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Misoprostol for non-alcoholic steatohepatitis: a randomised control trial

Mehreen Siyal ,[1] Zaigham Abbas,[1] Muhammad Ali Qadeer,[1] Alina Saeed,[1] Usman Ali,[2] Ambrina Khatoon[3]

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[1]Department of Gastroenterology and Hepatology, Dr Ziauddin Hospital, Clifton Campus, Karachi, Pakistan
[2]Department of Laboratory, Dr Ziauddin Hospital, Clifton Campus, Karachi, Pakistan
[3]Department of Molecular Medicine, Dr Ziauddin Hospital, Clifton Campus, Karachi, Pakistan

Correspondence to
Dr Mehreen Siyal;
drmehreensiyal@gmail.com

ABSTRACT

Introduction The management of non-alcoholic steatohepatitis (NASH) is an unmet clinical need. Misoprostol, a structural analogue of naturally occurring prostaglandin E1, has been reported to decrease proinflammatory cytokine production and may have a potential role in treating NASH. We aimed to evaluate the efficacy and safety of misoprostol in treating patients with NASH.

Methods In this phase 2, double-blind, randomised, placebo-controlled trial, patients with NASH were randomly assigned in a 1:1 ratio to receive 200 µg of misoprostol or placebo thrice daily for 2 months. The primary endpoint was an improvement in liver function tests (LFTs), interleukin-6 (IL-6) and endotoxin levels. The secondary endpoint was improvement in insulin resistance, dyslipidaemia, hepatic fibrosis and hepatic steatosis.

Results A total of 50 patients underwent randomisation, of whom 44 (88%) were males. The age range was 25–64 years (mean±SE of mean (SEM) 38.1±1.4). 19 (38%) patients had concomitant type 2 diabetes mellitus. 32 (64%) patients were either overweight or obese. At the end of 2 months' treatment, a reduction in total leucocyte count (TLC) (p=0.005), alanine aminotransferase (ALT) (p<0.001), aspartate aminotransferase (AST) (p=0.002) and controlled attenuation parameter (CAP) (p=0.003) was observed in the misoprostol group, whereas placebo ensued a decline in ALT (p<0.001), AST (p=0.018), gamma-glutamyl transferase (GGT) (p=0.003), CAP (p=0.010) and triglycerides (p=0.048). There was no diminution in insulin resistance, hepatic fibrosis (elastography) and dyslipidaemia in both groups. However, misoprostol resulted in a significant reduction in CAP as compared with the placebo group (p=0.039). Moreover, in the misoprostol group, pretreatment and post-treatment IL-6 and endotoxin levels remained stable, while in the placebo group, an increase in the IL-6 levels was noted (p=0.049). Six (12%) patients had at least one adverse event in the misoprostol group, as did five (10%) in the placebo group. The most common adverse event in the misoprostol group was diarrhoea. No life-threatening events or treatment-related deaths occurred in each group.

Conclusion Improvement in the biochemical profile was seen in both misoprostol and placebo groups without any statistically significant difference. However, there was more improvement in steatosis, as depicted by CAP, in the misoprostol group and worsening of IL-6 levels in the placebo group.

Trial registration number NCT05804305.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ NASH is a complex disease that is modulated by numerous mechanisms including metabolic, genetic, environmental and gut microbial factors. It is one of the leading causes of chronic liver disease worldwide.

⇒ Recent studies suggest that leaky gut syndrome can lead to portal translocation of microbial products, which leads to a cascade of events, ultimately resulting in liver fibrosis, cirrhosis and even hepatocellular carcinoma.

WHAT THIS STUDY ADDS

⇒ Evaluation of the efficacy of misoprostol in treating patients with NASH.

⇒ Evaluation of the safety of misoprostol in treating patients with NASH.

⇒ Evaluation of the role of misoprostol in targeting leaky gut syndrome and reducing inflammation in patients with NASH.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further studies are warranted to ascertain the efficacy and tolerability of misoprostol for treating patients with NASH by targeting leaky gut syndrome on a histological and molecular level.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), which is categorised into non-alcoholic fatty liver (NAFL) and NASH, is one of the leading causes of chronic liver disease worldwide. It is characterised by the presence of hepatic steatosis, detected either by imaging or histology, and a lack of secondary causes of hepatic fat accumulation such as excessive alcohol consumption, prolonged use of steatogenic medications and monogenic hereditary disorders.[1] Metabolic comorbidities associated with NAFLD include obesity, insulin resistance (IR), type 2 diabetes, dyslipidaemia and metabolic syndrome.[2] With the increasing global burden of obesity, the prevalence of NAFLD has also increased to 25.24%[3–6] and now it is the third most common cause of hepatocellular carcinoma

(HCC) and the second most common cause of liver transplantation.[1]

NASH is a complex disease that is modulated by numerous mechanisms including metabolic, genetic, environmental and gut microbial factors.[7] Over the past few years, gut-liver axis involvement in the pathogenesis of NAFLD has been emphasised. Recent studies suggest that leaky gut syndrome can lead to portal translocation of microbial products, which leads to a cascade of events, ultimately resulting in liver fibrosis, cirrhosis and even HCC.[8 9] Lipopolysaccharide (LPS) and proinflammatory markers are elevated in patients with NASH.[10 11]

Misoprostol, a synthetic prostaglandin E1 analogue is found to modulate LPS-induced cytokine metabolism, leading to a reduction of proinflammatory cytokines while simultaneously increasing anti-inflammatory ones, thus showing potential therapeutic implications for treating patients with NASH.[12]

This study aims to evaluate the role of misoprostol in targeting leaky gut syndrome and reducing inflammation in patients with NASH.

MATERIALS AND METHODS

This was a single-centre prospective double-blind randomised control trial conducted at Dr. Ziauddin University Hospital, Clifton, Karachi Pakistan, from 1 July 2022 to 31 December 2022. The study was registered on ClinicalTrails.gov having an ID: NCT05804305. 50 patients of NASH, visiting our outpatient department were randomised to receive either tablet misoprostol 600 µg per day in three divided doses or placebo for 2 months. The randomisation numbers were generated online by a random number generator. The placebo was an inactive compound, having no intrinsic therapeutic properties. Both the placebo and the drug were provided to the investigator by the sponsoring pharmaceutical company in white boxes, with no intention of promoting the drug. Only randomised numbers were mentioned on the white boxes. Neither the investigator, nor the patients, were aware of the group of enrolment. The groups were revealed by the pharmaceutical company at the time of data analysis. Allocation concealment was ensured. Compliance of the patients was ensured by contacting them over the phone fortnightly. The patients were called for a formal outpatient visit after 1 and 2 months of initiating treatment (figure 1). LFTs were repeated at the week 4 visit, whereas all relevant investigations including transient elastography were repeated at the end of 2 months of treatment. Written informed consent was taken from all the study participants.

Inclusion criteria were male and female patients between 18 and 80 years of age, having confirmed NAFLD as evident by a radiologic test like ultrasound, elastography (FibroTouch, Wuxi Hisky Medical Technologies, China) or CT scan, ALT level of 1.5 times the upper limit of normal, and if already known case of NAFLD then patient should be on stable doses of Vitamin E, oral hypoglycaemic or antilipidemic drugs, with no change in medication doses during 6 months before recruitment.

Exclusion criteria were patients with age less than 18 years or more than 80 years, women of childbearing age, clinically significant acute or chronic liver disease unrelated to NAFLD, evidence of hepatitis B and C, evidence of primary biliary cirrhosis, primary sclerosing cholangitis, or biliary obstruction, autoimmune hepatitis, drug-induced steatohepatitis (ingestion of drugs known to produce hepatic steatosis including corticosteroids, high-dose oestrogens, methotrexate, tetracycline or

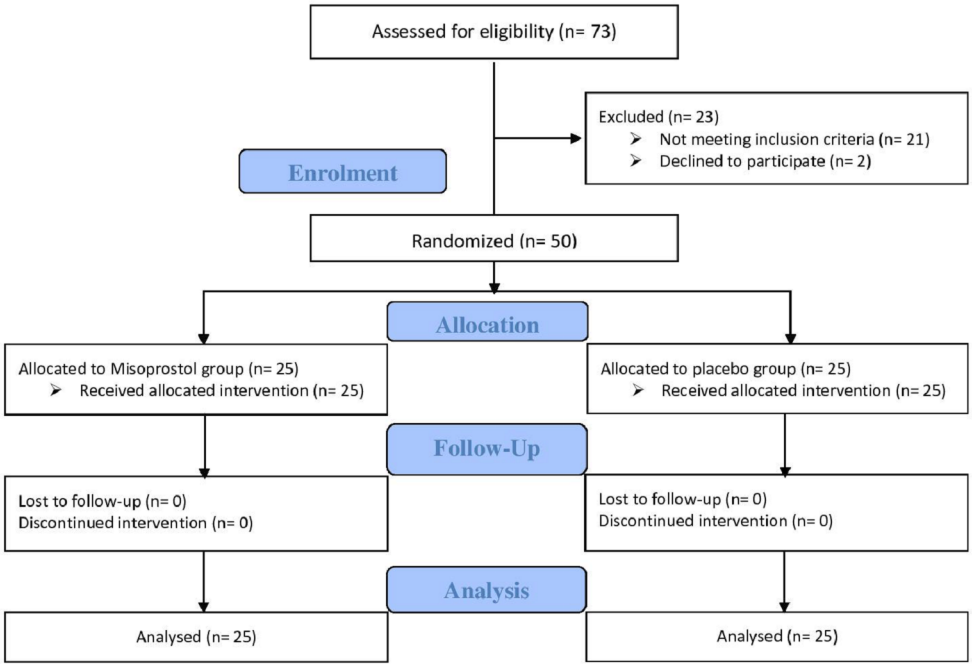

Figure 1 Consolidated Standards of Reporting Trials flow diagram of study patients.

amiodarone in the previous 6 months), any cardiovascular event or evidence of active cardiovascular disease, type 1 diabetes, those consuming alcohol of over 20 g/day for males and 10 g/day for females, severe end-organ damage, HIV infection, compensated and decompensated cirrhosis, patients with uncontrolled diabetes and mental instability or incompetence, pregnant females and patients who did not give written informed consent for getting recruited into the study.

Blood specimens for measurement of IL-6 and endotoxin were collected in serum separating tubes. The serum was separated from primary tubes by centrifuging for 10 min at 3000 g, was transferred to aliquots and stored at minus 70°C until testing. IL-6 was analysed by the electrochemiluminescence method on an automated Roche cobas immunoassay analyser, and the concentration of endotoxin was analysed by an ELISA kit: thermofisher scientific pierce chromogenic endotoxin Quant kit, catalogue No. A39553, with appropriate quality controls.

The primary endpoint of the study was to determine the change from baseline in LFTs, IL-6 and endotoxin levels in both groups. The secondary endpoints were the change from baseline in hepatic fibrosis measured in kilopascals, hepatic steatosis measured through CAP, dyslipidaemia by doing fasting lipid profile, IR by calculating homoeostasis model assessment-estimated insulin resistance (HOMA-IR). Adverse events were evaluated in both groups (online supplemental file 2). All the study patients were instructed for the life style modifications and better control of diabetes and lipid levels. An interim analysis was planned after recruiting 50 patients to make a decision about the continuation of the study.

Statistical analysis

Paired sample t-test was used to compare and appraise the improvement in LFTs, IL-6, endotoxin levels, hepatic steatosis, hepatic fibrosis, dyslipidaemia and IR from baseline. A p<0.05 was considered significant. IBM Statistical Package for Social Sciences (SPSS) V.23 was used for data analysis.

RESULTS

A total of 50 patients of NASH, as evidenced by a radiologic test such as ultrasound/FibroScan/CT scan were recruited in this study. 44 (88%) patients were males. There were three female patients in each group. The age range was 25–64 years (mean±SD: 38.08±9.64). 19 (38%) patients were diabetic, 10 (20%) had concomitant hypertension and 5 (10%) had a history of stable ischaemic heart disease with preserved ejection fraction. 34 (68%) patients were either overweight or obese.

At 1 month of treatment, in the misoprostol group, there was an improvement in total bilirubin from a mean of 0.83 to 0.72 mg/dL (p=0.009), indirect bilirubin from a mean of 0.56 to 0.47 (p=0.03), ALT from a mean of 89 to 67 U/L (p<0.001); whereas no improvement was observed in direct bilirubin (p=0.417), AST (p=0.065), ALP (0.467) and GGT (p=0.878). In the placebo group, there was an improvement in ALT from a mean of 92 to 69 U/L (p p<0.001), AST from a mean of 55 to 45 U/L (p=0.008), GGT from a mean of 70 to 56 U/L (p=0.011); whereas no improvement was observed in total bilirubin (p=0.623), direct bilirubin (p=0.384), indirect bilirubin (p=0.377) and ALP (p=0.297).

At the end of 2 months of treatment, in the misoprostol group, there was a reduction in TLC (p=0.005), ALT (<0.001), AST (0.002) and CAP (0.003); whereas in the placebo group, there was a reduction in ALT (p<0.001), AST (p=0.018), GGT (p=0.003), CAP (p=0.010) and triglycerides (p=0.048). However, misoprostol resulted in a significant reduction in CAP as compared with the placebo group (p=0.039) (online supplemental file 1).

DISCUSSION

NASH is a complex disease, modulated by various mechanisms including metabolic, genetic, environmental and gut microbial factors.[7] Treating NASH is imperative because approximately 20% or more of patients with NASH develop cirrhosis during their lifetime. In contrast, NAFL is believed to have a much more benign course with an estimated risk of progression to cirrhosis of less than 4%.[13]

The high clinical and economic burden of NASH strongly indicates a need for effective treatment options.[14] There are currently no approved pharmacological therapies to treat NASH. Various treatment options are available for decreasing steatohepatitis, thus leading to an improvement in LFTs including vitamin E, metformin, pioglitazone, etc. The results of these treatment options are often unsatisfactory, warranting the need for effectual treatment for improving the liver-related parameters in patients with NASH.

The role of misoprostol in reducing the proinflammatory cytokines while simultaneously mounting the anti-inflammatory ones has been proposed by previous studies conducted on animals with promising results.[12 15] Salam *et al* suggested hepatoprotective effects of misoprostol in carbon tetrachloride-induced liver damage in rats.[16] This randomised control trial is the first study conducted on humans for gauging the effect of misoprostol on the leaky gut in patients of NASH. In our study, we used the standard cytoprotective dose of misoprostol. Pretreatment and post-treatment IL-6 and endotoxin levels remained stable in the misoprostol group while in the placebo group, an increase in the IL-6 levels was noted (p=0.049).

The majority (88%) of our patients were males. The reason for this assortment is the fact that the use of misoprostol during pregnancy may incur miscarriages, premature labour or birth defects.[17] Six females recruited in this study were either postmenopausal or had completed their families.

IR in both liver and adipose tissue is believed to be a key driver in the pathogenesis of NASH.[18] All of our patients had IR, as evident by the elevated HOMA-IR.

This parameter was neither improved in the misoprostol group nor the placebo group.

A recent study has suggested hepatoprotective effects of misoprostol on doxorubicin-induced liver injury in rats by increasing HDL cholesterol levels, whereas considerably decreasing serum ALT, AST, LDL cholesterol, triglycerides and total cholesterol levels in serum.[15] In our study, there was an improvement in some parameters in both groups. This may be related to instructions given to all study patients about lifestyle modifications and better control of diabetes. However, the reduction in hepatic steatosis was comparatively more in the misoprostol group.

Hepatic lipotoxicity is a prominent driving force of fibrosis progression.[19] Our results showed that misoprostol led to a greater reduction in hepatic fat content as compared with the placebo, suggesting the potential to halt fibrosis progression.

Six (12%) patients had at least one adverse event in the misoprostol group, as did five (10%) in the placebo group. However, these events were transient, and frivolous, and did not lead to any dropout in this study. The most common adverse event in the misoprostol group was diarrhoea. No life-threatening events or treatment-related deaths occurred in each group.

Our study had several strengths. First, it was the first randomised double-blind placebo-controlled trial conducted to ascertain the effect of misoprostol for treating patients of NASH. Second, patients were closely followed up in OPD at 4 weeks and then at 8 weeks. Third, there were no drop-outs. All the study participants completed the study duration of 2 months. Fourth, the presence of any leaky gut was targeted by measuring the serum IL-6 and endotoxin levels. Fifth, an adverse event form was provided to the patient for proper documentation of any discomfort felt by the patient during the study period. Sixth, the effect on not only LFTs but also on hepatic steatosis, fibrosis, IR and dyslipidaemia were ascertained. Seventh, the chemiluminescent immunoassay technique, which is the most sensitive method, was used for measuring IL-6 levels. Our study was not without limitations. The sample size was small because a preliminary data analysis was performed at 50 sample size, and as the differences in results of both groups were not statistically significant, the study was concluded. Histological changes were not ascertained, and a cross-over trial was not conducted. Further studies with a higher dose of misoprostol are warranted.

CONCLUSION

Our study concludes that both misoprostol and placebo led to an improvement in biochemical profile without any statistically significant difference. However, there was more improvement in the hepatic steatosis, as depicted by CAP, in the misoprostol group and an increase in the IL-6 levels in the placebo group.

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Contributors MS and ZA conducted major data collection, analysis, manuscript writing and manuscript editing. MAQ helped with idea generation, data collection and arrangement of kits for measuring IL-6 and endotoxin levels. AS assisted with data entry and interviewing the patients regarding drug compliance over the phone. UA conducted the laboratory test for the measurement of IL-6 and endotoxin levels. AK helped with the proper storage of centrifuged serum samples of patients. All authors reviewed the manuscript and approved the final version. MS is the article guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Ethics Review Committee of Ziauddin University (reference code: 3280221MSGE). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

ORCID iD

Mehreen Siyal http://orcid.org/0000-0001-8294-0183

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