ORIGINAL ARTICLE

Probiotic and Lifestyle Modification in Obese Pediatrics with Non-Alcoholic Fatty Liver Disease

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Abstract

Background: Non alcoholic fatty liver disease is an upcoming causes of chronic liver disease in pediatric population in Asia. **Aims & Objective:** To evaluate the potential of probiotic VSL#3 and Lifestyle modification in obese pediatrics with Non alcoholic fatty liver disease (NAFLD). **Material & Methods**: we conducted clinical trial in 106 obese children in age group of 5 to18 years and divided in to four groups;VSL#3 plus lifestyle intervention (n=26), VSL#3 (n=27), Lifestyle intervention (n=26) and Placebo (n=27) received interventions for four months. To identify NAFLD by ultrasonography, Body mass index (BMI), mid arm circumference (MAC), waist circumference (WC) and triceps skinfold thickness (TSF) were done. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol(LDL-c),high-density lipoprotein cholesterol (HDL-c), triglyceride (TG), cholesterol, fasting blood glucose(FBG), high sensitivity C-reactive protein (HSCRP), uric acid, obesity hormones were measured along with their dietary intake at baseline and post trial. **Results:** VSL#3 plus lifestyle intervention significantly the most pronounced therapy for reducing fatty liver grades, anthropometric with biochemical parameters and beneficial impact on obesity hormones ($p<0.001$) as compared to single therapy of VSL#3 and lifestyle intervention alone. **Conclusion:** Combined therapy of VSL#3 plus lifestyle intervention is promising treatment for management of NAFLD in Indian obese children.

Keywords

NAFLD; Probiotic, Lifestyle modification; Obese Children

Introduction

Obesity influences gastrointestinal health and changes in gut microbiota composition, increased permeability of intestine with bacterial overgrowth in small intestine (SIBO) that leads to non alcoholic fatty liver disease (NAFLD).It has reported in several studies that diet of obese children rich in fructose and fat that impairs the intestinal barrier function causes “leaky gut” and also bacterial translocation with release of gut derived products: Pathogen-associated molecular patterns (PAMPs) and Danger-

associated molecular patterns (DAMPs) that reach first in liver cells stimulate fat accumulation in hepatocytes and secondly fibrosis followed by necrosis.(1,2) Previous studies demonstrated that alteration in the composition of gut microbiota is a new evidence for treating NAFLD through gut liver axis.(3)Therefore probiotics therapies are promising treatment for prevention of gastrointestinal diseases.VSL#3 is live freeze-dried lactic acid bacteria and bifidobacteria, is the most studied probiotic in NAFLD in adults. Currently, Alisi *et al*. described significant effect of VSL#3 vs. Placebo in improving fatty liver with 8.1% weight reduction.(4) Lifestyle interventional has also been an effective tool for preventing NAFLD.(5) Healthy lifestyle may improve hepatic steatosis. In children multi-target therapeutic approach could be successful by combining two treatments lifestyle interventional approach with probiotic VSL#3 for treating the advanced stage of NAFLD.

Aims & Objectives

Potential effects of probiotic VSL#3 and lifestyle modification in obese child with NAFLD

Material & Methods

Study Type: Randomized controlled Trial, **Study Population**: Obesity child with non-alcoholic fatty liver disease, **Study Area**: Jagraon and Daudhar, Punjab and all biochemical parameters were performed in fasting at Babe Ke Medical Hospital Moga, Punjab. Study Duration: January, 2017-October,2018 **Sample Size calculation**: Sample size formula applied for medical studies; n= Z2 P(1-P)/d2 Inclusion Criteria:, Obesity child age ranging from 5-18 years with BMI age and gender using WHO standard reference and without alcohol intake. Exclusion criteria; Secondary obesity, wilson disease, patient on medications, hepatitis B(HBV), hepatitis C(HCV), hepatitis A&E, autoimmune hepatitis (AIH). **Strategy for Data collection**: Self-reported questionnaire. **Ethics**: Ethics committee of Post Graduate Institute of Medical Education and Research Chandigarh, India ethically approved the study on 27 October, 2016 (protocol no. 2016/2608) and registered in Clinical Trials Registry India (CTRI/2017/12/010997).**Consents**: Informed written consents were taken from their parents before assessment and all principles in Declaration of Helsinki were followed for conducting all clinical investigations during entire study. SPSS version-22 used for statistical analysis of variables.

The scheme of study (Consort diagram) is presented in [Figure-1].

Diagnosis: Ultrasonography (USG) (convex transducer 2-5MHz probe) was performed by expert radiologist by using the following standard criteria: Mild steatosis (Grade-1), Moderate steatosis (Grade-2) Severe steatosis (Grade-3). USG detected (n=106) obese children with NAFLD, selected randomly through computer generated randomization and they were randomly divided in to four groups to receive interventions: 1. VSL#3 capsules with lifestyle intervention (Diet +physical activity) (n= 26), 2. VSL#3 capsules only (n=27), 3. Lifestyle Intervention only (n=26) and 4. Placebo (corn flour) (n=27). They were instructed to avoid all junk food and other medications during study period.

Nutritional Assessment:

Anthropometric measurements: Dr. Diaz digital weighing scale was used to the nearest 0.1kg with the capacity of 180. Standard measuring tape was used for measuring height, mid arm circumference (MAC) and waist circumference (WC) to the nearest of 0.1cm. BMI (body mass index) was calculated by weight (kg)/height (m2).Z-BMI (z score) was calculated from WHO reference data.(6)TSF(triceps skinfold) thickness was measured to the nearest 0.2 mm by skinfold caliper.

Biochemical parameters such as Serum alanine aminotranferase (ALT), aspartate aminotranferase (AST), gamma-glutamyl-transferase (GGT) low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride (TG), cholesterol, high sensitivity C-reactive protein (HSCRP), uric acid and fasting blood glucose(FBG) were measured using standard laboratory methods. Obesity hormones: serum ghrelin was estimated by using ELISA kit; Qayee bio for life sciences, with reference range: 3.7-120 ng/ml and serum leptin was also estimated by DRG Leptin normally range from 10-16ng/ml.

Three day dietary recall was taken by registered dietician (RD) to evaluate their dietary intake. Average three days intake of calorie, protein, fat, carbohydrate (CHO) and fiber were calculated by using Diet-Cal, software a tool for dietary assessment and planning (version-1). Self-reported questionnaire was used to collect data on their dietary intake.

Probiotic VSL#3 intervention:

Probiotic VSL#3 contains 112.5 billion lactic acid bacteria and bifidobacteria. Dosage form is

lyophilized powder contained in individual capsules. It is mixture of eight probiotic strains Bifidobacteria [B. breve, B. infantis, B. longum], Lactobacillus acidophilus (L. plantarum, L. paracasei, L. delbrucki subsp. bulgaricus) and Streptococcus thermophilus. Parents were instructed to keep capsules in refrigerator and avoid any other probiotic or antibiotic medications. Placebo capsules were identical of experimental capsules in shape, color and size. Children were randomized to receive treatment in each group. Duration of treatment was four months. No side effects or health hazards of VSL#3 in children during study were observed

Lifestyle intervention:

Hypocaloric diet consisting carbohydrate of 50-60%, fat 23-30%, protein 15-20% of total calories referring the protocol described by Jonathan *et al*,(7) Refined carbohydrate and excess saturated or trans-fatty acids were restricted including recommendation of ample amount of fresh fruits and vegetables. Lifestyle interventional group and VSL#3 plus lifestyle intervention group advised to follow diet plans and aerobic exercise was recommended initially for at least 3 days a week and gradually increased in to 5 days a week.

Statistical analysis: Data were abnormally distributed. Normality was checked by Shapiro-Wilk test. Non parametric test applied to all variables. SPSS version-22 was used for statistical analysis. All data has been expressed in mean±SD (standard deviation). Pearson Chi-square test was used for categorical data. Wilcoxon signed rank test was applied to analyze before and after intervention outcomes. Kruskal Wallis test used to determine the comparison within different interventional groups. p value<0.05 was considered statistically significant.

Results

In present study the NAFLD prevalence rate is 66.2% in obese children. Minimum and maximum age range was 8-16 years. Average age was not significant in group 1. (12.06±1.76),group 2. (11.7±2.21),group 3. (11.4±1.05),group 4. (11.0±1.20) (p= 0.181). Primary outcome: In VSL#3 plus lifestyle interventional group had 38.5% none fatty liver disease, probiotic VSL#3 only had 33.3%, Lifestyle intervention had 30.8% and Placebo group had 0%at completion of the study as shown in [Table 1].

The anthropometric and biochemical parameters at before and after intervention in different groups have shown in [Table 2]. VSL#3 plus lifestyle intervention group had weight loss of 9%, Probiotic VSL#3 only group had 4.2%, Lifestyle intervention had 5.3% and placebo group had 0% weight loss respectively. All biochemical parameters AST, ALT, GGT, HSCRP, LDL-c, cholesterol, triglyceride, FBG, uric acid were most significantly reduced and increase in HDL-c in VSL#3 plus lifestyle intervention group(<0.05) as compared to VSL#3 and lifestyle intervention group alone.

[Table 3]. explains the pre and post intervention outcomes of obesity hormones (leptin and ghrelin). Both obesity hormones were significantly changed in different intervention groups after treatment (p<0.001).Leptin levels was declined most in VSL#3 plus lifestyle intervention group (-4.40 ng/ml), in another groups i.e. probiotic VSL#3 only (-2.75), lifestyle intervention group (-2.85) and (+1.24) increase in placebo group respectively. Ghrelin (orexigenic hormone) levels was most increased in VSL#3 plus lifestyle intervention group (+6.71ng/ml) as compared to another groups i.e. probiotic VSL#3 only (+4.11), lifestyle intervention group (+5.20) and (-1.22) decreased in placebo group respectively.

The nutrients intake of calorie, protein, fat, CHO that was more significantly reduced and increased intake of fiber in three interventional groups(p<0.05) as compared to placebo at the end of study. [Table 4]

Discussion

In present study combined therapy of probiotic VSL#3 with lifestyle intervention is most effective novel treatment for the management of NAFLD in obese children as compared to single therapy of VSL#3 and Lifestyle intervention (diet+ physical activity). Combined therapy group had much weight loss (5.5 kg), BMI; 27.2 to 24.7kg/m2 and also had 38.5% none fatty liver disease at the end of study [Table 1&Table 2].In similar study Alisi *et al*. showed probiotic VSL#3 was effective for reducing BMI; 27.1 to 24.9 kg/m2 including 21% had none fatty liver and also increased in GLP-1 hormone secretion at completion of trial but they have not found any significant differences in TG, ALT and HOMA.(4) In our study all anthropometric measurements; BMI, MAC, TSF, WC most significantly reduced and also blood parameters; AST, ALT, GGT, LDL-c, cholesterol, TG, HSCRP, uric acid, FBG were also most reduced and increase in HDL-c in VSL#3 plus lifestyle intervention group as compared to VSL#3 alone and lifestyle intervention group. Obesity hormones, leptin and ghrelin regulates food intake in human

that also significantly changed after treatment [Table 3]. In meta-analysis probiotic therapy is cornerstone management of NAFLD with the reduction of AST, ALT, total cholesterol, ameliorate IR and (tumor necrosis factor -alpha)TNF-α. Because it modulates the gut microbiota a new evidence for treating gut health problems through targeting gut liver axis.(8) In another meta analysis of RCT's resulted that probiotic therapy is effective for reducing lipid profile and FBG.(8) Another study concluded that probiotic is effective for treating NAFLD with reduction of WC, AST, ALT, lipids in pediatrics.(9) A study observed significantly decline in cholesterol with consumption of 200g yoghurt enriched with potential bacterial strains had hypocholesterolemic effect and showed benefits effects in lipid profile.(10) Moreover, another study showed significantly increased in HDL-c levels with long time consumption of 300g yoghurt enriched with B. Longum and lactobacillus acidophilus strains.(11,12) and Bukowska *et al*. found L. plantarum reduced fibrinogen and pro-atherogenic biomarkers.(13) Rajkumar *et al*. observed VSL#3 was effective for reducing HSCRP, cholesterol, and increased HDL-c in overweight adults.(14) They also pronounced that BSH (bile salt hydrolase) gene might responsible for reduction of cholesterol but its concerned mechanism is unknown. In present study, increased intake of fiber with probiotics VSL#3 improved digestion and absorption of nutrients in the gut and that enabled to compete with various higher biomarkers i.e. liver enzymes, lipid profile and also (IR).The proposed mechanism of VSL#3 is associated with production of short-chain fatty acids (SCFA) by beneficial bacteria especially butyrate has weight loss effect. Yadav at al. demonstrated that butyrate enhanced the GLP-1 secretion from L-cells of intestine that suppressed food intake and prevent weight gain in mice.(15) Firmicutes are the largest proportion in the gut of obese person with NAFLD and diabetes.VSL#3 contains 8 strains of beneficial bacteria that enhanced the proportion of bacteriodetes and balanced the ratio of firmicutes and bacteriodetes, bifidobacteria in the gut. In mice model VSL#3 modulated the gut microbiota composition, improved the inflammatory state, increased metabolic efficiency that protects obesity, IR with NAFLD.(15) In our study VSL#3 alone therapy group reduced their food intake and had weight loss (2.5 kg) because of improvement in gut functions and satiety hormones [Table 3]. In one study postulated that enhance in GLP-1 levels are related with higher energy expenditure in resting state with increased fat oxidation rates in humans.(16)Another study concluded that probiotic and prebiotic supplement alongwith diet plus exercise had beneficial impact on glucose homeostasis and leptin levels than lifestyle intervention alone.(17) According to our findings VSL#3 plus lifestyle group had much better impact on leptin and ghrelin levels including IR than other single therapies groups. Wang *et al*. demonstrated that a lifestyle intervention significantly improved liver enzymes and decreased IR. In another study lifestyle intervention (diet restriction plus 1hour moderate activity/day) reduced average 7.1 kg weight in 10-weeks of weight loss camp.(18) In our study lifestyle intervention (diet restriction plus moderate aerobic exercise of 30 minutes/day) group had 3.1 kg weight loss in 16 weeks and improved liver function tests. Behavior modifications for weight loss programme needs tact and time especially children. Less compliance in obese children for lifestyle modification facilitates other treatment approach. We could not get consent for liver biopsy for histology examination but ultrasonography technique is accurate and non-invasive for diagnosis.(19) CK18 M30 and leptin are practical tests for elucidating disease extremity in children with NAFLD(20). We have not done serum CK18 M30 but leptin levels were done in pediatric NAFLD. High calorie diet with mixture of high carbs with more fat led to increase the pathogenesis of NAFLD.(21) In present study, Diet counseling with exercise sessions conducted and their intake of fat and CHO decreased including increased intake of fiber as compared to baseline intakes [Table 4].This is the first study done in India to see the effect of probiotic VSL#3 and lifestyle intervention on leptin, ghrelin hormones levels in pediatrics NAFLD

Conclusion

In our study Probiotic VLS#3 and lifestyle intervention are effective for managing NAFLD in obese child but VSL#3 plus lifestyle intervention significantly the most pronounced therapy for reducing serum AST, ALT,GGT, LDL-c, HSCRP, cholesterol, TG, uric acid, FBG and increase in HDL-c and also significantly most effective for leptin and ghrelin hormones. Multi-target therapy of probiotic VSL#3 plus lifestyle intervention could a novel approach for treating NAFLD, further studies are required to see the effect of VSL#3 on all obesity

hormones and other inflammatory markers in obese children with advance stage NAFLD.

Recommendation

Hence, it is recommended that Probiotic VSL#3 plus lifestyle intervention is most potent therapy for managing NAFLD and NASH in obese children when single therapy of diet with exercise is less effective because of lessen compliance in children.

Limitation of the study

Presently, we did not perform the following diagnostic tests: NEFA (non-esterified fatty acid), OGTT (oral glucose tolerance test) and HOMA (homeostasis model assessment) which can indicate the substantial mechanistic link between obesity and insulin resistance, T2DM (type-2 diabetes mellitus) and metabolic dyslipidemia and hence, predicting the risk of NAFLD. The diagnosis of NAFLD was based upon USG, we could not get written consent for liver biopsy.

Relevance of the study

Probiotic VSL#3 and Lifestyle intervention have beneficial impact on leptin (anorexigenic) and ghrelin (orexigenic) hormones.

Authors Contribution

All the authors contributed appreciably to the design, accession of data, analysis, interpretation, drafting of the article and final approval.

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Tables

TABLE 1 LIVER GRADES IN PERCENTAGE BEFORE AND AFTER INTERVENTION

Fatty Liver Grades	VSL#3 plus lifestyle intervention		Probiotics VSL#3		Lifestyle intervention		Placebo	
	Before	After	Before	After	Before	After	Before	After
None (0)	0%	38.5%	0%	33.3%	0%	30.8%	0%	0%
Grade (1)	38.5%	53.8%	37%	51.9%	38.5%	53.8%	40.7%	44.4%
Grade (2)	50.0%	7.7%	51.9%	14.8%	50.0%	15.4%	48.1%	44.4%
Grade (3)	11.5%	0%	11.1%	0.0%	11.5%	0%	11.2%	11.1%
***p value**	p<0.001		P<0.001		P<0.001		p= .317	
*Wilcoxon Singed Rank test p<0.05								

TABLE 2 ANTHROPOMETRIC AND BIOCHEMICAL PARAMETERS OF OBESE CHILDREN WITH NON-ALCOHOLIC FATTY LIVER DISEASE

Variables	VSL#3+ Lifestyle (n=26)		Probiotic VSL#3(n=27)		Lifestyle intervention (n= 26)		Placebo(n=27)	
	Before	After	Before	After	Before	After	Before	After
Weight(kg)	61.6±12.2	56.1±11.7**	60.7±12.3	58.2±12.1**	59.2±8.12	56.1±8.00**	57.5±8.33	57.3±8.43NS
BMI(kg/m2)	27.2±3.74	24.7±3.83**	27.1±4.07	26.0±4.06**	27±3.57	25.6±3.46**	27±3.23	26.9±3.17NS
Zscore	2.61±0.30	2.15±0.34**	2.64±0.40	2.52±0.38**	2.63±0.39	2.49±0.39**	2.63±0.44	2.63±0.43NS
MAC(cm)	33.7±2.39	32.0±2.52**	33.3±1.93	32.4±2.06**	33.8±3.23	32.8±3.47**	32.8±2.81	32.8±2.81NS
TSF(mm)	20.7±1.97	18.7±2.04**	20.4±2.00	19.0±2.29**	20.7±2.51	19.2±2.75**	19.3±2.32	19.4±2.38*
WC(cm)	87±1.54	84.5±1.68**	86.4±1.55	85.3±1.46**	86.3±1.94	84.8±1.92**	85.3±2.23	85.6±2.28**
AST (IU/L)	47.3±5.22	37.3±3.08**	46.5±5.97	40.2±4.68**	49.5±5.99	45.5±5.20**	46.4±6.45	47.1±6.55NS
ALT (IU/L)	58.1±7.36	45.2±4.95**	50.9±8.36	44.4±9.22**	51.5±9.72	47.1±9.18**	47.6±10.4	47.5±10.4NS
GGT(IU/L)	21.4±3.07	18.0±2.48**	21.7±3.28	18.6±3.44**	21±2.29	20.1±2.61**	20.8±2.22	20.8±2.18NS
LDL-C mg/dl	102±9.64	97.3±8.35**	100±8.09	96.9±7.95**	106±10.3	106±10.1NS	99.4±10.6	99.3±10.4NS
HDL-C mg/dl	38.9±3.90	41.9±3.49**	39.9±4.02	42.1±3.51**	40.2±5.02	40.3±4.92NS	40.1±3.37	40.2±3.30NS
Cholesterol	172±8.45	164±7.06**	170±9.51	165±8.42**	169±8.14	169±7.58NS	173±6.73	174±6.55NS
TG mg/dl	147±7.15	137.2±5.38**	151±3.82	146±3.81**	150±6.53	147±7.33*	150±4.07	149±4.08NS
HSCRP mg/L	2.46±0.40	1.33±0.41**	2.39±0.46	1.28±0.55**	2.36±0.30	2.11±0.13**	2.34±0.33	2.44±0.41*
FBG mg/dl	95.1±7.45	88.4±7.22**	98.4±5.73	94.7±5.66**	102±13.1	99.8±11.7**	99.14±5.46	98.10±4.83NS
Uric acid mg/dl	5.51±0.85	3.50±0.48**	5.10±0.90	3.84±0.81**	5.08±0.78	4.15±0.45**	5.08±0.94	5.15±0.94NS
Wilcoxon Signed Ranks Test p<0.001**, p<0.05*, p>0.05NS (not significant), Data are presented in mean±SD								

TABLE 3 PRE AND POST LEVELS OF OBESITY HORMONES IN OBESE CHILDREN WITH NON-ALCOHOLIC FATTY LIVER DISEASE

Interventional groups	Pre Ghrelin n=26	Post Ghrelin n=27	Pre Leptin n=26	Post Leptin n=27	Changes in Ghrelin	Changes in Leptin
Vsl#3+Lifestyle intervention	25.1±3.12	31.8±3.05	23.0±5.24	18.6±4.96	6.71±1.81	-4.40±0.66
Probiotic VSL#3	25.1±2.80	29.2±3.51	22.5±3.01	19.7±2.96	4.11±3.10	-2.75±0.46
Lifestyle intervention	25.5±2.87	30.7±3.27	23.8±3.68	21.0±3.54	5.20±1.44	-2.85±1.33
Placebo	25.5±3.07	24.3±3.16	23.5±3.83	24.7±3.89	-1.22±2.08	1.24±1.75
P value	0.824	<0.001**	0.490	<0.001**	<0.001**	<0.001**
Kruskal- Wallis test p<0.05, Data are presented in mean±SD, p<0.001**						

TABLE 4NUTRIENT INTAKE OF OBESE CHILDREN WITH NON-ALCOHOLIC FATTY LIVER DISEASE IN DIFFERENT INTERVENTION GROUPS

Nutrients	Before	After	Before	After	Before	After	Before	After
Energy(kcal)	2496±317	2045±269**	2475±370	2269±346**	2435±357	2134±357**	2420±366	2400±367**
Protein(g)	62.4±7.55	56.2±7.42**	61.8±9.27	59.8±9.66**	62.5±5.35	58.6±9.83*	60.5±11.7	60.7±11.1*
Fat(g)	91.8±9.63	70.9±9.35**	90±13.0	82.3±13.2**	88.9±3.93	71.1±11.9**	89.1±5.62	87.8±5.82*
CHO(g)	369±40.9	295±38.9**	354±52.3	324±52.4**	370±29	314±52.7**	383±69.2	389±71.6*
Fiber(g)	13.42±1.81	20.0±2.11**	12.44±1.28	15.77±2.11**	13.23±1.79	18.53±2.08**	12.05±1.13	12.40±1.36ns
Data are presented in mean±SD, Wilcoxon Signed Rank Test, P<0.001**, P<0.05*,P>0.05 ns(non significant)								

Figures

FIGURE 1 FLOW DIAGRAM OF STUDY