

Dysbiosis in Ukrainian Children with Irritable Bowel Syndrome Affected by Natural Radiation

Mohammad-Reza Sheikh Sajjadi^{*1,2}; Larisa V Kuznetsova¹, PhD, and Vadim B Bojenko², MD

1. Department of Clinical Immunology & Allergology, National Medical Academy for Post Graduate Education, Kiev, Ukraine
2. Ukrainian Specialized Dispensaries of Radiation Defend Population, Kiev, Ukraine

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Abstract

Objective: Microbiota has an important role in human metabolism, nutrition, immunity, and protection against colonization by pathogenic microorganisms. Radiation can harm the beneficial members of the gastrointestinal tract flora.

Methods: Our study included 75 rural children aged 4-18 years, who lived in contaminated area exposed to natural environmental radiation with clinical symptoms of irritable bowel syndrome and 20 healthy urban participants aged 5-15 as control group. The intestinal bacterial microbiota was examined from stool samples.

Findings: Our results indicated the population levels of microbiota such as Enterobacter, Enterococcus, Lactobacillus and Bifidobacterium in caecal contents in 61 subjects (81.3%) was significantly less than in control group.

Conclusion: We investigated alternation of the intestinal microbiota affected by ionizing radiation in children with clinical symptoms of irritable bowel syndrome.

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Key Words: Microbiota; Immune Status; Irritable Bowel Syndrome

Introduction

The gastrointestinal tract harbors a huge diversity of aerobic and anaerobic bacteria that interact in a complex ecosystem. This microflora comprises 400 to 500 metabolically active bacterial species, which have a pronounced impact on the host's intestinal function and health^[1]. Intestinal bacteria can be grouped into species that have detrimental effects on the host and species that have beneficial effects. The detrimental effects include diarrhea, infections, liver damage, carcinogenesis, and

intestinal putrefaction^[2]. Stimulation of the immune system, improvements in the digestion and absorption of essential nutrients, and vitamin synthesis are examples of the protective effects brought about by the intestinal microbiota^[3].

Microorganisms of the genera Bifidobacterium and Lactobacillus perform a variety of functions important for the host's health. Whereas microorganisms of the genus Bacteroides have beneficial as well as detrimental effects^[4] by producing acetic and lactic acids, which lower intestinal pH, that prevents the development of

* Corresponding Author;

Address: National Medical Academy for Post Graduate Education, Kiev, Ukraine

E-mail: info@ufhgc.com

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yeasts like *Candida albicans*^[5]. In addition, they can stimulate cells of the immune system, inducing the production of IL-12 by macrophages and B cells^[6]. Ionizing radiation is known to increase the development of irritable bowel syndrome^[7] and it is clear that radiation can harm the beneficial members of the gastrointestinal tract flora. Recent interest has been directed to the potential role of intestinal microbiota in the pathophysiology and symptom generation of irritable bowel syndrome (IBS).

The aim of this study was to determine the population of beneficial members of the gastrointestinal tract flora in Ukrainian children exposed to natural radiation with clinical symptom of IBS.

Subjects and Methods

The research has been conducted on 75 rural patients aged 4 to 18 who lived in a contaminated area at a distance of 60 to 90 km from Chernobyl Nuclear Power Plant and exposed to natural environmental radiation with pain or discomfort in the abdomen and an altered bowel habit without any inflammation or bacterial infection and did not use antibiotics in the period of study. All the patients were undergoing an outpatient radiation treatment program at Ukraine-based specialized hospitals and were directed to our hospital for health care monitoring from March to June 2009 (Kiev, Ukraine). The control group included 20 healthy urban participants aged 5 to 15 from Kiev. Clinical diagnosis of IBS was based on the Rome III criteria. The local ethics committee has approved this research. Parents were willing to allow their children to take part in medical research.

Radiometric analysis

The internal whole body radioactivity due to ¹³⁷Cs has been measured by g-ray spectrometry with scintillation detector (Gamma plus, Moscow, Russia). The micro curie is used to express the activity of ¹³⁷Cs in a period of time and then

converted to Becquerel^[8].

Examination of caecal bacterial flora:

The intestinal bacterial microbiota was assessed with the method proposed by Sutter et al^[9] with adaptations. Stool samples were collected from study groups which did not use antibiotics since 4 weeks before microfloral analysis, interval between sample collection and laboratory handling did not exceed 1 h. A 1-g aliquot was taken from each sample and transferred into Stuart transport medium. After homogenization, CPLX agar was used for selective isolation of Bifid bacterium. LBS agar was used for selective isolation of Lactobacillus; COBA agar was used for selective isolation of Enterococcus; DHL agar (Tokyo, Japan) was used for selective isolation of Enterobacter.

Once inoculated and identified, the plates were placed into GasPak anaerobic jars and incubated at 37°C for 48 h in a low-oxygen and high-carbon dioxide atmosphere generated by an Anaerobac system. After that, the plates were evaluated for bacterial growth and colony aspect. Colony-forming units (CFUs) were counted for each plate, and the mean values for each type of microorganism were calculated. Microorganism concentration was expressed as log₁₀ CFU/g of feces. Observations related to colony morphology, Gram staining, and catalase testing were recorded for each plate.

Mean values and standard deviations of log transformed data and mean values at the original scale were calculated for Enterobacter, Enterococcus, and Lactobacillus and Bifid bacterium counts.

Findings

The mean age of the participants in study group was 10.6±5.1 and in control group was 10.2±3.3 years. The mean value of the radioactivity of ¹³⁷Cs in control group was estimated as 1.11±0.01 Bq.106. In study groups, the values were 1.86±0.51 Bq.106. Study groups had significantly higher amount of radioactive material

Table 1: Mean age and internal radiation activity

Parameter	Control group (n=20)	Study group (n=75)
Age	10.2(3.3)	10.6(5.1)
Internal radiation activity (Bq)	1.11(0.01)	1.86 (0.51 *)

* $P < 0.01$

and proportions compared with the control group at $P < 0.01$ (Table 1).

Dysbiosis was found in 81.3% of study group. Table 2 shows that in the group study, the population levels of Enterobacter, Enterococcus, Lactobacillus and Bifid bacterium counts concentration in cecal contents in 61 subjects was significantly less than in control group (2.93 ± 0.16 , 6.15 ± 0.17 , 5.24 ± 0.13 , 6.03 ± 0.15 log CFU/g cecal contents, respectively, $P < 0.01$).

Discussion

The present study establishes the effect of low internal radiation depended to ^{137}Cs on the population of beneficial members of the gastrointestinal tract flora in Ukrainian children exposed to natural radiation with clinical symptoms of IBS.

Dysbiosis is an unbalanced condition in the gastrointestinal tract, resulting in fewer than normal "friendly" bacteria and an over abundance of potentially harmful bacteria, yeast, or parasites. This unbalanced condition is often the result of broad-spectrum antibiotics use, radiation therapy or exposure, stress, dramatic changes in altitude, ingestion of different organisms, or changes in diet. Occasionally, usually suppressed components of the indigenous microflora can allow potential pathogens or toxinogenic strains to colonize and to multiply thus causing diarrhea, flatulence, or different variations of colitis^[10].

Gastrointestinal dysbiosis may also be involved in conditions such as IBS. Cremon et al showed that the intestinal flora of patients with diarrhea predominant IBS diverged significantly not only from controls but also from other IBS subgroups^[11]. The results by them further support the hypothesis that intestinal microbial flora has a

role in IBS pathophysiology, and foster the idea that abnormal microbiota may act by triggering local and systemic immune responses linked to symptom generation^[11]. Some evidence suggests that IBS is affected by the immune system which fights infection in the body. Many of the health benefits associated with lactic acid bacteria and probiotic supplementation are derived from increased immunological activity. This would be expected because of the close association between the gastrointestinal tract and the immune system. For example, several strains of Lactobacilli were shown to enhance the serum and intestinal IgA response to rotavirus induced gastroenteritis in children^[12]. In another study involving 25 elderly patients, oral doses of Bifidobacterium and *L. acidophilus* were shown to significantly increase B cell frequency in peripheral blood and reduce colonic inflammatory infiltration compared with placebo controls^[13]. Bifidobacterium supplementation increased phagocytosis of *E. coli*, a general measure of increased immune response^[14,15]. A prevalent theory, derived from hypotheses that were first postulated by Metchnikoff a century ago, proposes that individual members of the microbiota might influence the balance between pro-inflammatory and regulatory host responses and that alterations in the composition of the microbiota (a process that is known as dysbiosis) could jeopardize host immune responses and promote the development of various inflammatory disorders.

Gerhard Reuter has obtained the median value of logarithm for Bifidobacterium and Lactobacillus of cecum/descending colon. Based on his study, the median values of logarithm for Bifidobacterium, Lactobacillus and Enterobacter were obtained as 10.86/g, 8.8/g and 3.9/g^[10]. Our results indicated in 81.3% of study group, the population levels of microbiota such as Lactobacillus, Bifidobacterium and Enterococcus in cecal contents was significantly less than in control group, but level of

Table 2: Population of bacteria in cecal contents in Ukrainian children with IBS

Organisms	Number of bacteria (log CFU/g cecal contents)	
	Control group (n=20)	Study group (n=61)
Enterobacter sp.	1.05 (0.12)	2.93 (0.16)*
Enterococcus sp.	7.41 (0.35)	6.15 (0.17)*
Lactobacillus sp.	6.71 (0.11)	5.24 (0.13)*
Bifidobacterium sp.	8.57 (0.21)	6.03 (0.15)*

Results are expressed as log CFU/g cecal contents, the mean (SD) per one gram of cecal contents

* $P < 0.01$.

Enterobacter compared to control group was highly significant statistically at $P < 0.001$ (Table-1). Many factors can harm the beneficial members of the intestinal flora, including antibiotic use, psychological and physical stress, radiation, altered intestinal peristalsis, and dietary changes. It is clear that radiation is in common associated with the development of dysbiosis. Our modeling data shows that internal radiation activity of whole body in rural population is higher than in urban population. This arises from better shielding features of urban buildings and different occupational habits, also, as the urban population depends less on local agricultural products and wild foods than rural population.

In our previous study, we have shown that immune status in children with IBS has changed, which may be associated with the potential role of microbiota on pro-inflammatory and regulatory immune responses and to establish whether the composition of the microbiota can influence the development of inflammatory diseases in and beyond the gut. Our modeling data show that internal radiation activity of whole body in rural population is higher than in urban population and showed to significantly increase B cell frequency (CD22) in peripheral blood and serum immunoglobulin levels have reduced compared to control group in subjects with clinical symptoms of IBS like cramping, abdominal pain, bloating, constipation, and diarrhea that cannot be explained by structural abnormalities^[8]. In the innate immune status, percent of CD16 cells in children with IBS increased significantly in comparison to control group ($P < 0.05$). Concentration of circulating immune complexes increased significantly in all study subjects compared to control group ($P < 0.001$). Phagocytes activity and phagocyte index decreased

significantly in all study subjects in comparison to control group at $P < 0.001$ ^[16]. In cellular immune status, percentage of CD3 and CD4 in all study subjects decreased significantly in comparison to control group ($P < 0.001$). Percentage of CD8 increased significantly in all study subjects in comparison to control group at $P < 0.05$ ^[7].

IBS is thought to arise owing to a combination of genetic and environmental factors that result in dysregulated immune responses to the gut microbiota and the subsequent development of gut inflammation. Compelling evidence from a variety of studies has shown dysbiosis in patients with IBS compared with healthy controls.

Conclusion

We suggest that a trade-off is established between the host immune system and the bulk of the microbiota. In a healthy individual, intestinal colonization stimulates and regulates host immune system, and in study group imbalance between the microbiota affected by natural radiation changed immune response, and this suggests a causative role for dysbiosis in generating clinical symptoms of IBS.

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Conflict of Interest: None

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