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Prospective study on safety and efficacy of trimethylglycine in patients with non-alcoholic fatty liver disease with or without fibrosis

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ABSTRACT

Recognized as a prominent contributor to liver-related morbidity and mortality, Nonalcoholic Fatty Liver Disease (NAFLD) holds significance due to its potential progression to cirrhosis and liver failure. Scientific literature indicates that dietary supplements containing betaine, a naturally occurring chemical compound, demonstrate efficacy in reducing hepatic fat accumulation. The primary objective of our study is to assess the impact of trimethylglycine (TMG) in patients diagnosed with NAFLD, with or without fibrosis.

Methods: Approval for this study was obtained from the local ethics committee, and participants were enrolled based on a diagnosis of NAFLD. This hospital-based interventional investigation involved 244 NAFLD patients, aiming to evaluate the safety and efficacy of TMG. Trimethylglycine, administered orally, consisted of 2 sachets TDS (three times a day) for 1 month, followed by 1 sachet TDS for the subsequent 2 months (each sachet containing 3 gm Betaine). Ultrasonography with elastography was conducted on NAFLD subjects before and after trimethylglycine treatment to assess liver grade, stiffness, and spleen size.

Results: Significant positive correlations were identified between BMI and ultrasonography with elastography parameters, including spleen size (p value = 0.002), liver stiffness (p value < 0.001), and visceral fat thickness (p value < 0.001). Abdominal circumference also exhibited positive correlations with spleen size (p value = 0.020), liver stiffness (p value = 0.002), and visceral fat thickness (p value < 0.001).

Conclusions: These findings suggest a crucial role for betaine in the treatment of NAFLD. However, further validation of its effectiveness is imperative through extensive, multicenter randomized studies.

Keywords: betaine, trimethylglycine (TMG), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), SAM (S-adenosyl-L-methionine), metabolic disease

1.INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) constitutes a comprehensive spectrum, ranging from simple steatosis to steatohepatitis that may progress to cirrhosis of the liver and hepatocellular carcinoma [1]. In 1980, Ludwig et al [2] introduced the term Non-Alcoholic Steatohepatitis (NASH) to characterize a form of liver injury histologically consistent with alcoholic hepatitis but occurring in obese, diabetic females who deny alcohol use. The estimated prevalence of NAFLD and NASH in the general population varies between 10% and 24%, and 1% to 5%, respectively. In the United States, NAFLD prevalence ranges from 57.5% to 74% in obese individuals and is reported as high as 90% in morbidly obese individuals [3] [4] [5]. In India, NAFLD prevalence is approximately 9% to 32% of the general population. NAFLD is not confined to hepatic manifestations; it is a multisystem disorder affecting extra-hepatic

organs and regulatory pathways. It escalates the risk of type 2 diabetes mellitus, cardiovascular and cardiac diseases, chronic kidney disease, and establishes a causal link with sleep apnea, colorectal cancers, osteoporosis, psoriasis, and various endocrinopathies such as polycystic ovary syndrome [6] [7].

A direct correlation between body mass index (BMI) and the prevalence and severity of NAFLD is evident. Lonardo and colleagues conclude that emerging high-quality evidence suggests a complex and bidirectional interaction between metabolic syndrome components and NAFLD [8]. Most NASH patients are asymptomatic, with liver enzyme elevation noted incidentally in biochemical investigations. While alanine aminotransferase and aspartate aminotransferase levels are commonly elevated, they do not reliably correlate with hepatic injury, inflammation, or cirrhosis in NAFLD. Early diagnosis and risk stratification are crucial for effective management.

Current imaging methods, such as ultrasound, CT, and MRI, have proven valuable as noninvasive imaging biomarkers to assess NAFLD progression [9]. However, they remain somewhat limited in detecting inflammation, which is more critical than steatosis in terms of its high risk for fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). In our study, we utilized ultrasonography with elastography to compare changes at the beginning and end of trimethylglycine (TMG) treatment.

In addition to its complex pathological nature, there is currently no FDA-approved therapy for NASH treatment. Nevertheless, several therapeutic options with varying efficacy are available for certain patients. These include Pioglitazone, Vitamin E, Pentoxifylline, and betaine, all of which have demonstrated positive results [10, 11, 12]. Our study aims to investigate changes observed in the liver and adjacent organs in patients after trimethylglycine (TMG) administration.

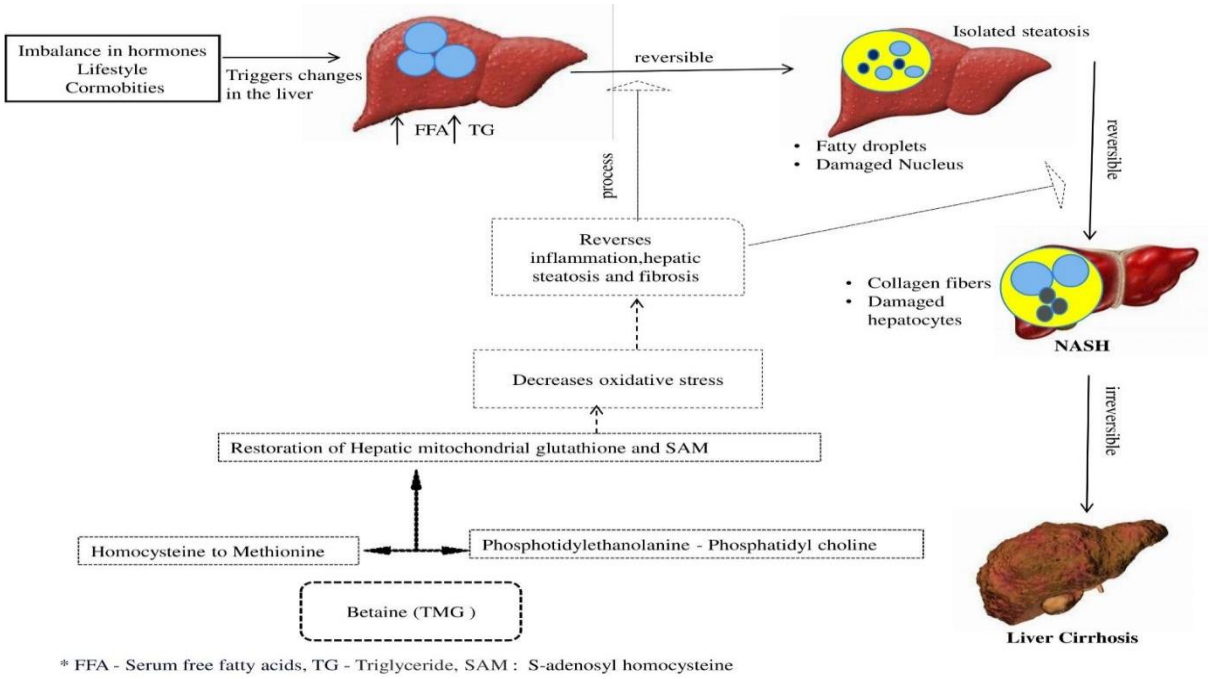

Figure 1: Pathogenesis of liver disease and mechanism of action of betaine at reversible stages.

2.MATERIALS & METHODS

A hospital-based interventional prospective study was conducted in the Department of General Medicine at Bhaktivedanta Hospital and Research Institute, Mira Road, Mumbai, between August 2019 and August 2021. The study enrolled a total of 244 patients with fatty liver disease, with or without fibrosis.

Based on the literature [13], the overall prevalence of non-alcoholic fatty liver disease was observed to be between 15% and 40% in Western countries and 9% to 40% in Asian countries. For the determination of our sample size, considering an assumed overall prevalence of Non-alcoholic fatty liver disease at 20%, and allowing for a 5% allowable variation, the calculated sample size at a 95% confidence level is 246 patients. Thus, the final sample size of 246 patients, accounting for a 5% dropout rate, brings the total enrollment in the study to 260 patients.

Step wise calculation of sample size:

$N = [Z^2 * p * (1 - p)]\ e^2$

Here N is sample size, Z^2 =1.96, p=0.20, (1-p) =0.80 and e^2 = 0.05

$N = [1.962 * 0.20 * (1 - 0.20)]\ 0.052 = 244$ patients

This led to the determination of an initial sample size of 244 patients.

Patients (>18 years) diagnosed on elastography enrolled for the study. Those with advanced stages of liver conditions and known diagnosis of malignancy were excluded from the study. Post Ethics Committee approval, clinical history, anthropometry, and physical examination were conducted. Apart from fasting and postprandial blood sugar, liver function test, HbsAg (Hepatitis B surface antibody), Anti HCV, and fasting lipid profile for patients diagnosed with metabolic syndromes (diagnosed as per NCEP ATP 3 criteria) [14], liver elastography for early detection of NASH, visceral fat thickness (VFT), and NAFLD. Abdominal girth measurements were taken midway between the umbilicus and lower costal margin and blood pressure, measured in sitting posture in both the upper limbs.

Trimethylglycine is 2 sachet TDS (three times a day) for 1 month and 1 sachet for TDS (three times a day) for 2 months (Each sachet =3 gm Betaine) was administered for study patients.

2.1 STATISTICAL ANALYSIS

The numeric data summarized descriptive statistics like; n, Mean ± SD, median, minimum, maximum. Normality test was performed before applying for any statistical test. For statistical significance of numeric data t-test was used. For ordinal type data, the analysis was done by non-parametric test like Wilcox on Signed-Rank test. The categorical data was summarized by frequency count and percentage and significance, analyzed using Chi-square/ fisher exact test. A p-value less than 0.05 was considered statistically significant. An SPSS version 26 was used to calculate the results.

3.RESULT

Amongst the total identified subjects of 260, 16 subjects were not willing to participate, hence a total of 244 NAFLD subjects post consenting were included as study participants. (Table: -1) 150 (61.5%) were male while 94 (38.5%) were female. And a majority were in the age group of 41-50 years (n=81; 33.2%) followed by 31-40 years (n=52; 21.3%) and 51-60 years (n=49; 20.1%). Only 3 (1.2%) subjects belonged to less than 20 years. The participants' mean SGOT level was 34.74±14.28 IU/L and SGPT level was 40.78±19.03 IU/L in NAFLD patients.

Table 1: -Demographic details of the study participants

Parameter	Categories	Number of study subjects	Percentage
Age	Up to 20 years	3	1.2
	21-30 years	14	5.7
	31-40 years	52	21.3
	41-50 years	81	33.2
	51-60 years	49	20.1
	61-70 years	31	12.7
	>70 years	14	5.7
Gender	Male	150	61.5
	Female	94	38.5
BMI	Upto 22.99 kg/m2	18	7.4

	23.0-27.5 kg/m2	65	26.6
	>27.5 kg/m2	161	66.0
Risk Factor	Diabetes mellitus	203	83.2
	Hypertension	197	80.7
	Dyslipidaemia	209	85.7
	Cardiovascular disease	48	19.6

3.1 Changes in fatty liver and fibrosis grade

In our study, it was observed that NAFLD patients after providing treatment with betaine had a mean decrease in fatty liver grade i.e. 0.72±0.61 (Table: - 2). The proportion of patients in the trimethylglycine group, in whom fatty liver grade improved by ≥1 grade was 66.4%, remained unchanged in 32.8%; and worsened by ≥1 grade in only 2 patients. We have also reported liver fibrosis grade based on Metavir score in NAFLD study subjects before and after trimethylglycine (Table 3).

Table 2: - Fatty liver grade in NAFLD study subjects before and after trimethylglycine treatment (n=244)

Fatty Liver Grade	**Baseline**	**At end of treatment**
Grade 1	64(26.2%)	218(89.3%)
Grade 2	160(65.6%)	25(10.2%)
Grade 3	17(7.0%)	1(0.4%)
Grade 4	3(1.2%)	0
P value < 0.001		
Changes seen in the liver at the end of treatment		
Change in fatty liver grade	No of subjects	Percentage of subjects
Decreased - 1	149	61.1%
Decreased - 2	10	4.1%
Decreased - 3	3	1.2%
No change	80	32.8%
Increased	2	0.8%
P value < 0.001		

At baseline grade I fatty liver disease was seen in 64(26.2%) patients, grade II in 160 (65.6%) patients, grade III in 17 (7.0%) patients and grade IV in 3 (1.2%) patients. At the end of treatment by trimethylglycine grade II and grade III fatty liver were reduced to only 10.2% and 0.4% respectively while 89.3% were in grade I.

Table 3: - Liver fibrosis grade based on Metavir score in NAFLD study subjects before and after trimethylglycine treatment (n=244)

Liver fibrosis grade based on Metavir score	**Baseline**	**At end of treatment**
F0	136 (55.7%)	203 (83.2%)
F1	31 (12.7%)	6 (2.5%)
F2	64 (26.2%)	25 (10.2%)
F3	8 (3.3%)	6 (2.5%)
F4	5 (2.0%)	4 (1.6%)
P value < 0.001		

Description of scores: Fibrosis stage 1 (F1) Zone 3 perisinusoidal fibrosis; focally or extensively present, Fibrosis stage 2 (F2) Zone 3 perisinusoidal fibrosis with portal fibrosis, Fibrosis stage 3 (F3) Zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis, Fibrosis stage 4 (F4) Cirrhosis, Fibrosis stage 0 (F0) is the absence of fibrosis.

Above table 3 represents liver fibrosis grade based on Metavir score in NAFLD study subjects before and after trimethylglycine treatment. At baseline F0 grade was seen in 136 (55.7%) patients, grade F1 in 31(12.7%) patients, grade F2 in 64 (26.2%) patients, grade F3 in 8 (3.3%) patients and grade F4 in (2.0%) patients. At the end of treatment by trimethylglycine, grade F1 patients were reduced to 6 (2.5%) patients, grade F2 was seen in only 25 (10.2%), grade F3 was observed in only 6 (2.5%) and Grade F4 was observed in 4 (1.6%) patients respectively while 89.3% were in grade I while grade F0 were seen in 203 (83.2%) patients.

3.2 Results of Ultrasonography (USG) with elastography, Pre and Post Treatment of TMG

In our study we have done USG with elastography in NAFLD study subjects before and after trimethylglycine (TMG) treatment.

Table 4: -USG with elastography findings in NAFLD study subjects before and after trimethylglycine treatment.

	Baseline	End of treatment	P Value
Spleen (in cm)			
Mean ±SD	10.32±1.69	9.52±1.66	< 0.001
Median	10.1(9.1-11.5)	9.4(8.22-10.60)	
Range	6.1-16.4	6.2-15.7	
Liver Stiffness (Kpa)			
Mean	6.30±1.20	5.79±1.16	< 0.001
Median	6.32(5.65-6.80)	5.67(5.2-6.32)	
Range	3-13.21	3.0-12.6	
Visceral fat thickness (in cm)			
Mean	10.10±1.93	9.32±1.73	< 0.001
Median	10(9.01-11.1)	9.4(8.3-10.4)	
Range	4.45-15.80	4.4-14.4	

Above table 4 shows USG with elastography findings in NAFLD study subjects before and after trimethylglycine treatment. Mean spleen size before treatment was 10.32±1.69 cm which was significantly reduced to 9.52±1.66 cm ($p<0.001$) at the end of treatment. Mean liver stiffness at baseline in study subjects was 6.30±1.20 kPa which was significantly reduced to 5.79±1.16 kPa ($p<0.001$) at the end of treatment. Mean visceral fat thickness at the start of treatment was 10.10±1.93 cm which was also significantly reduced to 9.32±1.73 cm after betaine treatment.

Table: -5 Correlation of different clinical and biochemical parameters with USG -elastography parameters

		Abdominal Circumference (cm)	Splenomegaly (in cm)	Liver Stiffness (kPa)	Visceral Fat Thickness (cm)
Body Mass Index	p value	.000	.002	.000	.000
Total Cholesterol	p value	.374	.645	.435	.785
Triglycerides	p value	.873	.895	.953	.723
SGOT (IU/L)	p value	.015	.202	.042	.012
SGPT (IU/L)	p value	.057	.536	.972	.037
Abdominal Circumference (in cm)	p value	NA	.020	.002	.000
Splenomegaly (in cm)	p value	.020	NA	.000	.000
Liver Stiffness (k Pa)	p value	.002	.000	NA	.000
Visceral Fat Thickness (cm)	p value	.000	.000	.000	NA

Above Table: -5 shows correlation of different clinical and biochemical parameters with USG-elastography parameters. Significant positive correlation was observed between BMI and USG elastography parameters like spleen size (p value = 0.002); liver stiffness (p value < 0.001) and visceral fat thickness (p value <0.001). Abdominal circumference was also found to be positively correlated with spleen size (p value = 0.020), liver stiffness (p value = 0.002) and visceral fat thickness (p value < 0.001).

4.DISCUSSION

Nonalcoholic steatohepatitis is a common condition that progresses to cirrhosis in up to 20–30% of patients [15-16]. Several mechanisms have been proposed in the pathogenesis of NASH, including oxidative

stress, endotoxins, cytokines, chemokines, and nutritional deficiencies. Increasing evidence suggests that altered methionine/folate metabolism contributes to the development of hepatic steatosis [17].

Betaine, an important human nutrient obtained from a variety of foods, is absorbed from the intestine, and transported to the liver. In the liver, betaine serves as a methyl donor to homocysteine to form methionine, resulting in decreased concentrations of homocysteine and increased concentrations of methionine (Figure 1).The consequent increase in SAM (S-adenosyl-L-methionine) can trigger a cascade of events leading to the activation of the phosphatidyl ethanolamine methyltransferase pathway, phosphatidylcholine synthesis, formation of very low-density lipoprotein, export of triacylglycerol, and attenuation of fatty liver (Figure:1) [18-20]Decreased hepatic concentrations of homocysteine can attenuate endoplasmic reticulum (ER) stress, resulting in the downregulation of pro-apoptotic genes and, thus, the attenuation of apoptosis, inflammation, and fibrosis [21]. Down-regulation of another ER stress gene, sterol response element-binding protein-1, can reduce hepatic fatty acid synthesis, resulting in reduced fatty liver. Increased hepatic concentrations of SAM can activate cystathionine β-synthase and lead to the up-regulation of the transsulfuration pathway, increased synthesis of glutathione, and attenuation of oxidative stress [22]. Thus, betaine can potentially attenuate fatty liver disease by improving steatosis, inflammation, and fibrosis.

Although not extensively studied for NASH, betaine has been shown to attenuate alcoholic liver injury by increasing the concentrations of hepatic SAM and decreasing the concentrations of homocysteine and SAH (S-adenosyl-L-homocysteine) in animal studies [21,22]. By increasing hepatic SAM levels, betaine may provide protection against liver injuries by 1) Increasing hepatic glutathione, a potent antioxidant; 2) Down-regulating TNF-α and up-regulating IL-10 synthesis; and 3) By inhibiting the apoptosis of normal hepatocytes. An increase in hepatic SAM can restore the hepatic mitochondrial glutathione concentration [23], which is critical for maintaining mitochondrial function and rescuing mitochondria from free radical damage. In addition to protecting hepatocytes from oxidative stress, elevated glutathione concentrations have been shown to rescue hepatocytes from TNF-toxicity, such as necrosis [24].

Our study's findings are in line with a study by Tinohen K et al [25], in which the visceral fat thickness at baseline was 93.9±16.9 cm2, which improved to 92.6±16.4 cm2 after 12 weeks of betaine treatment. The liver/spleen ratio at baseline was 1.01±0.3, which increased to 1.03±0.2 after 12 weeks of betaine treatment. In a study by Miglio F et al[26], liver size was 1.41 ± 0.07 at baseline, which improved to 1.33 ± 0.07 after the end of treatment, a 6% decrease in liver size from baseline.

Different studies showed these risk factors in their study population. In our study, 83.2% of subjects were diabetic, 80.7% were hypertensive, and 85.7% had dyslipidemia, while cardiovascular diseases were present in 48 (19.6%) patients. In a study by Abdelmalek MF et al [27], diabetes mellitus was seen in 9 (33.0%) patients, hyperlipidemia in 18 (67%) patients while hypertension was present in 11 (41%) patients. Another study by Miglio F et al (2000) found 30% had hyperlipidemia, 35% had diabetes, and 24% of subjects were obese [28]. In Abdelmalek MF et al study (2001), six (60%) patients had hyperlipidemia, one (10%) diabetes, four (40%) fatigue, and six (60%) vague abdominal pain.

During our study period, two patients experienced mild adverse events (AEs) like nausea and slight abdominal discomfort but did not warrant stoppage of medication). Miglio F et al reported AEs during the treatment with betaine which were mild or moderate, regarded mainly the gastrointestinal tract and they were all reversible, did not require discontinuation of treatment [26]. Tinohen K et al reported 5 non-serious adverse events in three cases in

the betaine group and 12 non-serious adverse events occurred in five cases in the placebo group. Amongst them, there was only one adverse effect (loose stool) in the betaine group that could have causal relationship with the product. All the events were mild, and the symptoms resolved without interruption of the intervention.[25]

Our findings were encouraging, as a statistically significant improvement was observed on USG with elastography. The results of this study suggest that betaine holds promise as a therapy for patients with NAFLD. Furthermore, the study was conducted at a single center and included only the intervention group without a control group.

5.CONCLUSION

There is still an ongoing intensive search for the management of NASH especially in view of its deleterious cascading to end stage liver disease. In this scenario, our study renews its focus on trimethylglycine as a potentially effective nutraceutical molecule in the management of NAFLD.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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