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Effect of probiotics on the occurrence of nutrition absorption capacities in healthy children: a randomized double-blinded placebo-controlled pilot study

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Abstract. – OBJECTIVE: Recent advances in the translational research showed that dietary nutrients have critical importance to the microbioma balance in the gastrointestinal tract. Therefore, the alteration of the intestinal microbiota in order to achieve, restore, and maintain favorable balance in the ecosystem, and the activity of microorganisms present in the gastrointestinal tract is necessary for the improved health condition of the host. The objective of this translational study was to evaluate, in a pediatric population, the efficacy and safety of prophylactic probiotics for a better nutritional absorption capacity in the view to enhance their overall health and immunity.

PATIENTS AND METHODS: A total of 40 pediatric patients between the ages of 14 and 18 years were enrolled in the study and divided under two categories (treated/active group and placebo group). Three-time points clinical evaluations were performed: a baseline assessment (Time 0), a second evaluation at 5 weeks after the start of probiotic use (Time 1), and a final evaluation at the timeline after 10 weeks (Time 2). In the initial phase of the study, the recruited subjects underwent a panel of initial T0 clinical tests. For each of the patients, a blood sample was taken in order to evaluate the following biochemical measurements: Vitamin D, Vitamin A, Calcium, Zinc, and Iron. Moreover, an initial nutritional evaluation was carried out through which the nutritionist estimated the body composition of the subject (weight and body mass index), the caloric needs and dietary behaviour of each recruited patient.

RESULTS: Eligible participants were randomized into placebo (n = 20) or treated/active (n = 20) treatment conditions by random allocation using a computerized random number generator, ensuring all investigators remained blind to the treatment distribution. The data were compared within and between groups using statistical methods. The results confirmed that the probiotic supplementation was effective in increasing the overall blood biomarkers levels of vitamins, calcium, and mineral absorption from baseline to 10 weeks of treatment, compared with the placebo.

CONCLUSIONS: Probiotics may be suggested as supplements to improve biomarkers serum concentration if administered for a period of at least ≥5 weeks. However, further studies are required for optimal recommendations in patient treatment.

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Introduction

The use of dietary supplements, such as probiotics, has increased over time worldwide. Probiotics (comes from the greek term, and it means "for life") are nonpathogenic organisms (yeast or bacteria, especially lactic acid bacteria) in foods that can exert a positive influence on the host's health[1-4].

An approach for modulation in the gut microbiota is the use of oral viable strains of bacteria (probiotics), indigestible carbohydrates (prebiotics), or synergistic combinations of a probiotic and prebiotic (synbiotic)[5,6].

Results of clinical studies confirm the positive effect of probiotics on gastrointestinal diseases (e.g., irritable bowel syndrome, gastrointestinal disorders, elimination of *Helicobacter*, inflammatory bowel disease, diarrheas) and allergic diseases (e.g., atopic dermatitis). Many clinical studies have proven the effectiveness of probiotics for the treatment of diseases such as obesity, insulin resistance syndrome, type 2 diabetes, and non-alcoholic fatty liver disease. Furthermore, the positive effects of probiotics on human health have been demonstrated by increasing the body's immunity (immunomodulation)[3,4].

As well as improving nutritional status by mitigating the effects of gastrointestinal diseases, there is growing evidence[3] that probiotics and prebiotics could be used to improve the absorption of micronutrients (such as calcium and iron) from ingested foods.

Primary bacterial stimulation of gut-associated immune cells is essential for the development and maturation of the pediatric population immune response. This phenomenon has been affected due to a decrease in vaginal births (which are the first source of bacterial exposure after birth), the substitution of breastfeeding, in favor of almost sterile formulas and increase the use of antibiotics. In addition, increased hygienic measures and pasteurization perpetuate this decreased microbe-host interaction[7,8].

The consumption of certain probiotics has positive effects on gut barrier function and immune response, improving host-microbe interactions for health maintenance, harvest nutrients, and further substances, such as short-chain fatty acids, vitamins, amino acids, polyamines, growth factors, and antioxidants, and for the management of a number of illnesses[9-11].

Nowadays, different studies focuses on the current knowledge concerning the mechanisms in vivo and challenges in translational research on probiotics. A better understanding of the clinical and/or molecular mechanisms of probiotics, the effect of probiotic mixtures versus single strains, the effect of formulation of probiotics and the fate of ingested probiotics should help to clarify the value of immune assays as selection criteria for probiotics.

The purpose of the present translational study was to evaluate the efficiency of specific probiotics customized for children for better nutritional absorption capacity that can enhance their overall health and immunity.

Patients and Methods

This research was conducted in collaboration with the Elbasan University (School of Technical Medical Sciences, "A. Xhuvani"), Albania, and the University of Bari Aldo Moro as a randomized, double-blinded placebo-controlled study. The Institutional Ethics Committee of the Faculty of Technical Medical Sciences of Elbasan "ALEKSANDËR XHUVANI" has approved the application to conduct the clinical trial in the Faculty. Title of the Protocol: Probiotics efficacy and safety in humans. Protocol Identification: INTL_ALIT-COOP/Probiotics/INRES2019_w/a/c.

Subjects Recruited

A total of 40 paediatric patients between the ages of 14 and 18 years were enrolled in the study and divided under two categories. All patients' parents or guardians involved in this study were requested to read, understand, and sign an informed consent. The study was conducted in compliance with the "Ethical Principles for Medical Research Involving Human Subjects" of the Helsinki Declaration. Patients' parents or guardians received a verbal description of the clinical protocol to be followed in this proposal of a clinical study. Eligible participants were randomized into placebo (n = 20) or active (n = 20) treatment conditions group, ensuring that all investigators remained blind to the treatment allocation.

A total of 40 healthy paediatric patients, with similar body mass index (BMI) and age were randomly allocated to receive either (1 tablet/day)

a placebo (tablets looking similar but without probiotics) or a symbiotic containing a specific targeted probiotic (Hyperbiotics PRO-Kids – US Patent 8,007,777 and 7,150,623), composed of 4 different probiotics patented stains (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacter infantis*, *Bifidobacter Lactis*), a prebiotic fructo-oligosaccharides (FOS), lacose free, gluten free, patented for LiveBac® manufacturing process, and BIO-tract®, a protection and time-release delivery.

The tested formula contains 3 Billion Colony Forming Units per BIO-tract® pearl, which is equivalent to 45 Billion colony forming units (CFUs) of standard probiotic capsules.

The main inclusions criteria were: healthy similar body mass index (BMI) and age group in all enrolled subjects.

The exclusion criteria were the following: no antibiotics for the last 4 weeks, no nutritional supplements, no active drug treatment, no malnutrition condition.

Randomization

To have the unbiased and accurate clinical data, we followed a double-blind protocol for enrolment of the patients in terms of the treatment plan and further categorization into the study group. A computer-generated list of random numbers was selected to create a series of sequentially numbered envelopes covering equal assignments to either placebo or probiotics.

The study clinical biochemistry investigator was responsible for the randomization and the study immunologist investigator for delivery of the blinded supplements. The supplements, which were assigned letter A or B, are otherwise identical and the participants, investigators, outcome assessors, and data analysts were blinded after assignment to interventions[6].

Clinical Outcomes

The primary outcome was the assessment of supplementing digestive probiotics (Hyperbiotics PRO-Kids) in kids for better nutritional absorption capacity that should enhance their overall health and immunity.

The secondary outcome included the blood biomarkers levels of Vitamin (D and A), Calcium, and Mineral Absorption (iron and zinc) from baseline to 10 weeks (laboratory sampling between 7:00 and 9:00 am).

Approximately 5 ml of blood were taken *via* vein puncture on each of the testing days following the vein of each participant and collected in BD Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA). The blood samples collected in the BD Vacutainer® were centrifuged at 2500×g for 10 min, and the serum was separated for serum biochemical analysis.

Statistical Analysis

The mean values for Treaded and Control groups were analysed using the GraphPad Prism (version 6.01, La Jolla, CA, USA) and IBM Statistical Package for the Social Sciences (SPSS Inc. Version 16.0, Chicago, IL, USA) software. Statistical analyses were performed using the Statistical Product and Service Solution (SPSS) Statistics 21 software (IBM Corp., Armonk, NY, USA). Data were analysed with ANOVA tests. A value of $p<0.05$ was considered statistically significant.

Results

The purpose of this study has been to evaluate the efficiency of specific probiotics customized for the pediatric population for better nutritional absorption capacity to enhance their overall health and immunity. The study has been managed in different stages, as reported in Figure 1.

The participants who met the preliminary criteria were boys and girls aged between 14 and 18 years old. This randomized clinical trial included a first enrolment phase. All subjects were examined by a medical expert through clinical history and physical examination. The standardized basic assessment included the compilation of a format concerning demographic, clinical, and risk factors information.

Furthermore, on the first visit, the participants were instructed on the objectives and methods of clinical trials. The subjects who agreed to participate in the study, after having signed the informed consent, were randomly included and divided into two groups: a test group using probiotics, instead, to the other group was provided a placebo substance, without probiotics.

Totally, 40 subjects were enrolled as follows:

a) Treated (Active) group (Hyperbiotics PRO-Kids): 7 girls and 13 boys;

b) Placebo group: 4 girls and 16 boys.

The participants were asked not to alter their routine habits during the study.

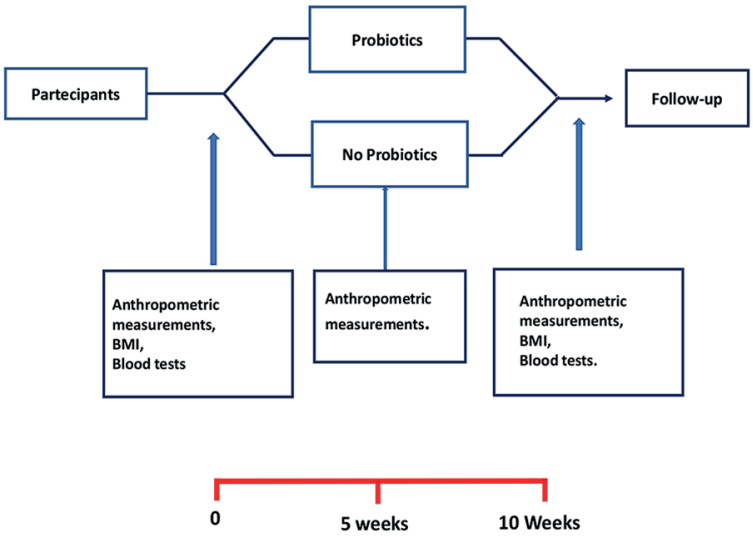

Figure 1. Summary of patient's flow diagram.

We selected three clinical evaluations of the study population: a baseline assessment (Time 0-T0), a second evaluation at 5 weeks after the start of probiotic use (Time 1-T1), a final evaluation at the timeline after 10 weeks (Time 2-T2). In the initial phase of the study, the recruited subjects underwent a panel of initial T0 clinical tests. For each of the patients, a blood sample was taken in order to evaluate the following biochemical measurements: Vitamin D, Vitamin A, Calcium, Zinc, and Iron (T0 and T2). Moreover, an initial nutritional evaluation was carried out through which the nutritionist estimated the body composition of the subject, the caloric needs, and the dietary behaviour of each recruited patient (T0, T1, and T2). During the visit, data of nutritional interest were acquired, namely: I-) • anthropometric measurements (body weight); II-) • body mass index (BMI).

As not all probiotics are helpful in overall conditions, a careful selection of proper strains of bacteria established on the desired clinical outcome is of vital importance. Therefore, we selected a combination of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacter infantis*, *Bifidobacter Lactis*, which have been shown in the current literature to improve health outcomes in humans, according to our findings (data shown in comparative charts figures).

In fact, there was a significant difference in the mean values, if we compare the two study groups ($p<0.001$). The results presented in the comparative chart figures show the effects of the probiotics supplementation *vs.* placebo supplementation in both groups at different interval times. Patients in the treated group show a weight increased from T0 to T2 of about 1.5%, the same situation is found in the placebo group; the BMI of the treated subjects remained unchanged and the same thing occurs for the placebo group (Figures 2 A-B and 3 A-B).

From the graphs, we can see that the levels of Vitamin A and Vitamin D between the two groups at different times have varied slightly. On one hand, the variations mainly concern the Treated group where we find an increase of about 9% in the levels of Vitamin A and 3.2% of Vitamin D, on the other hand in the placebo group we have a 1% increase in the levels of vitamin A and a 3% reduction in Vitamin D levels (Figures 4 A-B and 5 A-B).

From the comparative graphs, we can see how the levels of calcium have changed between the time T0 and T2 in the two different groups. In the treated group, there is an increase of 4%, on the contrary, in the placebo group we have a reduction of about 7% (Figures 6 A-B).

Zinc levels from time T0 to T2 increased by 1.7% in the treated group, while there was a remarkable reduction of about 3.5% in the placebo group (Figures 7 A-B).

From the comparison of the graphs we can see that the iron levels increased by 2% in the group treated from T0 to T2, while in the placebo group a reduction of 1% is shown (Figures 8 A-B).

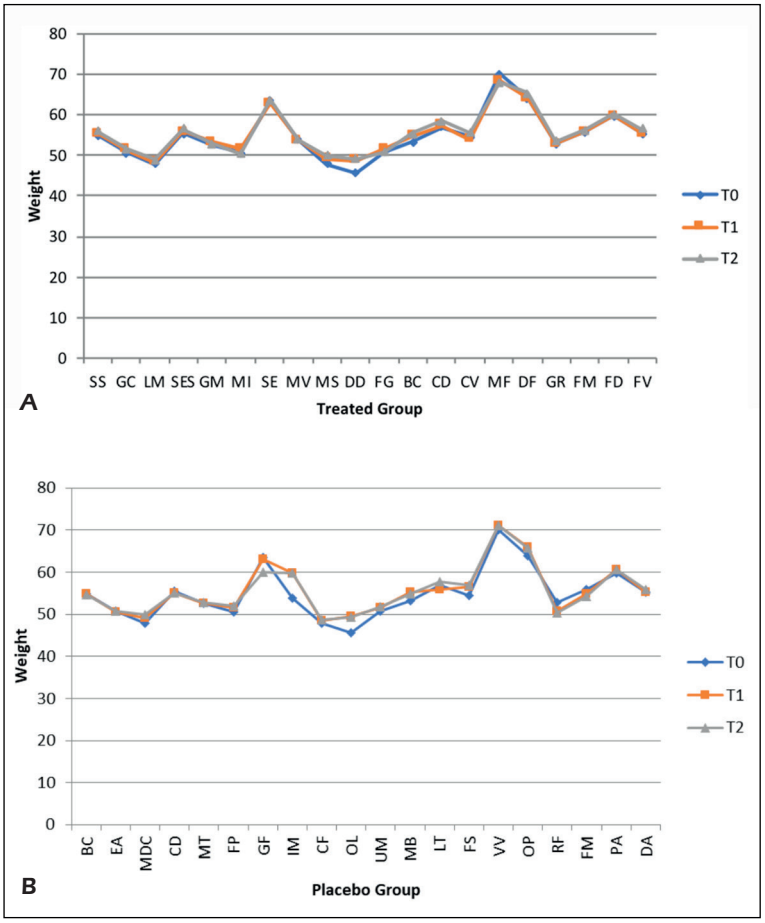

Figure 2. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Data of nutritional interest as anthropometric measurements (body weight) were recorded at baseline (T0) and after 5 weeks (T1) and 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (body weight) value.

No adverse events were reported as a result of the active or placebo treatment, or as a result of the study procedures.

Discussion

A meta-analysis conducted in 2015 and covering all studies (totally 23) conducted up to 2014, assesses the efficacy and safety of probiotics (any specified strain or dose) used for antibiotic-associated diarrhea (AAD) prevention in children[12]. These studies talked about the effects of probiotics on dyskinesia caused by the use of antibiotics on children and how they could improve or worsen the situation. Most studies dealt with probiotic treatments such as *Bacillus spp., Bifidobacterium spp., Clostridium butirricum, Lactobacilli spp., Lactococcus spp., Leuconostoc cremoris, Saccharomyces spp.* O *Streptococcus spp.;* these were administered in various doses and of different duration, combined or not. In 22 studies it was reported that a probiotic treatment improves the average duration of diarrhea. The effect combined with the use of antibiotics increases their efficiency. None of the studies had any serious side effects due to the administration of probiotics. In some cases, are reported adverse events such as skin rash, vomiting, and phlegm[12,13].

In a less recent study, conducted in 2002, researchers have shown, through a randomized clinical trial with a few patients in childhood, the efficacy of probiotic administration for the treatment of diarrhea. The subjects who received probiotics as a cure showed a frequency of diarrhea of 37%, by contrast, the control group showed a frequency of 80%[14].

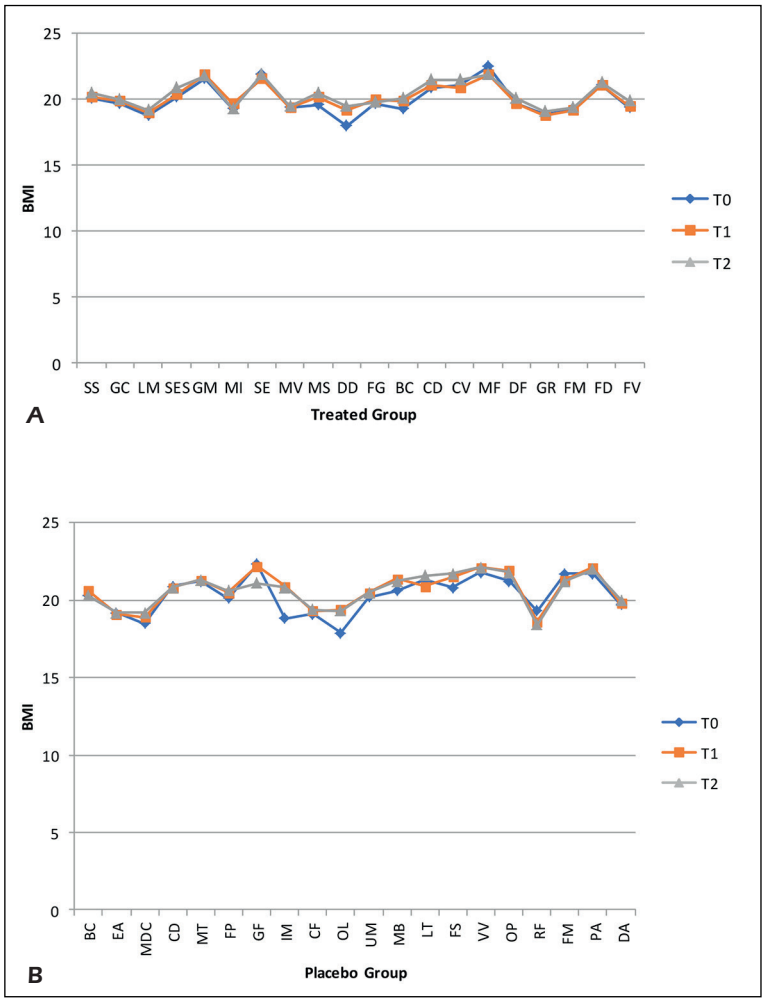

Figure 3. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Data of nutritional interest as body mass index (BMI) were recorded at baseline (T0) and after 5 weeks (T1) and 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (BMI) value.

A combination of *Bifidobacterium longum* PL03, *Lactobacillus rhamnosus* KL53A and *Lactobacillus plantarum* PL02, in a group of 78 children, aged 5 months 16 years, with otitis and various infections and treated with antibiotics, proved to be efficient. In the treated group formed of 40 children using the classic antibiotic, were added 10 colony-forming unit (CFU) of the combination of probiotics, while the other group with placebo effect was formed by 38 children. The administration of probiotics was carried out twice a day throughout the duration of the antibiotic treatment. The results showed that the patients receiving probiotics had a similar rate of diarrhea (≥3 loose or watery stools/day for ≥48 h occurring during or up to 2 weeks after the antibiotic therapy) as those receiving placebo (relative risk 0.5, 95% CI 0.06-3.5). The mean number of stools per day was significantly lower in the experimental group (mean difference -0.3 stool/day, 95% CI -0.5 to -0.07). The combined treatment with probiotics had no adverse effects, however this study did not show great results as while the frequency of feces per day decreased and the rate of diarrhea remained unchanged[15].

Probiotics have been described as "live microorganisms that, if dosed in adequate quantities, have a health benefit on the host", through their impact on the intestinal tract. The harmful action of antibiotics on microorganisms in the intestine justifies the idea of using probiotics to reduce the incidence of diarrhea consequently dyskinesias.

In a study conducted in August 2018, the authors showed that *Lactobacillus reuteri* (*L. reu-*

teri) DSM 17938 does not prevent diarrhea resulting from the use of antibiotics in children. The study was conducted on 250 children hospitalized for various infections. The children were divided into two different groups: one treated group and one group with a placebo effect. *L. reuteri* (at 2 × 10^8 CFU) was administered twice a day for the duration of the antibiotic treatment in the in treated patients. The data were taken even after the end of the treatment for a week. Stool frequency and stool consistency criteria were defined based on one of three definitions: (i) three or more soft or watery stools per day for ≥48 h; (ii) three or more soft or watery stools per day for ≥24 h; or (iii) two or more soft or watery stools per day for ≥24 h. Taking into consideration the most severe form of diarrhea the results obtained were the following: the occurrence of diarrhea in the *L. reuteri* group was 25 (20%) compared with 16 (13%) in the placebo group (absolute risk reduction -0.07 with values from -0.17 to 0.02). The results, therefore, showed that *L. reuteri* does not improve and does not prevent diarrhea in 4-month-old children[16].

The result of the study described above shows that not all probiotic strains are useful for reducing and combating the appearance of diarrhea following the use of antibiotics. For this purpose, in 2015 Canani et al[17] compared 5 types of different probiotics. In this randomized clinical trial, 571 Italian children were elected, from 3 to 36 months, to whom *Lactobacillus rhamnosus GG* was administered per group; *Saccharomyces boulardii; Bacillus clausii*; mixture of *Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum*; or *Enterococcus faecium* SF6. Only one rehydration solution was distributed to a control group. According to the experimental design, the administration of probi-

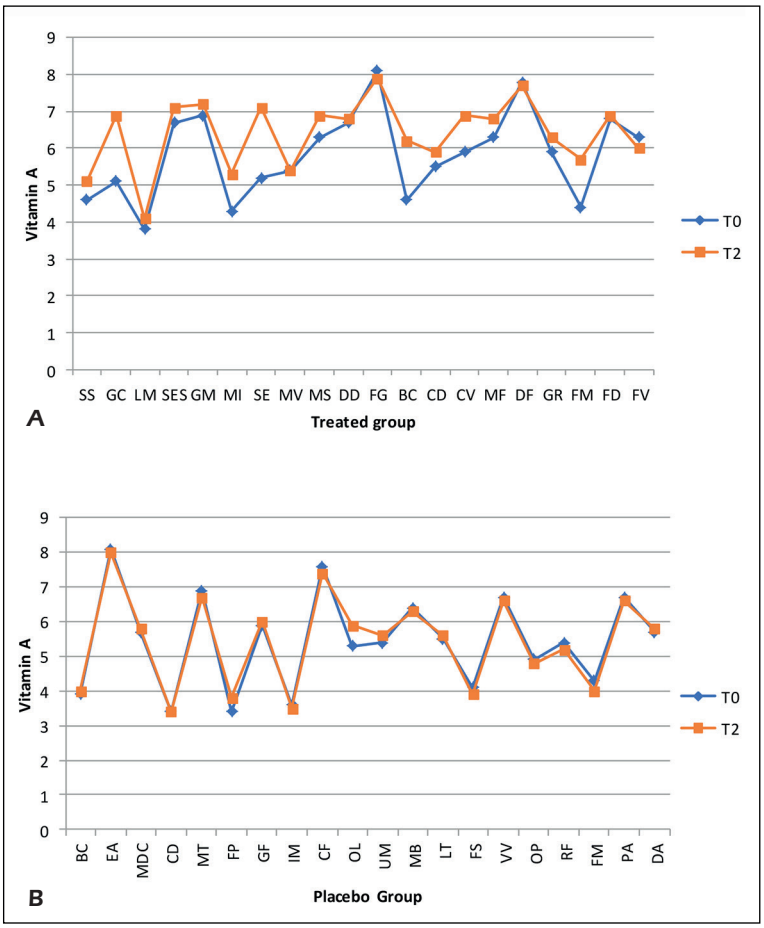

Figure 4. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Blood biomarkers levels of Vitamin A were recorded from baseline (T0) to 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (Vitamin A) value.

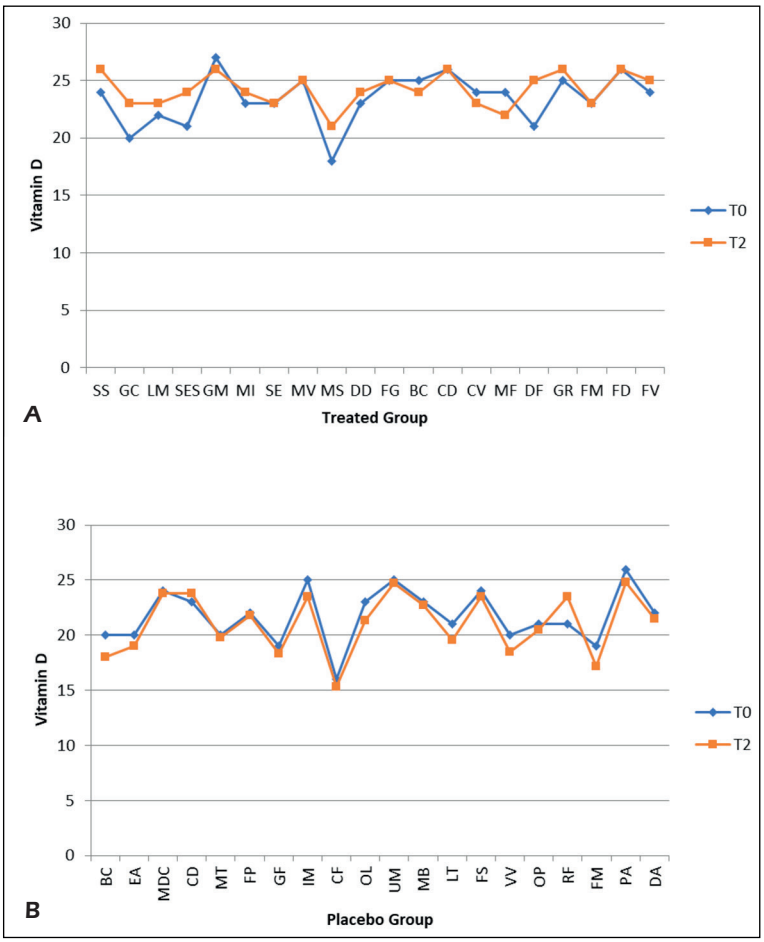

Figure 5. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Blood biomarkers levels of Vitamin D were recorded from baseline (T0) to 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (Vitamin D) value.

otics lasted for 5 days and was carried out orally, the solution was dissolved in 20 ml of water.

Group 1 took only a rehydration solution. The results of the study were evaluated based on the duration of diarrhea and stool consistency. Stool consistency was evaluated through a score system and feces were graded as 1 (normal), 2 (loose), 3 (semiliquid), and 4 (liquid). The results of the study were evaluated based on the duration of diarrhea and stool consistency.

The shortest duration of diarrhea occurred in the treated group number 2 and 5 or the one with the mix of bacteria compared to patients treated with the only rehydration solution. The other three probiotic strains had no effect either on the duration or on the stool consistency, with an effect very close to the rehydration solution served as controls. Stool consistency, rated by the scoring system, differed significantly ($p<0.001$) with preparations 2 and 5 compared to the other groups. Microbiological investigations were required only in some cases and the results did not provide useful information. None of the subjects who participated in the study had any side effects. In conclusion, the researchers stated that not all probiotics have an effective action against diarrhea resulting from the use of antibiotics. *Lactobacillus rhamnosus* GG has been associated with a shorter duration of diarrhea, *Streptococcus faecium* strain SF68 has resulted in clinical improvement in children with diarrhea associated with respiratory infection and treated with parenteral antibiotics[17].

In a double-blind, randomized, placebo-controlled study was evaluated the effectiveness of probiotic yogurt on diarrhea caused by the use of antibiotics in children. The study included two different groups; one group was given probiotic

yogurt, while to the other group was given a normal pasteurized yogurt. The study included 72 children who used antibiotics for various infections. The children were divided into two groups: a treated group and a placebo effect. Probiotic yogurt contained *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium lactis* (Bb-12) and *Lactobacillus acidophilus* (La-5) and was administered for a week with a dosage of 200 g per day. The results were evaluated through the frequency and consistency of the feces. At the end of the study there were no cases of severe diarrhea in the probiotic group while the severe cases were reported in six children in the placebo group. Only one episode of minor diarrhea was recorded in the probiotic group compared to 21 in the placebo group. The subjects in the treated group had fewer undesirable effects while in the placebo group there were 11 negative episodes including headache, vomiting, and abdominal pain. The researchers concluded the study by confirming the validity of probiotic yogurt add with LGG, La-5, e Bb-12 on diarrhea caused by the use of antibiotics in children aged 1-12[18].

The combination of *Bifidobacterium lactis* and *Streptococcus thermophilus* has positive effects in children. In a randomized clinical trial, the researchers assessed their combined efficacy. In the study, 80 pediatric children were enrolled, already subjected to various antibiotic treatments and assigned to two groups: a treated group where the children received 10 CFU of *Bifidobacterium lactis* and 10 CFU of *Streptococcus thermophilus* for two weeks, twice a day, and a group with a placebo effect. The total duration of the study was 30 days.

The children were checked daily. The recorded data was based on the hardness and frequency

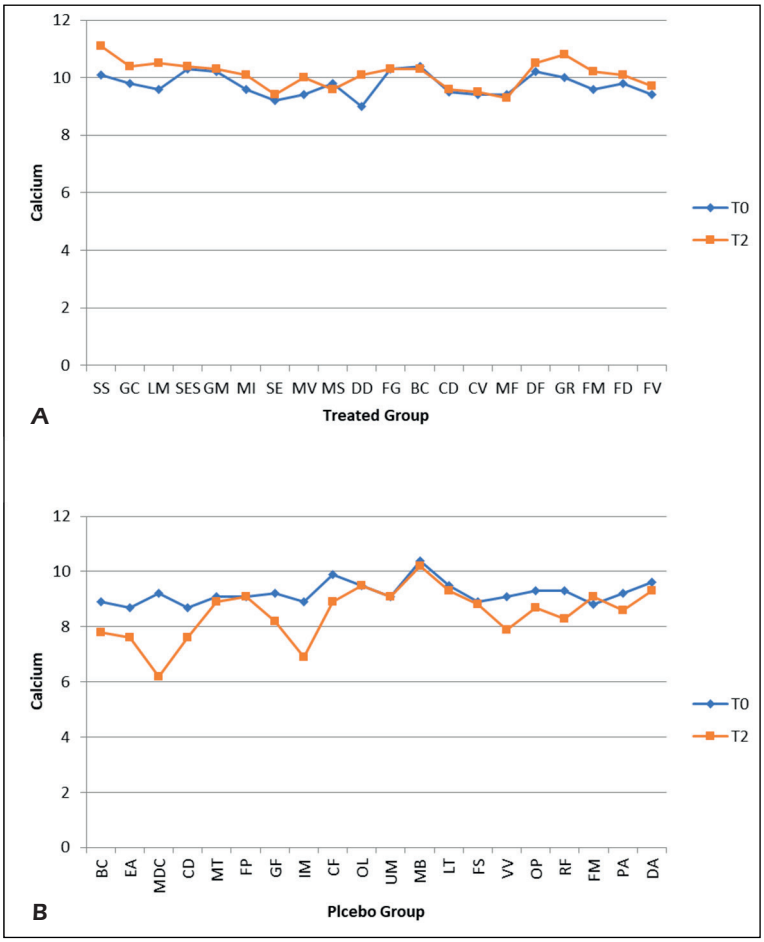

Figure 6. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Blood biomarkers levels of Calcium were recorded from baseline (T0) to 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (Calcium) value.

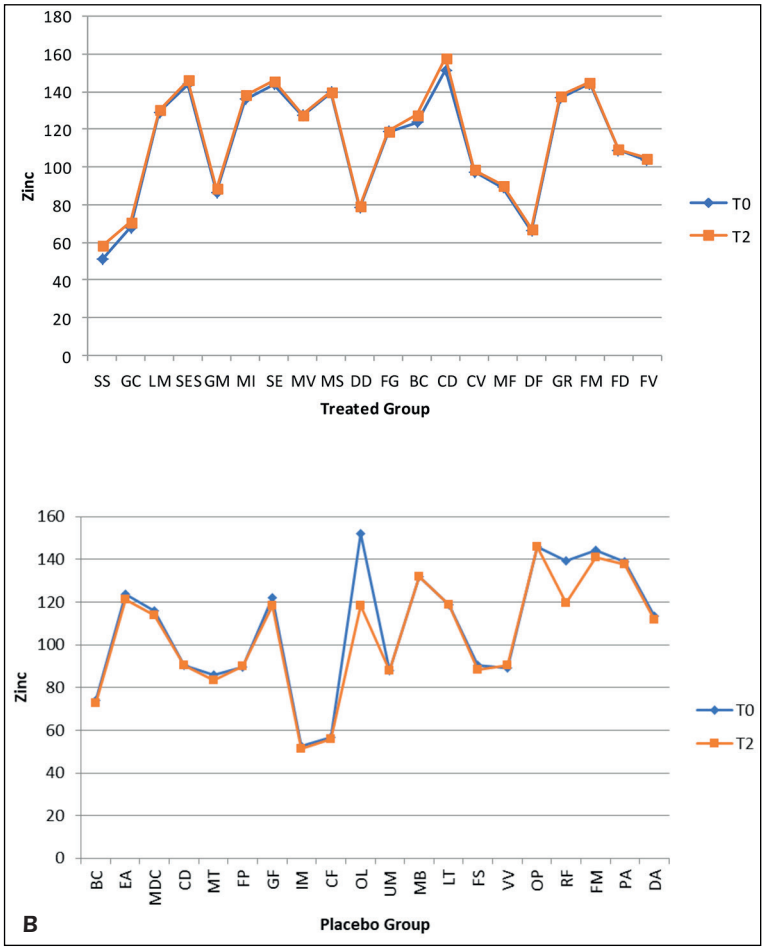

Figure 7. Comparative charts of Active/Treated **(A)** *vs.* Placebo (**B)** groups: Blood biomarkers levels of Mineral Absorption of Zinc were recorded from baseline (T0) to 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (Zinc) value.

of the faeces. Stool frequency was decreased in treated children compared to those in the placebo group. Therefore, these researchers have confirmed that the oral intake of probiotics containing *B. lactis* and *S. thermophilus* reduces and prevents diarrhea[19].

The use of antibiotics causes, as mentioned, an alteration in the intestinal flora, thus causing disorders such as diarrhea and vomiting.

The use of probiotics seems to have positive results to counteract and prevent such disorders in diarrhea caused by antibiotics. The analysis of the literature shows that the parameters considered to determine whether probiotics are effective in preventing diarrhea are inadequate: there are not currently available data to understand to what extent probiotics reduce clinically significant forms of diarrhea, leading in this way to suspending antibiotic treatment. The problem lies mainly in the definition of the outcomes considered: some studies do not even define diarrhea because it varies from a minimum of 1 evacuation of abnormal faeces per day to 3 or more evacuations of liquid stool for at least 2 days.

Numerous studies are still in progress, not only to explain their effectiveness but also to understand the interaction between the frequency of the faeces and the use of the probiotic. In a recent study conducted in 2018 and still in progress, the studies are testing different types of probiotics. A total of 350 children aged 6 months to 18 years, undergoing antibiotic treatment, were randomly allocated to receive either a MP consisting of two strains of *Bifidobacterium* (*B. bifidum* W23 and *B. lactis* W51) and six strains of *Lactobacillus* (*L. acidophilus* W37, *L. acidophilus* W55, *L. paracasei* W20, *L. plantarum* W62, *L. rhamnosus* W71 and *L. salivarius* W24) at a total dose of 10^{10} col-

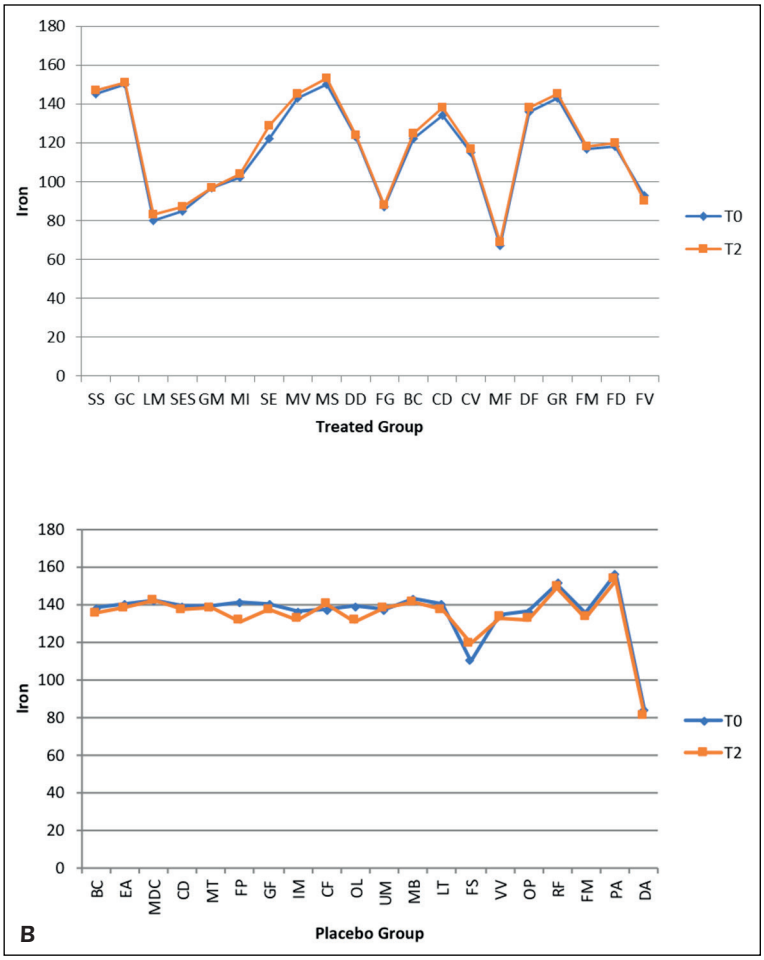

Figure 8. Comparative charts of Active/Treated **(A)** *vs.* Placebo **(B)** groups: Blood biomarkers levels of Mineral Absorption of Iron were recorded from baseline (T0) to 10 weeks (T2) of probiotic's/placebo supplementation. In abscissa axis were reported both the patient's initials (proper name, family name) and in the ordinate axis the outcome (Iron) value.

ony-forming units daily, or a placebo, from the first day of antibiotic treatment until 7 days after antibiotic cessation, up to a maximum of 17 days.

The statistical analysis was carried out by evaluating the consistency and frequency of the stools every day, during and after the use of the antibiotic. A statistical analysis was performed by using the Student's *t*-test or Mann-Whitney U test[20].

Articles dealing with the prevention and treatment of diarrhea from antibiotic use are increasing. In 2017 in an article have been established the guidelines regarding the use of combined use of probiotics with antibiotics for the prevention of diarrhea in children.

This systematic review found only two probiotic strains with sufficient evidence (efficacy demonstrated in more than 2 well-designed randomized trials); these strains are LGG and *S. boulardii*. However, examining LGG in 5 randomized clinical trials for a total of 445 children and administration in children reduced the risk of diarrhea associated with the use of antibiotics from 23% to 9.6%, regardless of why antibiotics and probiotics were used.

Similarly, *S. boulardii* used in children reduced the risk of diarrhea due to the use of antibiotics from 20.9% to 8.8%. Furthermore, *S. boulardii* has greater resistance to antibiotics used for bacterial infections[21].

Our study has several limitations. First, we have limited cases with many stronger outcomes. Second, there is a lack of fecal microbiome analysis. Since the gut microbiota of humans is highly variable, this may affect the response of subjects to the probiotic. The strength of this study lies in the selection of a product containing the strains of probiotics that have been indicated in the previous studies to affect overall health[22-25].

Conclusions

The results of this translational pilot study suggest that a prophylactic probiotic intervention with a selected strain combination, should be a useful tool for pediatric population for improving biochemical parameters serum concentration, in addition to a better nutritional absorption capacity that can enhance their overall health and immunity if administered for a period at least ≥5 weeks. However, further studies may provide better insights regarding the optimal recommendations in patient prevention or treatment.

Authors' Contributions

A.B. participated in the whole study design and supervised the experiments and data analysis. A.G. participated in biochemical analysis and coordination. S.S. supervised the manuscript and gave the final approval of the version to be published. S.C. performed the experiments, analysed the data and drafted the manuscript. A.S. and C.G.I. contributed to manuscript writing. G.D., L.S. and T.S. participated in bibliographic research. D.D.V., F.I., and R.S. made substantial contributions to the conception of the study. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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