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LETTER TO THE EDITOR

PROBIOTICS AND EPICOR® IN HUMAN HEALTH

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Altered gut microbiota may favor the production of effector over regulatory T cells, thereby disrupting the balance between them and contributing to the development of autoimmune disorders (1).

Probiotics are non-pathogenic microorganisms able to interact with the gut microbiota and provide health benefits; their use has recently been exploited to dampen immunological response in several experimental models of autoimmune diseases (2). Probiotics also have varying effects on the immune system during different stages of life and have different benefits for certain age groups, which is similar to how the microbiota in our gut is constantly changing over the course of life (1).

Lactobacilli and *Bifidobacteria* are the most common probiotics, and possess substantial health-promoting properties such as modulating the population and composition of gut microbiome and improving the intestinal barrier function (1-3). Furthermore, these microorganisms facilitate the production of metabolic parameters such as short-chain fatty acids (SCFAs) and reduce gut permeability which ultimately leads to improved immune responses and decreased inflammation (2).

Orally ingested probiotic bacteria are able to modulate the immune system. Conversely, alterations occur in the immunomodulatory properties of different probiotic strains. Prior studies achieved *in vitro* advise that EpiCor® fermentate has prebiotic-like properties, being able to positively benefit immonomodulation in non-vaccinated humans in terms of a meaningfully reduced incidence and length of cold and flu-like symptoms.

Therefore, the aim of the present study was to evaluate, in healthy adults, the effects of probiotic supplementation to the diet (*Hyperbiotics Immune*)

Key words: probiotic bacteria; prebiotics; synbiotics; human health; immunomodulation; translational medicine; dietary supplementation

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on variations in the immune response through regulating some immunomodulation markers.

MATERIALS AND METHODS

Participants

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" approved the application to conduct the clinical trial in the Faculty. Title of the Protocol: Probiotics efficacy and safety in humans. Protocol Identification: INTL_ALITCOOP/Probiotics/INRES2019_w/a/c.

A total of 20 healthy adult human volunteers (10 females and 10 males) with ages ranging from 30 to 50 years (mean age 42.7 years) were included in the study. Exclusion criteria were lactose intolerance, recent antibiotic treatment, frequent gastrointestinal disorders or metabolic diseases. The study was carried out according to the Helsinki declaration and informed written consent was obtained from all the subjects.

Experimental design

A randomized double-blind, placebo-controlled, parallel group design was used for this study. After completing baseline measures, participants were stratified by sex and randomized into one of two groups. The study consisted of three phases: a pre-treatment period (2 weeks/2 tablets twice per day), a treatment period (4 weeks/ 2 tablets once per day) and, finally, a wash-out period (2 weeks). The participants were asked not to alter their routine habits during the study and were randomly allocated to receive either a placebo (tablets looking similar but without probiotics) or tablets containing a specific targeted synbiotic (Hyperbiotics Immune), containing 5 different probiotic patented stains (*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium infantis*, *Lactobacillus fermentum*, *Lactobacillus reuteri*), a prebiotic fructo-oligosaccharides (FOS), Vitamin C (500 mg), Echinacea (100 mg), and Chelated Zinc (10 mg), patented for LiveBac® manufacturing process, BIO-tract®, a protection and time-release delivery and EpiCor®, a fermented yeast superfood with antioxidant properties that have been clinically studied to support the immune system on multiple levels, soy free, gluten free, non-GMO. The tested formula contains 4 Billion Colony Forming Units per BIO-tract® pearl, which is equivalent to 60 Billion colony forming units (CFUs) of standard probiotic capsules.

Blood collection, immunoglobulin and leukocytes/lymphocytes (White blood cells, CD56+ natural killer cells) measurements

After an overnight fasting of at least 10 h, blood samples were taken using ethylenediaminetetraacetic acid (EDTA)-containing vacutainers from the volunteers just before and after the treatment period. All measurements were taken between the hours of 7:00 and 11:00 a.m. Blood was collected at baseline and at 8 weeks for immunoglobulin (IgA) and leukocytes/lymphocytes (White blood cells-WBC and CD56+ natural killer-NK cells) measurements.

Approximately 5 ml of blood were taken *via* vein puncture on each of the testing days from each participant and collected in a BD Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA). The blood samples collected were centrifuged at 2500×g for 10 min, and the plasma was separated for serum biochemical analysis and stored at −80°C until analysis and compared if in the ranges of those for haematologically normal Caucasian adults.

Statistical analyses

The outcome values for Treated and Control groups were analysed using the a *t*-test for paired samples for pre–post differences with time as the factor and IBM Statistical Package for the Social Sciences (SPSS Inc. Version 16.0, Chicago, IL, USA) software to detect significant differences between pre-test and post-test scores.

RESULTS

To evaluate and study how and whether probiotics had a modulatory action on the human immune system, we performed a randomized study on 20 adult subjects aged between 30 and 50 years. The randomized study was completed through a computerized random number generator that selected 10 females and 10 males who were separated into two different groups. Group A was the experimental group (3 females and 7 males),

while group B was the control group (7 females and 3 males). The experimental group compared to the control group used probiotics during the study period. The study was divided into 3 phases. The first phase was before the treatment and lasted 2 weeks, the second phase lasted 4 weeks and was the treatment phase, and finally the last phase, the third, was the wash-out phase (2 weeks).

In light of the data analysis during the several phases of study, in the experimental group A it was highlighted that an increasing parameter was the WBC count, with a variation of 20%, compared to the values in the control group B in the different phases (Fig. 1 A-B). In particular, as shown in Fig. 1 B, we

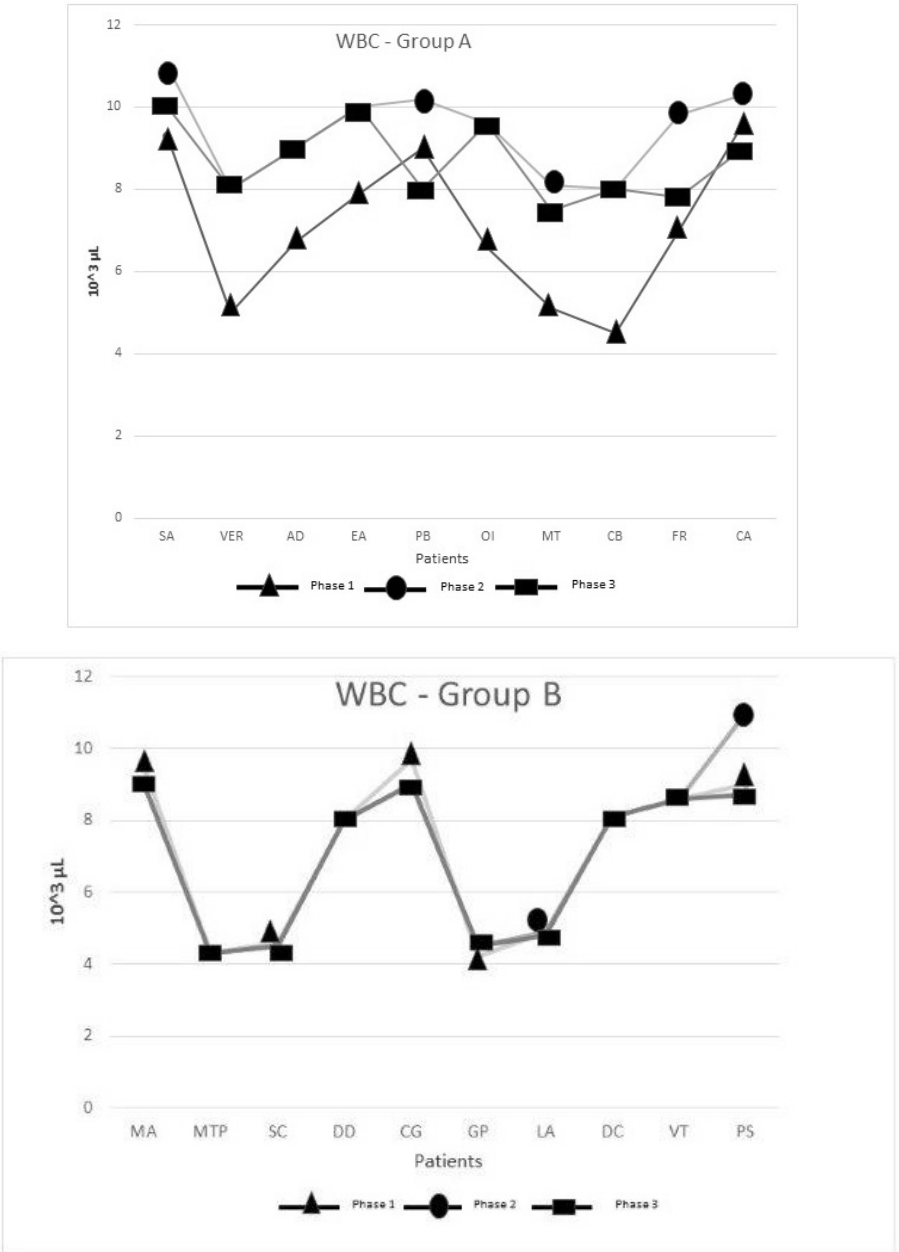
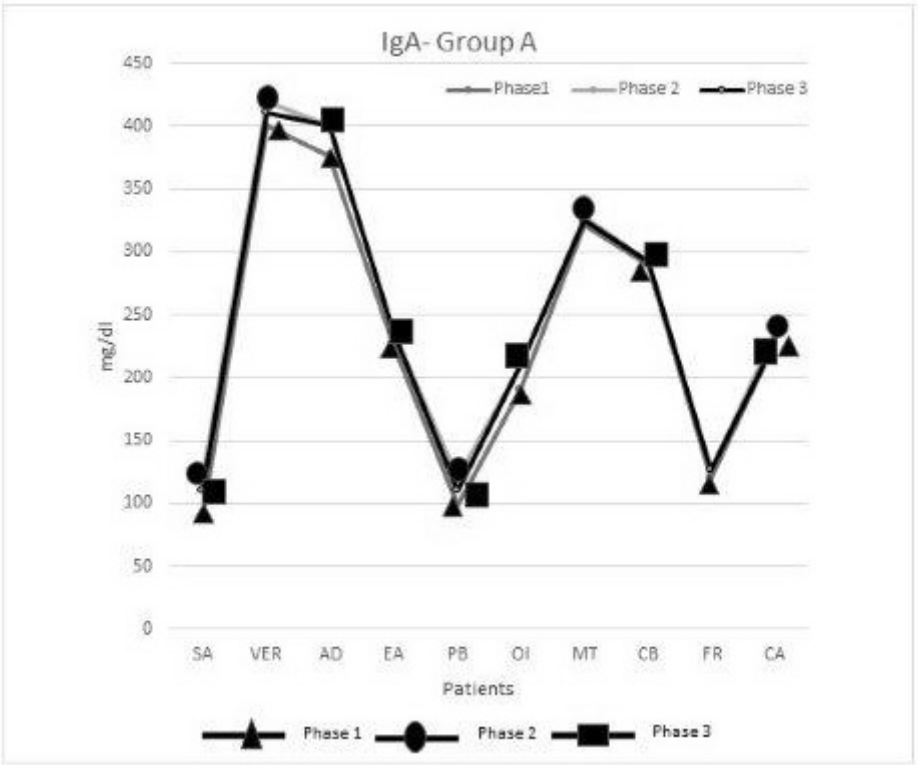


Fig. 1. ***A-B)*** *Comparative charts of Experimental/Treated (**A**) vs Placebo (**B**) groups. Blood levels were recorded from baseline to first two weeks (Phase 1) and after 4 weeks (Phase 2) and 2 wash-out weeks (Phase 3) of probiotic/placebo supplementation. In abscissa axis are shown both the patient's initials (proper name, family name) and in the ordinate axis the outcome (WBC count) value.*



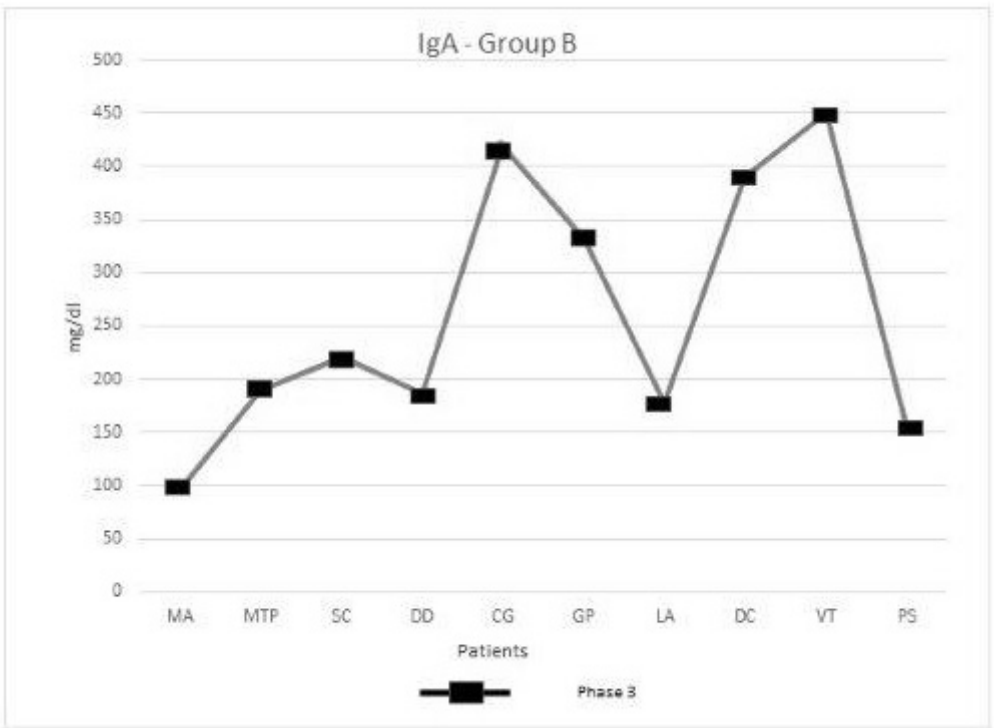


Fig. 2. ***A-B)*** *Comparative charts of Experimental/Treated (**A**) vs Placebo (**B**) groups. Blood levels were recorded from baseline to first two weeks (Phase 1) and after 4 weeks (Phase 2) and 2 wash-out weeks (Phase 3) of probiotic/placebo supplementation. In abscissa axis are shown both the patient's initials (proper name, family name) and in the ordinate axis the outcome (IgA levels) value.*

can state that in the control group the values remained unchanged, in fact, we found variations around 1%; only in the participating subject in phase 2, did we find an increase in WBC count which was, however, within the normal parameters in phase 3. Another value taken into consideration in both groups was IgA. Fig. 2 A-B shows a stationary condition in the control group B in the different phases with a variation of 0.2%, while there was an increase in the levels of IgA (≈ 5%) in the experimental group in phase 2 supportive of the data that the use of probiotics induces the increase of cells producing IgA.

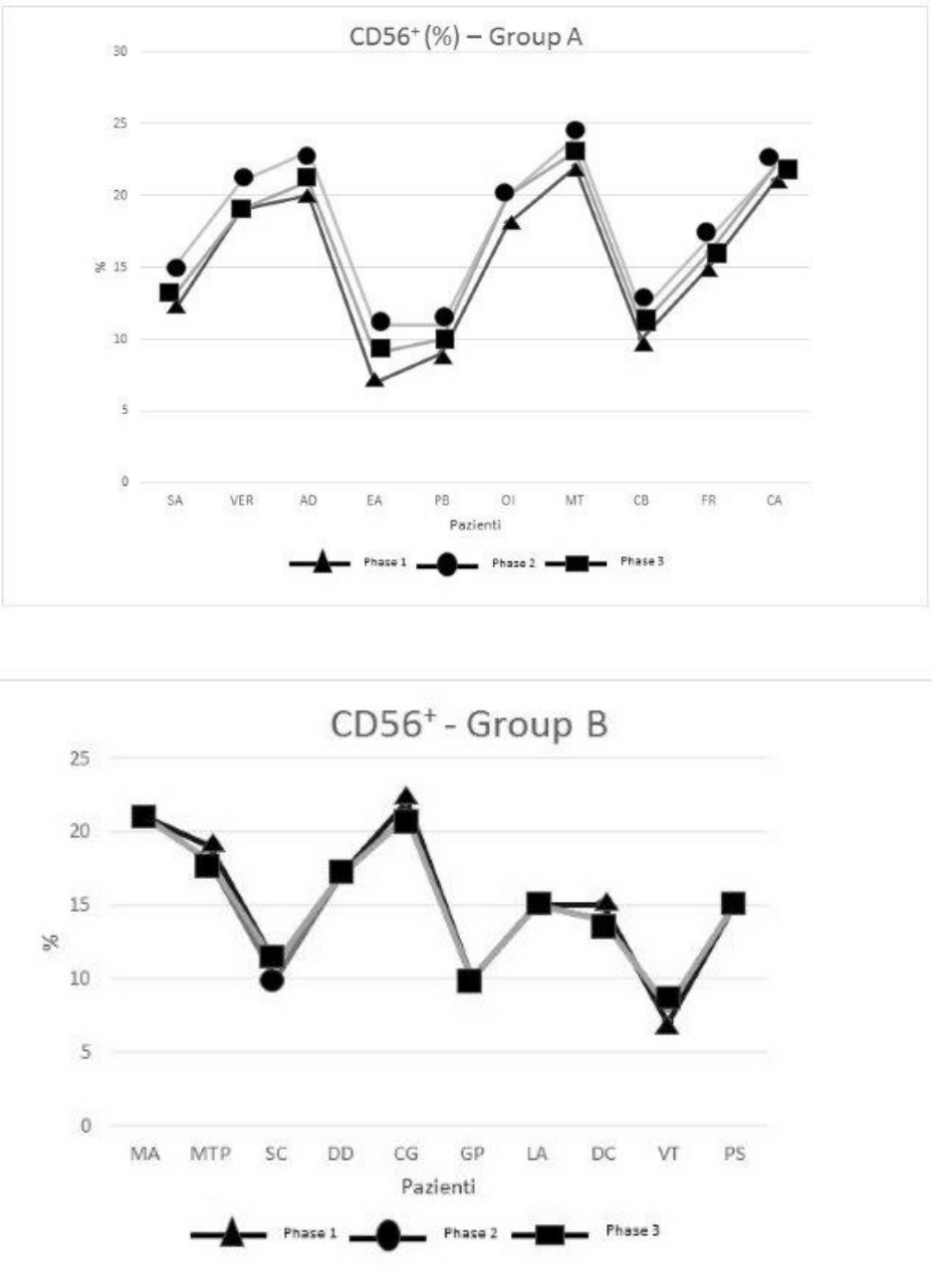


Fig. 3. ***A-B****) Comparative charts of Experimental/Treated (****A****) vs Placebo (****B****) groups. Blood levels were recorded from baseline to first two weeks (Phase 1) and after 4 weeks (Phase 2) and 2 wash-out weeks (Phase 3) of probiotic/placebo supplementation. In abscissa axis are shown both the patient's initials (proper name, family name) and in the ordinate axis the outcome (CD56⁺ count) value.*

Because several sub-populations belong to immuno-modulatory response, we took into consideration lymphocytes and, in particular, $CD56^+$ natural killer cells. Notably, in the experimental group (A) at different phases, an increase of 10%in the levels of natural killer $CD56^+$ cells can be seen, probably because the intake of targeted probiotics induces an increase in lymphocytes and NK-cells (Fig. 3 A-B). Moreover, no adverse events were reported as a result of the active or placebo treatment, or as a result of the study procedures.

DISCUSSION

The potential impact of the therapeutic effect of probiotics on a dysbiotic situation could not be seen without taking into consideration a change of lifestyle. Diet habits, stress, poor health conditions, and lack of exercise can significantly impact the microbiota stability (1-3).

In recent years, scientific studies have focused on the anti-carcinogenic effects of natural substances, such as probiotics (4). In particular, these studies were aimed at developing an effective drug with the lowest possible side effects (5). The most common risk factors for cancer include tobacco, alcohol, obesity, chronic virus infections, chemicals (6), environmental toxins, ionizing radiation and hormonal changes (7-10). In the current decade, intensive cancer research involving genomics and proteomics has improved the knowledge regarding cancer as well as promoting public awareness (11, 12). As not all probiotics are helpful in overall conditions, a careful selection of proper strains of bacteria established for the desired clinical outcome is of vital importance. Therefore, we selected a combination of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium infantis*, *Lactobacillus fermentum*, *Lactobacillus reuteri*) a prebiotic FOS, Vitamin C (500 mg), Echinacea (100 mg), and Chelated Zinc (10 mg), patented for EpiCor®, which have been shown in the current literature to improve health outcomes in humans, according to our findings (1). Our study has several limitations. Firstly, we have limited cases with many stronger outcomes. Secondly, 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 in addition to EpiCor® that have been indicated in previous studies to affect overall health. Probiotics act on a wide variety of cells in the intestine to modulate the immune system towards a pro-or anti-inflammatory action, depending on the strain, setting and immunological parameters measured, and the type of cells being acted upon. It is necessary to further evaluate potential changes in the gut microbiota composition that may occur following the immunomodulatory effects of these probiotic strains in humans.

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