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Original Research Article

A randomized, open-label and prospective study to compare the efficacy and safety of synbiotics and rosuvastatin along with concomitant ursodeoxycholic acid in non-alcoholic fatty liver disease in North India

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disorder without significant alcohol consumption with a global prevalence of 32%. It is strongly associated with dyslipidemia, obesity, insulin resistance and gut dysbiosis with no current USFDA approved pharmacotherapy. Thus, this study aims to compare the efficacy and safety of Synbiotics and Rosuvastatin along with concomitant Ursodeoxycholic Acid (UDCA), in treatment of NAFLD.

Methods: An interventional, randomized, open-label, prospective and parallel study of 12 weeks with patients randomly divided into two groups- A and B of thirty each. Group A was prescribed Synbiotics (Velgut) 5 billion CFUs BD and Group B was prescribed Rosuvastatin 20 mg OD along with UDCA 300mg BD in both the groups. Patients were followed up every 15 days and mainly assessed on hepatic profile, ultrasound grading, FibroScan, fibrosis indices and lipid profile along with safety profile and compliance.

Results: On comparison, Group A showed significant improvement in hepatic parameters ($p<0.001$) whereas Group B showed better improvement in lipid profile ($p<0.001$). In case of ultrasonography for hepatic steatosis and assessment of liver stiffness by FibroScan, both Group A and B showed comparable improvement over 90 days ($p=0.143$ and $p=0.722$, respectively) with no worsening of any grades. Both groups performed similarly in terms of safety ($p>0.05$) and patients showed good compliance ($p>0.05$).

Conclusion: Combination of Synbiotics and UDCA (Group A) seems to be more efficacious than Rosuvastatin and UDCA (Group B) in North-Indian NAFLD patients over a period of 3 months. Further extensive research with more sample size and studies with longer duration are needed to validate the role of these combination therapies in NAFLD.

Keywords: Gut dysbiosis, FibroScan, Probiotics, Statins, Ultrasound liver

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a complex, chronic liver disorder and is defined as the accumulation of excessive fat in the liver, as demonstrated by imaging or by histology, in the setting of no significant alcohol consumption (<20g/day in males and<10g/day in females) and the absence of any secondary cause.[1] It has emerged as the most common chronic liver condition with an increasing global prevalence of 32% with prevalence in India ranging from 6.7% to 55.1%, affecting both adults and children.[2,3] It is increasingly being associated with metabolic dysfunction such as obesity, dyslipidaemia and insulin resistance, thus giving rise to the term 'MAFLD'

(metabolic dysfunction-associated fatty liver disease).[4] If left untreated, NAFLD can progress to Non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and even hepatocellular carcinoma (HCC), thus making it a significant health concern. The pathogenesis of NAFLD is multifactorial, involving a number of complex mechanisms including-gut dysbiosis, dyslipidaemia, bile acid metabolism, oxidative stress and hepatocyte degeneration.[5]

The management of NAFLD remains challenging, with lack of options in terms of approved pharmacotherapy by USFDA, EMA and DCGI (with only Saroglitazar being approved in India for NASH). Current treatment strategies mainly focus on lifestyle modifications, including weight loss through diet and exercise. However, patient adherence to these interventions is often poor in the long-run.

As per recent literature: Synbiotics (a combination of prebiotics and probiotics), which targets gut dysbiosis by modulating the gut microbiome and improving intestinal barrier function, Rosuvastatin, because of its pleiotropic effects and lipid-lowering ability, and Ursodeoxycholic acid (UDCA), because of its cytoprotective, anti-apoptotic and anti-inflammatory effects on the liver, have shown a promising therapeutic potential in the management of NAFLD.[6-8]

While these treatments have shown promise individually, their combined efficacy in NAFLD management remains unexplored. But, because of lack of definitive clinical trials as well as availability of insufficient and variable data regarding their safety and efficacy, there is a need for exploring combination therapies, targeting multiple aspects of disease prevention and progression. This becomes more relevant in the North Indian population because of high prevalence of NAFLD (72.4%) and dyslipidaemia in the northern states, especially in Punjab (27%), which might be attributed to a fat-rich diet.[9]

This study aims to compare the efficacy of Synbiotics and Rosuvastatin, both in combination with UDCA, in treating NAFLD patients. Moreover, the comparison of Synbiotics and Rosuvastatin alongside UDCA allows for a nuanced evaluation of how targeting different pathogenic mechanisms–gut dysbiosis, dyslipidaemia and insulin resistance may synergistically improve outcomes in NAFLD patients.

By focusing on a population with a significant disease burden, this study has the potential to provide valuable insights into effective management strategies for NAFLD, tailored to the regional demographic pattern.

Primary objective

To compare the efficacy of Synbiotics and Rosuvastatin along with concomitant UDCA in the treatment of North Indian patients having NAFLD.

Secondary objective

To compare the adverse effects of all the drugs. To assess the compliance of the treatment given.

METHODS

Study design

Randomized, open-label and prospective study**.**

Study place

Department of Pharmacology, Department of Medicine and Department of Radiodiagnosis, Government Medical College, Amritsar, Punjab, India.

Study duration

The duration of the study was of 90 days.

Study population

Diagnosed cases of NAFLD defined according to the International Classification of Diseases 10th Revision (ICD-10) of either sex, between the age group of 18-45 years.

Sample size

The size of the sample was N=60 (30 in each group).

Inclusion criteria

NAFLD diagnosed by suggestive imaging findings (ultrasound) with abnormal aminotransferase levels (AST/ALT 50-150 U/l (≥1 to 3 times the upper limit of normal). Subjects with concomitant dyslipidaemia. Age between 18-45 years. Alcohol consumption<20g/day i.e. <60ml of 40% spirits or<2 standard drinks/day (1 standard drink=10 g alcohol=30 ml of 40% spirits)] in males and <10 g/day (i.e<1 standard drink/day) – confirmed by at least two family members. Non-Diabetic individuals

Exclusion criteria

Any known hypersensitivity to UDCA, Synbiotics and Rosuvastatin. Patients with any evidence of liver cirrhosis (on imaging/ histology), alcoholic liver disease, autoimmune hepatitis, hepatocellular carcinoma or any secondary causes of NAFLD (surgery or drugs). Patients with age group<18 years and >45 years. Patients with type 1 diabetes and type 2 diabetes. Patients with other chronic hepatic, renal or cardiovascular co-morbidities. Patients with any history of drug or alcohol abuse, taking lipid lowering drugs or any other drugs that may cause fatty liver changes, in the past 3 months. Pregnant and Lactating mothers. Patients refusing to give written informed consent.

Ethical approval

Before starting the study, approval of Institutional Ethics Committee (No. 10742/D-26/2021) and Thesis Committee was taken. 66 diagnosed cases of NAFLD, defined according to ICD-10, of either sex, between the age group of 18-45 years who met the inclusion criteria, were recruited in the study. A written informed consent was taken from all the patients prior to enrolment and after explaining the study particulars in easily understandable vernacular language.

Sample size calculation

Minimum sample size to be taken in each group, calculated by a formula using mean and standard deviation values from previous studies, was n=24. Patients were randomly divided by simple randomization technique, using a computer software (Random Allocation Software) into 2 groups of equal distribution, consisting of 30 patients each (after considering the total dropouts which were 6)

Group A was prescribed: UDCA 300 mg BD and Synbiotics (Velgut) 5 billion colony forming units (CFUs) BD (containing L. acidophilus, L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum, B. infantis, S. thermophilus, Saccharomyces boulardii and 100 mg of fructooligosaccharides) Group B was prescribed: UDCA 300 mg BD & Rosuvastatin 20 mg OD. After recruitment of patients in the study groups, a detailed history was taken and general physical examination was done. Patients underwent assessment of anthropometric parameters (weight, BMI, waist and hip circumference and waist-hip ratio) every 45 days and hepatic parameters (serum bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase and serum protein) as well as lipid profile (total cholesterol, serum triglycerides, LDL, HDL and VLDL) at the start of the study and then followed up every 30 days. Ultrasound of liver (for the grade of steatosis) and FibroScan (to measure the liver stiffness) were assessed at 0 and 90th day.

Non-invasive scores for prognosis of hepatic fibrosis such as NAFLD Fibrosis Score (NFS), Fibrosis-4 Index (FIB-4), AST to Platelet ratio (APRI) and BARD score were also assessed at 0 and 90th day. Safety profile (with causality assessment of adverse drug reactions by WHO-UMC system and severity assessment by Modified Hartwig and Seigel's severity assessment scale) and compliance to treatment (by pill count method and MARS-5 score) were also assessed over the course of 90 days.

Statistical analysis

The efficacy and safety data were recorded and analysis was done for patients who completed 90 days of the study phase. Data generated from the study was tabulated with respect to all parameters at specific intervals and results were expressed as Mean±SD of each variable. For categorical data, comparison between Group A and Group B was done by Chi square test. For continuous data, comparison between the groups was done using Unpaired T-test. Paired T-test was applied for intragroup comparison at different time intervals. Data analysis was conducted with the help of licensed SPSS software version 23.0 (Chicago, Illinois). A p value of<0.05 was taken as statistically significant and that of<0.001, as highly significant.

RESULTS

In the present study, both the groups were comparable at baseline among various parameters. It was observed that the prevalence of NAFLD increased with increasing age-18-25 years (3.3%), 26- 30 years (10%), 31-35 years (15%), 36-40 years (36.6%) and in >40 years (35%), affecting more males than females (55% males vs 45% females). It also showed that NAFLD was more prevalent in patients with a sedentary lifestyle (n=49, 81.6%) with 24 patients (80%) in group A and 25 (83.3%) in Group B.

Anthropometric parameters

Group A showed a highly significant decrease (p<0.001) in weight as well as waist circumference at 45 & 90 days but a significant (p<0.05) decrease in BMI was seen only at 90 days. On the other hand, Group B showed a highly significant (p<0.001) decrease in weight only at 90 days. On comparison, both Group A and Group B were comparable in terms of improvement of anthropometric parameters (all p>0.05) (Table 1).

Hepatic parameters

There was a highly significant improvement (p<0.001) in liver function tests, especially ALT, AST, ALP AND GGT in Group A throughout the study period of 90 days. Whereas, Group B showed a highly significant (p<0.001) reduction in AST and ALT levels only after 60 days and at 90 days respectively.

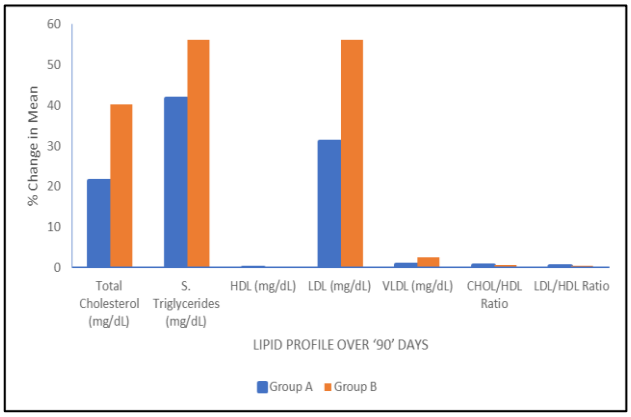

Figure 1: Comparison of the change in mean lipid profile over '90' days between group 'a' and 'b'.

p>0.05: Not significant *p<0.05: Significant, **p<0.001: Highly significant. (p value: Unpaired t-test.)

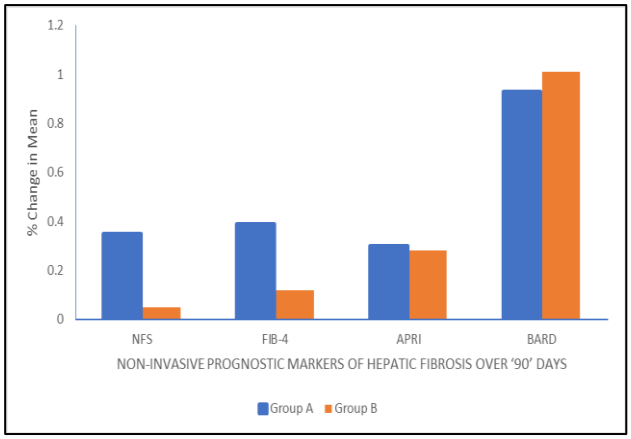

Figure 2: Comparison of change in mean non-invasive prognostic markers of hepatic fibrosis over '90' days between group 'a' and 'b'.

p>0.05: Not significant *p<0.05: Significant, **p<0.001: Highly significant. (p value: Unpaired t-test).

On comparison, group A showed a highly significant decrease in AST (p<0.001) and ALT (p<0.001) over 90 days of treatment and a statistically significant decrease in serum bilirubin (p=0.023), ALP (p=0.036) and GGT (p=0.048) as compared to Group B. (Table 2)

Lipid parameters

In group A, improvement in total Cholesterol and HDL became statistically highly significant (p<0.001) only at 90 days whereas mean reduction in serum Triglycerides and LDL was highly significant (p<0.001) after 60 days. On the other hand, group B had a more pronounced effect in the improvement of lipid profile, which was statistically highly significant (p<0.001) throughout the study period of 90 days. On comparison, group B was better in improving the lipid parameters as compared to group A (p<0.001) (Figure 1).

Radiological parameters

In ultrasonography, both Group A and B showed comparable improvement (p=0.143) over a period of 90 days of treatment i.e. In Group A, 53.3% patients showed improvement in grades while 40% patients showed improvement in Group B.

There was no worsening of ultrasound grades in any group (Table 3). On FibroScan assessment of median liver stiffness, both groups showed a highly significant improvement (p<0.001) at 90 days (mean values were in the normal range both at baseline and 90 days). Although mean reduction in median liver stiffness was more with Group A (1.72±0.53) as compared to Group B (1.47±0.89), it was statistically non-significant (p=0.722) and thus, were comparable.

Non-invasive prognostic scores of hepatic fibrosis

In case of non-invasive prognostic markers of hepatic fibrosis, Group A showed a highly significant (p<0.001) improvement in NFS, FIB-4, APRI as well as BARD scores over the course of 90 days only. Whereas, Group B showed a highly significant improvement (p<0.001) only in APRI and BARD scores at 90 days only.

On comparison, Group A was significantly better in improving NFS and FIB-4 index (p=0.045 and p=0.002), respectively (Figure 2).

Safety profile and compliance

Expected adverse effects with Synbiotics, Rosuvastatin and UDCA were 15, 20 and 13 respectively, over the course of 90 days. Nausea (15), vomiting (11) and other gastrointestinal disturbances like flatulence (6), indigestion (10) and abdominal pain (5) were common in both the groups.

As per the causality assessment, almost all the ADRs were possibly related to the drugs under research with level 1 severity, as assessed during the study period. Both the groups were comparable in terms of safety profile (p>0.05) and therapy was well tolerated in both the groups as none of the patients had serious adverse effects requiring withdrawal from study.

Mean compliance by pill count method decreased over 90 days of treatment from >90% in both Groups A and B to<85%, which was statistically non-significant (p >0.05).

Table 1: Comparison for the change in mean anthropometric parameters over '90' days between group 'a' and 'b'.

Anthropometric parameters	Group A	Group B	P value
	Change in Mean±SD	Change in Mean±SD	
Weight (kg)	4.00±0.83	3.97±1.49	0.920
Waist circumference (cm)	4.43±2.64	4.41±1.77	0.962
Hip circumference (cm)	4.030±2.25	5.00±2.48	0.115
Waist-hip ratio	0.03±0.11	0.008±0.024	0.266
BMI (kg/m^2)	1.03±1.89	1.41±0.55	0.283

BMI: Body Mass Index, p>0.05, Not significant *p<0.05, Significant, **p<0.001, Highly significant (p value: Unpaired T-test).

Table 2: Comparison of the change in mean hepatic parameters over '90' days between group 'a' and 'b'.

	Group A, Change (Mean±SD)	Group B, Change (Mean±SD)	P value
S. Bilirubin (mg/dl)	25.59±20.08	15.27±13.53	0.023*
AST (U/l)	49.26±8.92	38.80±10.20	<0.001**
ALT (U/l)	61.01±10.20	53.90 ±11.41	<0.001**
ALP (IU/l)	10.03±10.73	5.43±6.19	0.036*
GGT (IU/l)	8.10±4.89	5.13±2.54	0.048*
Total S. protein (gm/dl)	0.23±0.82	0.08±0.75	0.555
A/G Ratio	0.05±0.48	0.01±0.39	0.441

S. Bilirubin: Serum bilirubin, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline Phosphatase, S. Albumin: Serum albumin, S. Globulin: Serum Globulin, p>0.05, Not significant *p<0.05, Significant, **p<0.001, Highly significant (p value: Unpaired T-test).

Table 3: Comparison of effects in group 'a' and 'b' on grading in ultrasound liver over '90' days of treatment.

Grade	Group A		Improvement in grading over 90 days (%)	Group B		Improvement in grading over 90 days (%)	P value
	0 day N (%)	90 days N (%)		0 day N (%)	90 days N (%)		
Grade 0	0 (0.0)	2 (6.7)	6.7	0 (0.0)	6 (20.0)	20	0.143
Grade I	17 (56.7)	23 (76.7)	20	18 (60.0)	18 (60.0)	0	
Grade II	13 (43.3)	5 (16.7)	26.6	12 (40.0)	6 (20.0)	20	
Grade III	0 (0.0)	0 (0.0)	0	0 (0.0)	0 (0.0)	0	
TOTAL	30 (100)	30 (100)	53.3	30 (100)	30 (100)	40	

p>0.05: Not significant *p<0.05: Significant, **p<0.001: Highly significant (p value: Unpaired t-test).

Mean total MARS-5 score was ≥20 in both the groups A and B over 90 days of treatment, which indicated a better adherence as per the MARS-5 score assessment. Comparison of MARS-5 scores between Group A and B were statistically non-significant on all follow ups over 90 days of treatment (p>0.05).

DISCUSSION

Upon extensive literature search, there was a lack of studies investigating the role of combination therapies in NAFLD, especially involving Synbiotics, Rosuvastatin or UDCA. Moreover, no equivalent studies were found comparing the efficacy and safety of these drugs in NAFLD in Punjab, where the present study was conducted.

The comparison of Synbiotics and Rosuvastatin alongside UDCA allows for a nuanced evaluation of how targeting different pathogenic mechanisms gut dysbiosis, dyslipidemia and hepatocyte protection–may synergistically improve outcomes in NAFLD patients. Thus, the present study investigates a novel combination of Synbiotics and Rosuvastatin with UDCA as a baseline drug in NAFLD patients.

Evaluation of demographic profile in this study showed that the prevalence of NAFLD increased with increasing age which is in accordance with a study (n=924) by Kalra S et al, (2013) that demonstrated the rising prevalence of NAFLD with age (45.8% in age group of 25-50 years and 54.2% among those aged 51-60 years and 61.8% in 61-70 years age group) in the Indian population.9 This could be attributed to the fact that older adults are more likely to have risk factors for developing NAFLD, such as hypertension, diabetes, high cholesterol and obesity, which can increase their risk for the disease. The present study also showed that NAFLD was more prevalent in males i.e. (55% males vs 45% females) which is likely because estrogen in premenopausal females has a protective effect against NAFLD and males tend to accumulate more visceral fat.[10] It was observed in the present study that prevalence of NAFLD was more in patients with a sedentary lifestyle (n=49, 81.6%) with 24 patients (80%) in group A and 25 (83.3%) in group B. This observation is in accordance with a study (n=13,518) conducted by Joo et al (2020), wherein a highly significant (p<0.001) link was found between sedentary lifestyle (sitting time >10 hours a day) and an increased risk of NAFLD in adults aged≥20 years.[11]

As per known literature, sedentary lifestyle and physical inactivity are strongly correlated with decreased insulin sensitivity, accumulation of visceral fat, altered gut microbiome, hormonal imbalance as well as systemic inflammation, which are some known contributing factors in the development of NAFLD.[12]

Upon extensive literature search on PubMed, Google scholar and other databases, no studies could be found comparing the efficacy and safety of concomitant UDCA plus Synbiotics and UDCA plus Rosuvastatin in NAFLD, at doses which are equivalent to the doses of drugs used in our study. Thus, the present study will be discussing and correlating the results based on the studies involving monotherapy (variable doses) with Synbiotics, Rosuvastatin and UDCA and their effects on various parameters in NAFLD management.

Anthropometric parameters

Our results are in concordance with a study (n=80) by Asgharian et al, (2017), wherein Synbiotic supplementation (500 mg capsule containing 7 species of probiotic bacteria *L. acidophilus, L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum, Saccharomyces boulardii* and FOS) for 8 weeks in the intervention group caused a significant (p=0.001) reduction in body weight and BMI, as compared to placebo, at the end of study.[13] A reduction of 2.47±0.98 in mean weight (kg) and 0.89±0.32 in BMI (both p<0.001) with Rosuvastatin in 12 weeks was also observed by Rana et al, (2016) (n=98) in a study at Lucknow, which was in accordance with the present study.[14] Another study (n=174) by Nadinskaia et al, supported our results, wherein UDCA monotherapy (15mg/kg/day) caused a significant (p<0.001) weight loss (> 5%) in 31% patients along with a statistically significant (p<0.001) reduction in BMI over 6 months.[15]

Modulation of gut microbiota can influence carbohydrate metabolism, improve insulin resistance and enhance satiety (causing reduced caloric intake), all of which can ultimately influence body fat accumulation, causing reduction in body weight, waist circumference and BMI. UDCA, through its indirect effects on lipid and glucose metabolism and Rosuvastatin, through its lipid lowering action, can similarly cause changes in body fat composition, thus supporting the observations in the present study.

Hepatic parameters

Our results are in agreement with a pilot study (n=52) conducted by Eslamparast et al in 2014, which evaluated the effect of oral supplementation with synbiotics (200 million CFUs BD containing *L. acidophilus, L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum* and inulin) vs placebo for 28 weeks wherein reductions in AST, ALT, ALP and GGT levels were significantly better (all p<0.001) in the study group.[16]

These findings are also supported by Abhari et al wherein synbiotic supplementation (1 billion CFUs/day containing *L. acidophilus, L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum, Saccharomyces boulardii, S. thermophilus* and FOS) in 53 patients for 12 weeks saw a decrease in AST, ALT and GGT levels in the study group as compared to placebo (p<0.05).[17]

This is potentially due to the fact that Synbiotics, which have shown promising results in improving liver function markers, enhance the integrity of intestinal barrier function and exert anti-inflammatory as well as antioxidant effects on liver health, thus, proving their effectiveness in managing NAFLD.[6]

Another study by Kim et al, in 2018 supports our results, in which 300 mg UDCA given twice daily for 8 weeks in patients with obesity and liver dysfunction, observed a significant (p<0.05) reduction of the liver enzymes (ALT, AST, GGT) after 4 weeks of treatment. This is potentially attributable to the hepatoprotective action of UDCA and its role in reshaping the gut microbiome thus having a synergistic effect with synbiotics.[18]

Lipid parameters

Our results are in accordance with Rana et al (n=98), wherein significant (p<0.001) improvement in the Rosuvastatin (10mg/day) group was seen in the lipid profile, including increase in HDL over 24 weeks.[14]

Another study by Kargiotis et al, in 2015 is in accordance with the fact that Rosuvastatin monotherapy (10mg/day) had a significant (p<0.05) effect on improving lipid profile, including increase in HDL, over a period of 12 months in 20 patients.[19] Similarly, in a study (n=36) conducted in 2014, NAFLD patients receiving UDCA (300mg BD) for 2 months observed a significant reduction in lipid profile at the end of the study (p<0.05).[8]

Longer half-life, increased reduction in LDL and rise in HDL levels by rosuvastatin contribute to the improvement of NAFLD in the present study. Above results also prove that rosuvastatin may have synergistic effects when combined with other NAFLD treatments.[7]

Radiological parameters

Ultrasound Liver: Our results are in agreement with Abhari et al, wherein Synbiotic supplementation (1 billion CFUs/day) in 53 patients for 12 weeks in NAFLD patients had a significant improvement in steatosis measured on ultrasound after 12 weeks (p<0.01).[17]

Similarly, in a study (n=126) by Ratziu et al, UDCA therapy (13-15 mg/kg/day) showed a significant (p<0.05) reduction in liver steatosis after 12 months.[20] Khan et al, observed a significant (p=0.011) reduction in hepatic steatosis on ultrasound after treatment with Rosuvastatin (10mg/day) over 6 months.[21]

Fibroscan

As per a study by Mofidi et al, a highly significant (p<0.001) improvement in liver stiffness measurement on synbiotic supplementation (200 million CFUs BD containing *L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum, B. infantis, Saccharomyces boulardii* and 100 mg of inulin) was observed after 28 weeks in 50 patients, which supported our results.[22] Similarly, Ratziu et al, demonstrated that UDCA therapy (13-15 mg/kg/day) in 126 patients showed a significant (p<0.05) reduction in liver fibrosis over 12 months.[20]On the other hand, in a study by Cho et al, in 67 patients, on receiving Rosuvastatin (20mg/day), no significant difference (p>0.05) was seen in liver stiffness after 6 months, which is in disagreement with our results.[23] Although, ultrasonography is a commonly used tool in clinical

practice to diagnose fatty liver, liver biopsy is the gold standard for definitive diagnosis, but it was not included in the present study as it is an invasive procedure and carries the risk of complications. Thus, non-invasive methods like ultrasonography and FibroScan, with a sensitivity of 53-76% and 86-92% respectively, were used in our study. Certain limitations regarding ultrasound and FibroScan, which could have led to improved results in our study, may have been, interoperator differences in interpretation of results and unreliable accuracy of readings which can be frequently observed in patients with obesity, older age and increased waist circumference.

Non-invasive prognostic scores of hepatic fibrosis

Our results are in line with a pilot study (n=52) conducted by Eslamparast et al, in 2014, which evaluated the effect of oral supplementation with synbiotics (200 million CFUs BD) vs placebo for 28 weeks wherein significant ($p<0.001$) improvements in the NFS and FIB-4 index values were observed, suggesting a positive impact of synbiotics on liver fibrosis in NAFLD patients.[16]

This is also supported by another study (n=104), by Scorletti et al, (2020), wherein synbiotic supplementation (10 billion CFUs/day containing *L. plantarum, L. casei, L. rhamnosus, B. breve, B. longum, B. infantis, S. thermophilus, Saccharomyces boulardii* and FOS) caused a significant reduction in NFS ($p<0.05$) over 24 weeks.[24]

Similarly, another study (n=240) by Elhini et al observed that UDCA (250mg/day) caused a significant ($p<0.05$) reduction in NFS and FIB-4 over 24 weeks.[25]

On the other hand, a study (n=81) by Khan et al showed a significant ($p<0.05$) reduction in NFS and BARD scores after treatment with Rosuvastatin (10mg/day) over 6 months.[21] But, in a study by Parikh et al, statistically non-significant ($p>0.05$) effect on fibrosis after 52 weeks of UDCA therapy (13-15 mg/kg/day), measured by NFS and FIB-4 index, was observed, which may not agree with our study results.[26] These indices often incorporate markers of liver function (like ALT, AST) and other parameters (like platelet count, albumin). Improvements in these underlying parameters due to the treatment given may have led to better scores on these indices at the end of the study.

Safety profile and compliance

On analysis of adverse effects in the present study, both the groups had a comparable safety profile. None of the groups had shown any serious adverse effect or the need to discontinue the treatment. Flatulence, nausea and diarrhoea were the most common adverse effects seen with Synbiotics whereas nausea/vomiting and headache were noted the most with Rosuvastatin. On the other hand, abdominal pain, nausea/vomiting and indigestion were the most commonly observed adverse effects with UDCA.

All the adverse effects observed were possibly related to the study drugs, as per the causality assessment done by WHO-UMC system and mild in severity (level according to the modified Hartwig and Siegel's severity assessment scale.[27,28] The present study showed that mean compliance at the first visit i.e., at 15 days, as assessed by pill count, was≥85% in both the groups, which was seen as a good compliance but at 90 days, it was below 85%. Similarly, in the present study mean total MARS-5 score at 15 days as well as 90 days was≥20 in both the groups, which indicated a better adherence as per the MARS-5 score assessment.[29] But it was also observed that the adherence decreased over the course of 90 days, which could be due to adverse effects or the need to take multiple pills, although MARS-5 score was still≥20 ($p>0.05$).

Novelty of the study

This is the first study to compare the efficacy and safety of combination of Synbiotics and UDCA with Rosuvastatin and UDCA in NAFLD as per available literature. This is the first study to evaluate the effects of the above-mentioned drugs in combination with UDCA as baseline drug in North-Indian NAFLD patients of Punjab. This is the first study to comprehensively evaluate combination therapy in NAFLD using a wide variety of parameters including hepatic, lipid, radiological and non-invasive fibrosis scoring assessment along with detailed assessment of safety profile and compliance using different scales.

As with the majority of the studies, the present study is also subjected to a few limitations which are small sample size (n=60), time constraint (3 months) and interoperator differences in interpreting the results of diagnostic imaging (ultrasonography and FibroScan), were the limitations of the study.

CONCLUSION

Synbiotics along with UDCA demonstrates superior efficacy, comparable safety and better compliance as a combination therapy as compared to Rosuvastatin and UDCA, in North-Indian NAFLD patients over 3 months. Additionally, it can be concluded that both Synbiotics and Rosuvastatin may have potential as an effective and safe therapy in management of NAFLD. Combination therapy provides a newer perspective in NAFLD management. Thus, a pressing need for exploring combination therapies through long-term studies and larger sample size, targeting multiple aspects of disease prevention and progression, are needed to further validate the role of these drugs in NAFLD, as this disease stands as a rising health concern globally.

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