutathione reductase levels.[24] Additionally, studies in animal models, such as those with carbon tetrachloride (CCl4)-induced fatty liver disease, have shown that NAC significantly reduces microscopic steatosis.[25] Contrary to our findings, some human studies have reported beneficial effects of NAC on liver enzymes. For instance, a recent study conducted by Babu Balagopal et al[26] has reported reductions in liver enzyme levels in 13 patients with NAFLD after 16 weeks of NAC treatment. Similarly, Khoshbaten et al[14] found that 12 weeks of NAC treatment at 1200 mg/day improved liver enzyme levels in 30 patients, while Pamuk et al[27] reported significant reductions in ALT and AST levels after four weeks of NAC therapy in 34 individuals. These discrepancies underscore the need for further research to elucidate the potential therapeutic role of NAC in MASLD and NAFLD, particularly in considering variations in dosage, treatment duration, and patient characteristics.

NAC on Glycemic Markers

Our analysis indicated improvement of glycemic indices, including a significant reduction in FBG, fasting insulin, and HOMA-IR levels in individuals following treatment with NAC. Consistent with our findings, Balagopal et al[26] implemented a pilot study demonstrating improved and decreased insulin resistance. These findings are supported by Rani et al,[28] who conducted a study on 35 individuals administering 1200 mg NAC, which revealed a significant reduction in FBG, FPI, and insulin resistance over six weeks. In the study of Fulghesu et al,[29] the daily administration of 1800 mg of NAC in patients with polycystic ovary syndrome led to a significant increase in insulin sensitivity and a significant decrease in insulin resistance, while it did not affect the lipid profile. The effect of NAC on glucose metabolism involves the regulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) genes, inhibition of enhancer binding protein beta, and modification of the PI3K/Akt insulin signaling pathway.[29]

NAC on Lipid Profile

Our study found that NAC did not significantly affect lipid parameters, with TG, TC, LDL-C, and HDL-C levels showing no notable changes between the NAC and placebo groups, consistent with previous studies.[27] However, some research, such as that by de Oliveira et al[30] reported that NAC has metabolic benefits, including reduced insulin resistance and increased HDL-C levels. While NAC has been suggested to inhibit lipid accumulation in liver cells,[31] its lack of effect on lipid profiles in our study may be due to the short treatment duration. This could be attributed to the complex regulation of lipid metabolism, which involves multiple pathways that may require prolonged intervention to show measurable changes.

NAC can increase the sensitivity of insulin receptors and decrease insulin resistance by increasing the expression of the PPAR-γ gene.[32] It may also have beneficial effects on glucose homeostasis parameters and the lipid profile by activating the insulin signaling pathway through AMPK/protein kinase, which increases cellular GLUT-4 and improves glucose uptake.[33]

NAC on Oxidative Stress and Inflammatory Marker

Our study found that NAC treatment significantly increased glutathione levels and reduced CRP levels, indicating its potential antioxidant and anti-inflammatory effects. However, there was no significant change in MDA levels or TAC. These findings align with previous studies, which reported a significant reduction in CRP levels following NAC administration.[26,34] Additionally, Jeremias et al[35] found that a daily dose of 1800 mg of NAC for four weeks in patients with type 2 diabetes led to a notable decrease in CRP levels. Similarly, Sayed et al[36] demonstrated that injectable NAC provided anti-

inflammatory benefits in cirrhotic patients undergoing liver resection. In contrast, Jeremias et al[35] reported no significant change in glutathione levels after four weeks of NAC treatment, suggesting that the effects of NAC may vary based on dosage, treatment duration, and patient population. The observed reduction in CRP levels may be attributed to NAC's ability to modulate oxidative stress and inflammatory pathways, potentially through its role in replenishing intracellular glutathione and regulating pro-inflammatory cytokine activity.[20]

Strengths and Limitations

This study's strengths include its double-blind, randomized controlled design, which reduces bias and enhances the reliability of results. The inclusion of well-defined MASLD patients and a standardized NAC dosing regimen provides a strong foundation for evaluating its effects on metabolic and inflammatory markers. Additionally, the assessment of multiple biochemical parameters, such as liver enzymes, glycemic markers, and oxidative stress indicators, offers a comprehensive insight into NAC's potential benefits. However, some limitations should be acknowledged. The relatively small sample size may limit the generalizability of the findings, and the short intervention period may not have been sufficient to capture long-term effects. Additionally, financial constraints prevented the measurement of key inflammatory biomarkers, such as interleukin-1, interleukin-6, and TNF-alpha, which could have provided further insights into NAC's anti-inflammatory mechanisms. Future studies with larger cohorts, longer treatment durations, and broader biomarker analysis are needed to confirm and expand upon these findings.

Conclusion

This RCT suggests that NAC may offer metabolic and anti-inflammatory benefits in patients with MASLD despite showing no significant effects on liver enzyme levels. NAC treatment resulted in improvements in glycemic markers and oxidative stress, as indicated by increased glutathione levels and decreased CRP levels. However, NAC did not significantly affect lipid profiles, liver enzymes, or the severity of hepatic steatosis. While the study results highlight NAC's potential as an adjunct therapy for metabolic regulation and inflammation control in MASLD, further research with higher doses and longer treatment durations is needed to fully understand its therapeutic role in liver health.

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Authors' Contribution

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Competing Interests

The authors declare that they have no competing interests.

Consent for Publication

All patients and the authors consented to the publication of this manuscript.

Data Availability Statement

Data are provided within the manuscript.

Ethical Approval

This study adheres to CONSORT guidelines. This study was conducted in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments, or with comparable ethical standards. This study was approved by the *Ethics Committee of Shahid Beheshti Hospital*, Kashan, Iran (Ethical code: IR.KAUMS.MEDNT.REC.1399.150, Ethical approval date: 20/12/2020).

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