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Effects of Probiotics on Clinical, Biochemical and Radiological parameters in Obese Children with NASH /NAFLD in Combination with Dietary and Lifestyle Modification: A Randomised Control Trial

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Abstract

Background: Childhood obesity is a global problem with metabolic abnormalities including NAFLD/ NASH. The gut liver axis is thought to play a major role in pathogenesis and altering it could affect the pathophysiological process. Probiotics are known to alter the gut microbiota and therefore could be a therapeutic option in the management of childhood obesity related complications. A double-blind, randomised, placebo control trial was conducted to evaluate the effects of probiotics on metabolic derangements in obese children with NAFLD/ NASH.

Methods and Results: Obese children from the nutrition clinic conducted by University Paediatric Unit at Lady Ridgeway Hospital, Colombo with NAFLD/ NASH were recruited. Anthropometry, body fat, metabolic derangements and ultrasound scan (USS) of liver were evaluated at the beginning and at the end of 6 months. Transient elastography (Fibroscan®) was performed in a subsample of patients. Eighty-four patients were recruited and randomised into probiotics group (43) and placebo group (41). Mean age of the probiotic group was 11.3 years (+/-1.9 SD) and placebo group was 12.1 years (+/-1.5 SD). Baseline parameters including USS stage of the liver, body fat percentage, fasting blood sugars, lipid profile, liver functions and CRP showed no statistical difference. In the Probiotic group, statistically significant reduction of BMI was noted from baseline. However, the reduction was not significant when compared with the Placebo group. There was significant reduction in triglycerides, AST, ALT, AST/ ALT and ALP in placebo group over treatment period. Even though the USS stage of fatty liver showed improvement from stage II - III to stage I in a small number in the probiotic treated group, transient elastography performed in a subsample did not demonstrate significant improvement in either group.

Conclusion: Our results indicate that there does not seem to be an advantage of probiotics over lifestyle modifications in improving obesity associated metabolic derangement in children.

Keywords: Obesity; Metabolic syndrome; NAFLD; NASH; Probiotics

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Introduction

Childhood obesity is a global health problem, which leads to metabolic derangements including insulin resistance, metabolic syndrome, impaired lipid metabolism and Non Alcoholic Fatty Liver Disease (NAFLD)/Non Alcoholic Steatohepatitis (NASH). Hence, prevention of obesity in the younger generation is of paramount importance. However, over the years most preventive methods have failed to decelerate the rapid growth of this health burden. Therefore, it is important to find new therapeutic options which could be used in addition to lifestyle modifications. It is documented that NAFLD prevalence in

children varies from 3% in the general population to 80% in obese children [1]. In children, NAFLD is commoner among males, during puberty and is associated with insulin resistance [2]. Several studies have estimated that NAFLD/ NASH would increase the 5-year medical costs by 26% [3]. In Sri Lankan children, the prevalence NAFLD in a suburban community was 8.4% [4], and the prevalence of presumed NASH was estimated to be about 18% among obese children [5]. The pathogenesis of NAFLD is unclear. Theories regarding its development are based on the '2- hit hypothesis', where the 'first hit' involves hepatic lipid accumulation, and insulin resistance is proposed to be the main contributing factor for the development of steatosis [6]. Then, oxidative stress followed by lipid peroxidation as well as the action of proinflammatory cytokines (e.g. TNF α), adipokines and mitochondrial dysfunction initiate the 'second hit', which leads to the progression of simple steatosis to NASH [7]. In addition, Dowman et al recently described a 'third hit', which is also caused by oxidative stress that inhibits the replication of mature hepatocytes resulting in an increased number of hepatic oval cells [8]. It has been reported that NAFLD might be linked to small intestinal bacterial overgrowth (SIBO), which is defined as an increase in the number and/ or alteration in the type of bacteria in the upper gastro-intestinal tract owing to the loss of more than one of the several endogenous mechanisms. SIBO induces liver injury by gut derived lipopolysaccharides (LPS) and TNF α production leading to steatohepatitis [9]. Solga and Diehl concluded that bacterial overgrowth, release of the LPS constituent of the gram-negative bacteria and impaired intestinal barrier integrity results in increased endotoxin absorption leading subsequently to liver toxicity [10]. The theory is supported by several studies. One of the studies by Madsen K et al has shown that SIBO is present in 50% of patients with non-alcoholic steatosis [11]. Probiotics are live organism that when consumed in adequate quantities, confer a health benefit to the host (WHO). They exert their anti-inflammatory effects through several mechanisms including intestinal barrier stabilisation, immunomodulation and SIBO alteration that can contribute to the clinical benefits in obesity related metabolic complications [12-17] Children with NAFLD are often asymptomatic or have non-specific symptoms. Although there are no specific biochemical tests, describing hepatic steatosis on imaging with an AST/ALT ratio of less than 1 suggest the diagnosis of NAFLD, with or without the development of hepatic fibrosis [18]. abdominal ultrasound scan (USS) is often used in screening for NAFLD as it has a predictive value of 84-94%, but USS cannot detect a fat load less than 30% in the liver, compared to histological examination [19]. However, recent large prospective paediatric cohort showed a good correlation between steatosis score assessed by USS and the severity of steatosis on liver biopsy, which is the gold standard study to diagnose NAFLD/ NASH [20]. However, liver biopsy is invasive, has a potential for sampling errors and inconsistent interpretation of the histopathology. Furthermore, recent studies demonstrate the benefits of the transient elastography to detect liver fibrosis in the paediatric age as a non-invasive method [21]. However, elastography is prone to fail in obesity, presence of ascites, liver congestion and with narrow intercostal spaces [21]. But, usage of algorithms such as the controlled attenuation parameter (CAP) help to minimise these errors [22]. At present there are limited management options to tackle obesity related metabolic derangements apart from losing weight through dietary modification and physical activity [23]. Unfortunately, the target of gradual and controlled weight loss is difficult to achieve by diet and physical exercise. An extremely low percentage of individuals are able to steadily lose weight through regular exercise and dietary modifications [24], warranting new therapeutic approaches. Considering the evidence of the possible role of gut microbiota in the development of obesity related metabolic derangements, probiotics may be utilized to modify gut microbiota as a preventive or therapeutic strategy. Malasanos et al shows that probiotics enhance the barrier function of epithelial cells and decrease intestinal permeability and endotoxemia in patients with liver disease [25]. showed that probiotic therapy significantly decreased alanine aminotransferase (ALT), aspartate transaminase (AST), total-cholesterol, high-density lipoprotein (HDL), TNF-α and improve insulin resistance in NASH patients. Also, a placebo controlled randomized study in histologically confirmed cases of NAFLD treated with daily Lactobacillus bulgaricus and Streptococcus thermophiles showed a decrease in ALT and γGT [26]. Another study showed that serum AST, ALT and ultrasound grading of NASH improved in the group treated with metformin and probiotic compared to the group treated with metformin alone [27]. The increase in the incidence of childhood obesity, and its related metabolic problems, has reached epidemic proportions in developing countries. However, existing medical and non- medical efforts to tackle this problem are currently inadequate prompting the investigation of safe and inexpensive novel strategies. Despite the limited number of randomized controlled trials, probiotics have shown promising results in treating the metabolic consequences of obesity. Hence, this study attempted to evaluate the effectiveness of probiotics in the treatment of obesity related metabolic derangement in a group of obese Sri Lankan children with NAFLD/NASH.

Methods

Trial design

A double-blind, randomised, placebo control trial.

Study population/ participants

Children between age 5- 15 years of age, with a BMI more than +2SD for age of WHO standards (2007) together with AST/ALT ratio less than 1 and ultrasound evidence of hepatic steatosis, including grade I to III, were recruited from nutrition clinic conducted by Professorial Paediatric Unit of University of Colombo at the Lady Ridgeway Hospital for Children, Sri Lanka. Children with an acute infection, on long term medication, chronic illness and on antibiotics within 2 months period of recruitment were excluded after studying past records and clinical evaluation.

Intervention

The two randomized groups were allocated treatment arms as follows:

Group 1: structured diet (Annex 1) + physical activity (Annex 2) + probiotics (Bio-Kult 14 strain probiotic capsule- Annex 3)

Group 2: structured diet (Annex 1) + physical activity (Annex 2) + placebo (a capsule without probiotic strains –Annex 3)

The dose was one capsule for children under 12 years and two capsules for children above 12 years of age on each day as per manufacturer's guidance. Both groups were followed up for 6 months ensuring they adhered to the prescribed diet, physical activity and treatment with compliance chart and direct questioning during the monthly interval follow ups.

Both groups were observed for possible side effects. However, none were reported

Outcome assessment

Outcome assessment was done after 6 months by acquiring anthropometric, clinical, biochemical and radiological parameters similar to baseline assessment. The primary outcome measures were liver transaminases (AST, ALT), USS assessment of hepatic steatosis and transient elastographic assessment of liver stiffness and steatosis quantification. The secondary outcome measures were gammaglutamyltransferase, lipid profile, glucose homeostasis, metabolic syndrome, body fat mass and anthropometric parameters. A research assistant, a physician and a radiologist evaluated the patients. No changes to trial outcomes were done after the trial commenced.

Sample size

Sample size was calculated to determine a statistically significant difference in the mean liver function test levels at baseline and after 6 months. Guided by the findings of Aller et al. [27], a standardized effect size of 0.55 was estimated to be seen after 6 months of treatment. Using

a α error of 5%, a β error of 20% (power of 80%) and a non-response rate of 10%, calculated sample size was 43 subjects per treatment arm.

Recruitment and randomization

Informed, written consent was obtained from the guardian and assent from the patient when he/ she was above 12 years of age. Participants, once registered for the trial were randomly allocated to two groups (receiving either probiotics or placebo) using a computer-generated, concealed allocation sequence. Both the subjects and the investigators implementing the protocol were blinded to the treatment.

Baseline Assessment

Baseline evaluation, comprising anthropometric parameters, body composition measurement using Bio Electrical Impedance (BIA - InBody®, South Korea), blood pressure measurement with sphygmomanometer (with age appropriate cut-off using standard mercury spigmomanometer) and pubertal staging was conducted by trained research assistants. Blood was collected for glucose, lipid profile, insulin, liver aminotransferase (AST/ ALT), gamma glutamyltransferase, alkaline phosphatase, high sensitive CRP and albumin, after 12 hours of overnight fast. Also, random blood sugar and insulin levels were measured two hours after 1.75g/kg (maximum 75g) anhydrous glucose challenge. In addition, detailed ultrasound scan liver was performed on each subject by a Consultant Radiologist categorising hepatic steatosis according to National Health and Nutrition Examination Survey (NHANES) III criteria, and in a sub sample (n= 27), elastography (Fibroscan®) was performed [28-30].

Additionally, chronic liver diseases in subjects were ruled out by performing hepatitis B surface antigen, hepatitis C antibody, hepatitis A antibody, serum ceruloplasmin and full blood count (total volume of 10-15ml of blood was processed). A positive test would have been a criterion to exclude from the study. None were positive in these screening tests.

Outcome assessment

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Data Analysis

Data analysis was performed by Statistical Package for Social Sciences (SPSS) software for windows. P value less than 0.05 ($p<0.05$) was considered as significant. Baseline characteristics of the treatment and control groups were compared using chi square test and independent samples T test or relevant non-parametric tests. Between the two groups, the anthropometric, metabolic and radiological parameters at 6 months as well as the pre-post difference in the parameters were compared using independent samples t-test or relevant non- parametric tests. Within the treatment and control groups the pre-post difference in the parameters were assessed using paired t-test or equivalent non-parametric tests. Intention to treat analysis was performed, substituting any missing values with the latest available measurement.

Ethical considerations

The study was designed appropriately to ensure scientific validity. Ethical clearance was obtained from Ethics Review Committee of Faculty of Medicine, University of Colombo (EC-16-030) and Lady Ridgeway Hospital for Children. Permission to conduct the study was obtained from the relevant authorities including Sub Committee on Clinical Trials (SCOCT) of Ministry of Health. The study was registered in the Sri Lankan Clinical Trials Registry (SLCTR/2016/021). Participation in the study was voluntary. Informed written consent was obtained after providing the necessary information and giving the patients / their guardians' adequate time and information to make a decision on their own. Personal details were collected in a separate data sheet that was detachable from the main questionnaire. All hard copies of data were kept under lock and key. The electronic database was password protected. Adequate privacy was maintained during history taking and all physical examination procedures.

Results

Eighty-four obese children with NAFLD/ NASH were randomised into probiotics group (n=43), who received structured diet plan and physical activity plan together with probiotic treatment according to the age or the control group (n=41) who received the placebo treatment in addition to similar diet and physical activity plan (Figure 1). The two groups were similar in age, gender and pubertal stage distribution. Table 1 summarises the baseline anthropometric, body composition, metabolic and ultrasound-related characteristics of two study groups, and there was no statistically significant difference in their baseline values. After the study period of 6 months probiotic and placebo treated groups showed significant reduction of BMI compared to baseline values (p= 0.023 and 0.001 respectively). However, there was no significant difference in BMI between the probiotic and placebo groups. The placebo group showed significant improvements in serum triglycerides, AST, ALT, AST/ALT ratio and ALP from baseline values. The probiotic group did not. However, the placebo did not demonstrate a significant advantage over probiotic treated group (Table 2). However, other metabolic parameters including, fasting blood sugar, oral glucose tolerance test, fasting insulin together with post prandial insulin, total cholesterol, high density cholesterol and low-density cholesterol did not demonstrate statistically significant improvement from baseline in either group. Furthermore, clinical parameters including waist circumference and body fat percentage did not show significant improvement over 6 months. The USS imaging of subjects, showed that in the stage I1 fatty liver category, 20% in probiotic arm and 44.2% in placebo arm down staging to a normal USS. The probiotic treated group showed 100% (n=4) conversion of USS stage I-II or II fatty liver to stage I fatty liver by 6 months (Table 3). In the placebo group all (n=3, 100%) who had fatty liver of stage I-II or II at baseline remained at the same stage at the end of 6 months. However, the numbers were too small for statistical analysis. Although USS studies showed some improvement of fatty liver in stage I-II or II with probiotic treatment, the limited subjects (n=27) in both groups who underwent transient elastography did not show statistically significant improvement in fatty liver parameters during the 6 months study period (Table 4).

Discussion

Obesity related metabolic derangements are reaching epidemic proportion in children worldwide leading to increased morbidity and health cost. The clustering of various cardio metabolic risk factors associated with insulin resistance underlies the concept of metabolic syndrome (MetS) and is closely related to the increasing levels of adiposity. Also, elevated rates of lipolysis inherent in adipose tissue, alterations in fatty acid fluxes and consequent ectopic fat deposition in skeletal muscle and liver are thought to be the mechanisms linking altered fat distribution and insulin resistance in MetS [31]. Out of the metabolic derangements in obesity this research aimed at studying NAFLD/ NASH in view of a novel therapeutic option. NASH was first

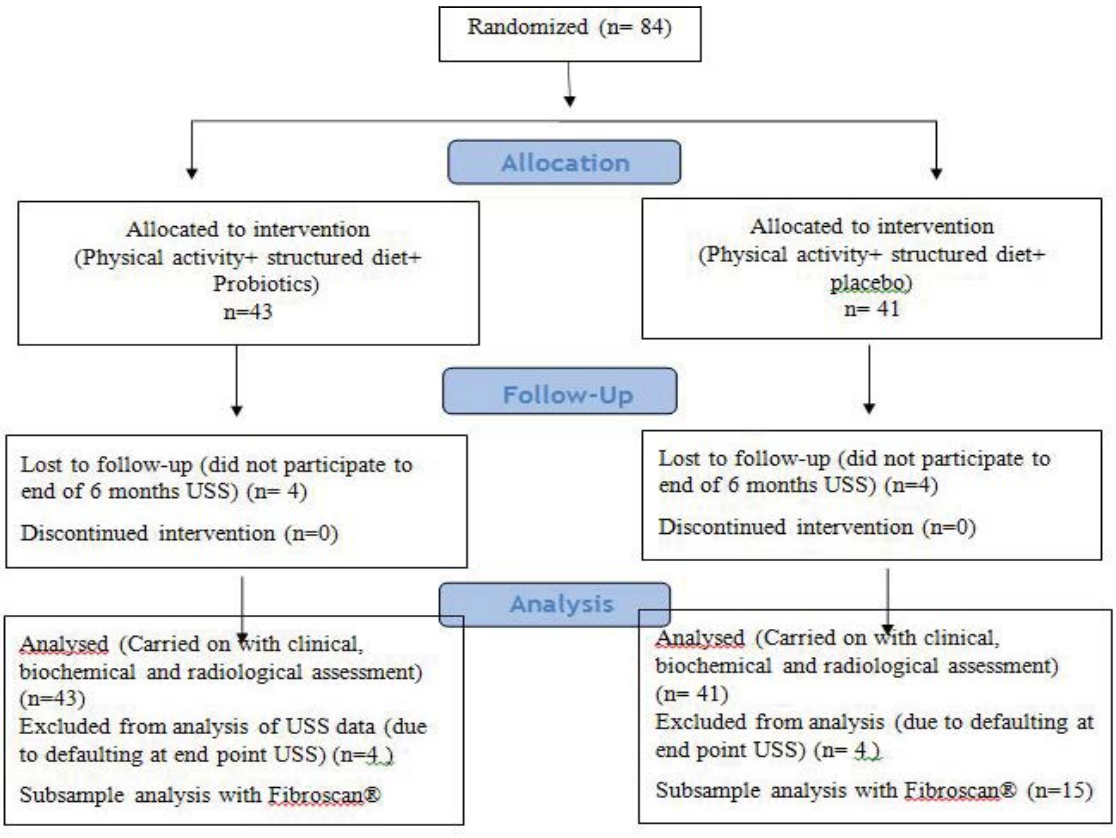

Figure 1 Participant's flow diagram.

Table 1: Baseline characteristics of the sample randomized in to the two treatment arms (n=84).

Parameter	Probiotic Group(n=43)		Placebo Group(n=41)		
	Mean	SD	Mean	SD	
Age	11.28	1.87	12.05	1.45	
Stage 2	9	20.90%	14	34.10%	
Stage 3	4	9.30%	5	12.20%	
Stage 4	1	2.30%	3	7.30%	
Liver USS* Stage I	39	90.70%	38	92.70%	NS
Stage I- II	2	4.70%	1	2.40%	
Stage II	2	4.70%	2	4.90%	
BMI SDS28	2.56	0.57	2.63	0.513	NS
Height SDS28	0.39	0.89	0.25	1.1	NS
WC SDS 29	2.86	0.5	2.91	0.46	NS
SBP SDS30	-1.61	1	-1.55	0.92	NS
DBP SDS30	0.84	0.75	1.15	0.8	NS
Fat Percentage	41.7	4.68	42.2	5.87	NS
FBS	75.59	11.79	78.35	14.1	NS
OGTT	107.85	18.28	101.77	20.79	NS
Fasting insulin	12.23	10.45	12.77	8.46	NS
2hr-insulin	93.56	80.91	80.49	68.77	NS
TC	203.84	41.83	190.32	44.36	NS
TG	125.47	52.52	123.86	58.38	NS
HDL	48.97	9.25	45.36	8.54	NS
LDL	129.55	37.47	118.67	33.57	NS
AST	37.87	26.81	48.42	57.25	NS
ALT	52.32	34.01	60.36	52.29	NS
AST/ALT	0.74	0.17	1.79	6.66	NS
GGT	28.42	27.19	25.88	12.86	NS
ALP	478.74	205.86	484.62	237.33	NS
Albumin	43.44	3.05	43.06	3.15	NS
CRP	3	2.69	3.12	2.71	NS

Table 2: Median Anthropometric and Metabolic Parameters at baseline and 6 months in the two treatment arms (n=84)

Parameter	Probiotic Group(n=43)			Placebo Group(n=41)			
	Baseline Median (IQR)	End of 6 months Median (IQR)	Sig*.	Baseline Median (IQR)	End of 6 months Median (IQR)	Sig*.	Sig**.
Height SDS	0.35 (-0.36- 1.03)	0.089 (-0.52-0.87)	**0**	0.11 (-0.33-0.79)	-0.047 (-0.57-0.67)	**0**	0.428
BMI SDS	2.51 (2.09- 2.92)	2.39 (2.024-2.90)	0.023	2.61 (2.24-2.97)	2.43 (2.17-2.88)	**0.001**	0.387
WC SDS	2.86 (2.51-3.31)	2.85 (2.41-3.16)	0.262	2.92 (2.63-3.2)	2.84 (2.59 -3.13)	0.69	0.778
Fat Percentage	42.3 (38.3-45)	41.7 (37.15-44.85)	0.196	42.6 (37.8-46.5)	41.8 (36.9-45.55)	**0.026**	0.7
SBP SDS	-1.99 (-2.42- - 1.08)	-1.5 (-2.37- -1.1)	0.345	-1.46 (-2.41-1.13)	-1.35 (-1.65- 0.42)	0.226	0.219
DBP SDS	0.44 (0.43-1.58)	0.44 (0.44-1.58)	0.274	1.52 (0.44-1.58)	1.57 (0.44-1.58)	**0**	0.05
FBS	72.7 (66.7-84.6)	72.9 (67.95- 79.45)	0.658	80.1 (68.52-87.75)	75.75 (70.1- 85.55)	0.57	0.379
OGTT	106.7 (92.3-121.75)	98.75 (84.65-109.6)	0.065	101 (90.0-121.4)	96.75 (90.43- 120.95)	0.681	0.394
Fasting Insulin	10.3 (4.93-17.25)	10.4 (4.65-16.19)	0.673	9.86 (6.6-19.0)	9.6 (7.53-15.4)	0.779	0.751
2 hr Insulin	65.65 (41.7-106.17)	61.7 (29.65-97.1)	0.187	64.3 (27.6-95.3)	44.5 (16.4-90.1)	0.202	0.167
TC	197 (180.45-233.5)	194.4 (168.3-227.6)	0.399	181.9 (158.95-229.5)	195.2 (156.5-220.4)	0.943	0.436
TG	120 (85.9-140.4)	116 (79-142.8)	0.699	107 (87-139.8)	90 (74.75-121.7)	**0.004**	0.081
HDL	46.6 (42.75-54.95)	50.2 (43.5-56.8)	0.882	45.7 (40.5-50.85)	48.1 (41.7-54.65)	0.067	0.438
LDL	120.7 (97.4-160.1)	120.7 (99.3-152.8)	0.236	115.3 (92.2-149.9)	119.6 (94.4-145.15)	0.704	0.613
AST	32.9 (21.25-43.5)	29.7 (22.75-40.5)	0.607	30.4 (22.35-53)	25.2 (21.1-36.9)	**0.014**	0.367
ALT	44 (29.875-62.25)	36.4 (23.92-52.97)	0.199	43 (25.7-76.25)	24 (17.9-49.2)	**0**	0.078
AST/ALT	0.78 (0.63-0.86)	0.84 (0.63-0.98)	0.081	0.75 (0.56-0.87)	0.896 (0.69-1.19)	**0**	0.148
GGT	22.4 (16.32-27.52)	22.95 (17.05-32)	0.135	22.5 (17.9-26.5)	22.2 (17.8-28.5)	0.845	0.91
ALP	423.3 (297-663.8)	577.9 (419.6-671.9)	0.158	434.8 (291.5-633.45)	560.5 (426.3-760.2)	**0.014**	0.576
Albumin	43 (41.9-45.6)	42.9 (41.8-44.7)	0.686	42.7 (41.5-45.1)	43.1 (40.7-44.4)	0.173	0.708
CRP	2.2 (0.875-3.9)	1.6 (1-3.2)	0.527	2.4 (1.1-4.0)	2.1 (1.05-5.25)	0.369	0.363

*Significance for the difference in medians within group from baseline to 6 months – Wilcoxan signed rank test **Significance for difference in median values between groups at 6 months – Mann-Whitney U test

Table 3: Changes in liver USS in the two treatment arms at baseline and six months.

USS at Baseline		Probiotic Group				Placebo Group			
		USS at six months*			Total	USS at six months*			Total
		Normal	I	I-II or II		Normal	I	I-II or II	
	No.	7	24	4	35	15	18	1	34
Stage I	%	20.00%	68.60%	11.40%	100.00%	44.20%	52.90%	2.90%	100.00%
	No	0	4	0	4	0	0	3	3
Stage I-II or II	%	0.00%	100.00%	0.00%	100.00%	0.00%	0.00%	100.00%	100.00%
	No	7	28	4	39	15	18	4	37
Total	%	17.90%	71.80%	10.30%	100.00%	40.50%	48.60%	10.90%	100.00%

Table 4: Comparison of the changes in liver Fibroscan in the two treatment arms at baseline and six months (n=27).

Parameters	Probiotic Group (n=12)		Sig.*	Placebo Group (n=15)		Sig.*	Sig.[a]
	Median	IQR		Median	IQR		
Fibroscan CAP Baseline	302	(289-331)	P=0.959	291.5	(263-319)	P=0.638	P=0.572
Fibroscan CAP Six months	295	(262-321)		287	(267 – 319)		P=0.880
Fibroscan E Baseline	5.5	(5.3-5.9)	P=0.474	5.3	(4.8 – 6.4)	P=0.722	P=0.599
Fibroscan E Six months	5.8	(4.7 – 6.8)		5.2	(4.5 – 6.8)		P=0.914
Fibroscan CAP Difference	0	(-15.8-32.5)		0	(-20 – 9)		P=0.792
Fibroscan E Difference	-0.15	(-0.3 – 1.35)		0	(-0.8 – 0.9)		P=0.719

described in obese children by Moran and Colab [32,33], after 3 years of describing the condition in adults in 1980. Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of conditions ranging from simple hepatic steatosis to steatohepatitis, which is characterized by hepatic inflammation, liver cell injury and fibrosis and cirrhosis. From a pathological point of view NAFLD is where there is excess accumulation of fat in the liver, in the form of triglycerides. Whereas, non-alcoholic steatohepatitis (NASH) has liver cell injury and inflammation in addition to fat deposition, which can progress to cirrhosis, and sometimes even result in hepatocellular carcinoma. Recent studies describe NAFLD as a hepatic manifestation of the metabolic syndrome [34], highlighting the importance of metabolic complications in obese children. The concept of using probiotics as a novel therapeutic option in children with NAFLD/ NASH is based on theory that SIBO [9] leads to the development of liver cell injury based on the 3 hit hypothesis [8]. When the intestinal barrier fails, bacterial translocation will occur, causing endotoxemia especially in the portal circulation initiating a proinflammatory cascade. However, under normal circumstances this endotoxemia is rapidly cleared by the liver's reticuloendothelial system. But, at times of liver disease or long-term exposure to hepatotoxins, a cascade of morphological and functional changes begins in the liver inducing an acute inflammatory response, which releases reactive oxygen metabolites, proteases and other enzymes from polymorphonuclear cells resulting in further damage to the liver [35]. Furthermore, it is known that obesity, diabetes and insulin resistance are associated with low-grade systemic inflammation [36] and metabolic endotoxemia [37]. On the other hand, evidence suggests that children generally show poor compliance with lifestyle modifications, such as dietary modification and exercise. However, large randomized control studies are needed to provide the required evidence for this potential benefit. Hence, we used probiotic treatment compared with a placebo in a double-blind randomised control trial for a period of 6 months. The study revealed reduction of BMI-SDS after 6 months in both arms. However, it failed to show an added advantage of probiotics in obese children. Similarly, placebo arm demonstrated improvement in serum triglycerides, AST, ALT, AST/ALT ratio and ALP without showing statistically significant benefit compared to the probiotics arm. Therefore, it is clear that lifestyle modifications play a role in improving the mentioned parameters. But, insignificant improvement in probiotic arm could be due to a) probiotic strains used b) insufficient treatment duration or c) sub therapeutic dosage used despite the compliance being assured during the study. Hence, future studies should be designed with different probiotics strains, lengthier treatment plans and higher probiotic dosages. It is noted that, Alisi et al showed supplement constituted an almost identical probiotics strains (VSL#3) to our study significantly improved NAFL in children in comparatively much higher dosages (1 sachet per day for children under 10 years of age and 2 sachets per day for others) Also, several important metabolic parameters including fasting blood sugar, oral glucose tolerance test, fasting insulin together with post prandial insulin and total cholesterol did not show significant improvement in both arms. This finding highlights the importance of finding different therapeutic modalities to manage metabolic syndrome in children. Therefore, further research should be focused on therapeutics options that could improve glucose and insulin metabolism in obese children. In contradictory to metabolic parameters, USS grading of liver showed marked improvement in probiotic arm especially in subject categorized as USS stage I-II or II at baseline while the control group failed to show such improvement. However, sample size was small in the concerned group indicating that power should be increased to draw definitive conclusion. Also, it was noted that both arms are beneficial in down staging US grade I fatty liver to normal USS. Transient elastography performed in selected group of subjects did not show significant improvement in both arms, again most probably due to the small sample size. Also, it may be due to its limited role in assessing NAFLD/ NASH in obese subjects. However, no adverse effect was reported during

the study period, which is promising in view of consideration of non-harmful therapeutic options in managing obese children with NAFLD/ NASH. In conclusion, probiotic treatment for 6-month duration is not superior to conventional management (when compliance to lifestyle measures is satisfactory) of obesity related NAFLD/ NASH. However probiotic treatment seems to reduce the BMI and improved the fatty liver USS grade I-II/II.

Conclusion

Future studies should be designed with more power, different probiotic strains, dosages and longer period of treatment. Also, improving glucose metabolism in obese children needs new therapeutic approaches since the current study failed to demonstrate significant improvement in glucose metabolism in both arms.

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