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Antibiotic Supplementation's Immunostimulatory Benefits on Asthma Patients: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Asthma is seen as an ongoing inflammatory condition of the airways. Living bacteria called probiotics are prevalent in the human stomach and have been shown to be protective against a variety of illnesses, including allergies. This study sought to better understand how probiotic treatment for asthma affected patients' clinical symptoms, changes in the expression pattern of specific microRNAs, and changes in plasma levels of IL-4 and IFN-.

Materials and methods: 40 asthmatic patients participated in the current investigation, which was a randomised, double-blind, placebo-controlled trial. Probiotics or a placebo was given orally once day for eight weeks. The expression of microRNAs, IL-4 and IFN- levels, and pulmonary function tests were evaluated before and after treatment.

Results: The findings demonstrated that probiotic supplementation dramatically decreased the expression of miR-16, miR146-a, and IL-4 levels in asthma patients while considerably increasing the expression of miR-133b. Furthermore, following taking probiotics, pulmonary function tests revealed a significantly improved Forced Expiratory Volume in 1 s and Forced Vital Capacity.

Conclusion: In our investigation, an 8-week probiotic supplementation regimen decreased IL-4 linked with Th2 cells and increased forced vital capacity and forced expiratory volume. The use of probiotics seems to be an option in addition to conventional asthma therapies.

Keywords: Asthmatic; Patients; Inflammatory

Introduction

One of the most prevalent chronic inflammatory disorders, asthma is characterised by airway inflammation, recurrent breathlessness, wheezing, bronchial hyperresponsiveness (BHR), and finally reversible airway obstruction. In recent decades, asthma has become more common. The microbial hypothesis, which contends that reduced microbial exposure upregulates T-helper cells type 2 (Th2) cytokine production, leading to an increase in allergy disorders, is one possible explanation for this high incidence. A correct assessment of asthma severity, the use of 2-adrenergic agonists, which are bronchodilators for acute reactions, and anti-inflammatory medications like inhaled corticosteroids are presently the main treatments for asthma. The World Health Organization defines probiotics as "living microbes" that, when given in adequate amounts, can have positive impacts on the host's health. It is still unclear exactly how allergic disorders develop in children. Intestinal microbiota, in which the make-up and structure of typical bacteria interact with the developing immune system, is one potential explanation. Such interactions may influence immune system development, which may result in allergic Th2-type reactions. Humans have probiotics in their intestines. They offer protection from a variety of illnesses include allergies, cancer, diabetes, gastrointestinal tract inflammation, and nervous system issues. Probiotics have a big impact through controlling the immune system, modulating cytokine gene expression, improving mucosal barrier function, and fighting off harmful bacteria. MicroRNAs (miRNAs) are a family of endogenous [1-7] non-coding RNAs that have evolved over time. They attach to the 3′ untranslated region of target mRNAs to control the activity of cells. MicroRNAs control several biological processes, including cell proliferation, signal transduction, differentiation, and apoptosis. They also control the function of immune cells, destroying or inhibiting the translation of mRNA in the process. Probiotics may alter the host's miRNA, which may have an impact on a variety of host processes. Maintaining gastrointestinal homeostasis depends on probiotics' capacity to control miRNA expression. There are, however, little research on the function of miRNAs in the control of the gut microbiota as a disease treatment. Additionally, it is yet unclear how miRNAs affect the host's ability to control the gut microbiota. The goal of the current study was to look at how probiotics affected asthma patients' clinical symptoms, changes in cytokines and microRNAs, and pulmonary function. This was done since probiotics may have positive effects on immune system-related miRNAs.

Materials and Methods

The investigation was a randomised double-blind placebo-controlled clinical trial. We utilised the usual formula recommended for parallel clinical studies to determine the sample size by taking into account type one error () of 0.05 and type two error () of 0.20 (power = 80%). We compared the probiotic-treated and placebo groups based on changes in the lung function test's parameters from a prior study. This meant that each group needed 20 people. A total of 40 asthmatics with a mean age of 38.62 10.49 and a history of mild to moderate asthma for at least a year were enrolled. A history of two or more bouts of wheezing within the previous six months and/or a bronchodilator test showing a positive response with a 12% rise in forced expiratory volume

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(FEV1) over one second were the inclusion criteria based on GINA (Global Initiative for Asthma) guidelines. Patients with lung infections like pneumonia, coronary heart disease, lung cancer, primary and secondary immunodeficiency, other chronic diseases, participation in other therapeutic studies within the previous six months, use of high-dose multivitamins and probiotics within the past three months prior [7-11] to screening, and SARS-CoV-2 infection during the trial were among the exclusion criteria. The probiotic and placebo groups were randomly assigned to the eligible patients. A basic random sequence was produced using Microsoft Excel, to put it briefly. With the use of numbered, opaque, and sealed envelopes, the distribution was decided. Each participant then chose an envelope after completing the fundamental measurements. A blind researcher who was not a part of the protocol carried out the entire randomization process. The identical medication was taken by the patients in both groups. Both the intervention and placebo groups had the same inclusion and exclusion standards. The sampling period (the arrival period) ran from December 2020 to June 2021. Patients' blood was drawn before and 60 days after the intervention and used for the following procedures. Patients were physically examined, their blood pressure, heart rate, and pulmonary function were recorded, and their asthma symptoms, asthma exacerbations, and adverse events were assessed at each session.

Outcome measures

Asthma control test (ACT) scores, forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), and forced expiratory volume in the first second (FEV1)/forced vital capacity (FVC) ratio were the main outcomes following treatment. Changes in AQLQ scores (quality of life questionnaire scores), miR-21, miR-155, miR-146a, miR-126, miR-16, and miR-133b gene expression in plasma, as well as IL-4 and IFN- plasma levels during the 2 months of intervention, were the secondary outcomes.

RNA isolation and cDNA synthesis

In EDTA tubes, blood was drawn from the intervention and placebo groups. Within 1 hour after collection, it was centrifuged for 10 min. at 500 g, 18 to 20 °C for plasma separation. For further investigation, the plasma was moved to a brand-new tube and kept at -80° C. According to the manufacturer's instructions, total RNA containing small RNA was extracted from 1 mL of plasma using the BIOzol Reagent (Stem cell technology research centre, Iran). 250 mL of chloroform were added after the plasma and BIOzol reagent were combined, and the mixture was centrifuged at 2000 g for 15 minutes at 4 ° C. The upper aqueous phase was aspirated, combined with 800 L of isopropanol, and incubated for an entire night at 20 ° C. After this, the mixture was centrifuged at 2000 g for 45 minutes at 4 ° C. The RNA pellet was then rinsed with 75% ethanol, centrifuged at 2000 g for 20 minutes at 4 ° C, and allowed to air dry before being dissolved in RNase-free water. Using a Nano Drop ND-1000 spectrophotometer, the total RNA concentration and purity were evaluated (Thermo Scientific, USA).

Quantitative real-time RT-PCR

Applied Biosystems' Step One Plus real-time PCR system and SYBR Green PCR master mix kit were used to quantify the quantity of the miR-16, miR-21, miR-126, miR-133b, miR-146a, and miR-155 gene transcripts in plasma (Stem cell technology research center, Iran). Utilizing U6 as an internal reference for target miRNAs, the 2ct technique was used to compute the relative expressions of miRNA.

Enzyme linked immunosorbent assay (ELISA)

The same technician used enzyme-linked immunosorbent assay (ELISA) kits from MabTagGmbH, Friesoythe, Germany, in accordance with the manufacturer's instructions to assess the concentrations of IL-4 and IFN- in the plasma of the intervention and placebo groups at the [10, 11] same time. IFN- and IL-4 had sensitivity levels of 24 and 2.3 pg/mL, respectively. In a nutshell, 100 L of capture antibody was applied to ELISA plates before being incubated at 4 °C overnight. The wells were blocked for 1 hour at room temperature with ELISA diluent. Standards or patient plasma (100 L) were added to each well for cytokine detection, which was then incubated at room temperature for 2 hours.

Statistical analysis

The mean SEM was used to express all data. The paired t-test, independent t-test, Wilcoxon, and Mann-Whitney tests were used to compare data within and across groups. Spearman's correlation analysis was used to examine the correlations between various variables. StepOne Software, Prism 8.0.2 (GraphPad v7, USA), and SPSS were used for data analysis (SPSS, v22, USA). A p-value of 0.05 or less was regarded as statistically significant.

Results

Baseline characteristics of participants

At the start and end of the trial, pulmonary function was measured using spirometry. After the study period compared to the baseline, the patients in the probiotic group demonstrated a substantial improvement in FEV1 and FVC. The FEV1/FVC ratio in the probiotic group did not significantly alter from the starting point, on the other hand. The findings revealed that the probiotic group's FEV1 and FVC at the end of the intervention were significantly greater than they were at the start of the trial (p 0.01) and (p 0.001), respectively. FEV1 and FVC in the probiotic group, however, did not differ significantly from those in the placebo group. Before and after receiving the placebo, there were no appreciable changes in the FEV1 and FVC levels in the placebo group.

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Conflict of Interest

For the research, writing, and/or publication of this work, the authors disclosed no potential conflicts of interest.

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