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Characteristics of the Gut Microbiota in Obese Children with Allergic Diseases

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Abstract

There are many evidences, that obesity increases the risk of asthma and atopic diseases. Therefore, misbalance in the gut microbiota is considered one of the micro ecological factors responsible for the increase of body mass and changes in energy metabolism. The aim of study was to investigate the characteristics of children gut microbiota, and to recognize relations with obesity, as well as allergic disorders. The study included patients of both sexes from 3 up to 17 years old: 43 with a Body Mass Index (BMI) from 23.16 to 40.28 with history of allergic diseases; and 24 children without allergies with BMI from 14.27 to 48.96. Children with allergic diseases we observed atopic dermatitis - 41.46%, asthma - 17.07%, allergic rhinitis -21.95%, food allergies - 9.76%. Condition of gut microbiota we evaluated by analysis of fecal samples and identification of microorganisms. Quantity of Enterococcus and Staphylococcus was higher than in children without allergies (56% vs. 33%; 44% vc. 17%, p=0,05 respectively) .The proportion of *S. aureus* negatively correlated with BMI (r=-0.39, p=0.047) in allergic children, while in non-allergic children of group of children number of Bacteroides and Bifidobacteria negatively correlated with BMI (r=-0.53, p=0.010; r=-0.42, p=0.046 respectively), but the proportion of Clostridia positively correlated with BMI (r=0.56, p=0.006). Thus, we identified and characterized the qualitative and quantitative values of gut microbiota in obese children with allergy.

Keywords: Microbiota; Allergic diseases; Obesity

Introduction

There is an increased growth of allergic, autoimmune and inflammatory diseases, as well as an increase in the population of people with overweight and obesity in developed countries. Moreover, there is a significant relationship with the development of obesity and worsening of allergy [1]. Researchers still have not managed to definitively establish the determining factors in the formation of allergic reactivity and obesity despite the considerable attention of the scientific community to environmental factors, including the eating habits in early childhood.

The hygiene hypothesis postulates that changes in the environment associated with the so -called "Western life" have led to a reduction of microbial exposure at an early age and thus misbalance the formation of the normal protective immunity by conditioning the formation of an allergic phenotype. The absence of a child of sufficient stimulation of the immune system of the intestinal mucosa may lead to the disruption of the formation of oral tolerance and the development of chronic inflammatory reactions, against which can form an allergic reaction to food (food allergy / food intolerance in irritable bowel syndrome), accompanied, as a rule, intestine dysbiosis.

The gut contains a large and diverse microorganisms that is, quantitatively, the most important postnatal source of microbial stimulation of the immune system [2]. The initial gut composition can significantly influence immune system development [3]. Hence, disruption of this process early on in life at a time of dynamic changes in the infant gut, may have long-term health effects and lead to chronic diseases [4,5]. Both asthma [6] and obesity often begin in early childhood when the gut microbiota is primarily developed [7] and still immature. Recent studies in animal models and in humans have found a relation among gut microbiota, atopic diseases (eczema, allergic rhinitis, and asthma) [8-10] and obesity [11]. Early-life factors (i.e., diet, medications, hygiene, antioxidants, and nutrients) associated with asthma and/or obesity6 may alter the gut milieu.

Many clinical studies have shown that the intestinal microbiota, by interacting with the lymphoid tissue associated with the intestine, provides a first line of defense of the host [12,13]. Recent data have shown that the intestinal microflora is also involved in the maintenance of body weight control and energy metabolism, affecting the two main causes of obesity: the consumption and energy storage, as well as contribute to the development of insulin resistance and the formation of an inflammatory state characteristic of obesity [14]. These results contribute to the development of the hygiene hypothesis and suggest that manipulation of early intestinal microbial communities can offer new strategy for prevention of allergic sensitization and excessive weight gain.

The study of the microbiota using metagenomics approaches estimates that microbiome tract in an adult human contains approximately 10^{12} microorganisms per milliliter of luminal content and harbors approximately 15,000 species of bacteria [15]. Account for more than 90% of all the phylotypes of colonic bacteria belong two dominant phylum Bacteriodetes and Firmicutes. Children exposed to a plurality of different environments of microbes immediately after birth and are colonized by microorganisms which are first facing, or during passage through the birth canal mother or from the skin, depending on the conditions of delivery child [16,17].

Babies born by caesarean section have an increased risk of asthma, obesity, diabetes type 1, while breast-feeding protects against these

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and other diseases, but not with 100% certainty [18]. In addition, children born by caesarean section, was significantly more likely to develop diarrhea and sensitization to food allergens than children born vaginally [19]. Recent studies suggest that bacteria and bifidobacteria species Bacteroides, appears to prevent the development of obesity [20]. Reduced the number of bifidobacteria and Bacteroides of babies born by Caesarean section, could contribute to the subsequent formation of excess body weight. Currently, there are not a lot of data of the association of the intestinal microbiota and obesity and therefore requires further study. However, in the case of confirmation of this hypothesis, the regulation of the intestinal microbiota (through the appointment of probiotics) may amount to an effective treatment method for preventing the development of obesity and allergic diseases.

Methods and Materials

This was a cross-sectional study of the association of gut microbiota in children with allergic diseases, depending on body weight. The study was approved by the Local Research Ethics Committee of the FGBU, Mechnikov Research Institute of Vaccines and Sera, RAMS. Informed consent was obtained from the parents for all study participants, and children were willing to participant in this study. No participant used any antibiotic, pre- or probiotics within the preceding 2 months before sampling. Children of any gender 3-17 of age with allergic diseases (n=43) and children without allergies (n=24) were recruited from children urban clinics in SEAD Moscow. Atopic disease was defined as reported doctor-diagnosed allergic rhinitis and/or dermatitis and/or asthma and/or food allergy in medical cards. Body Mass Index (BMI) was calculated per standard definition (kg/m^2). Each child was weighed in light clothing to the nearest 0.1 kg using a calibrated scale. Height was measured without shoes to the nearest 0.1 cm using a vertical ruler. Further, the Body Mass Index (BMI) was calculated as weight (kg)/ square meters of height. Overweight was defined as BMI scores at or above the 85th percentile for age and gender, obesity at or above the 95th percentile.

Approximately 10 g of stool sample was collected into sterile plastic containers by parents and immediately stored in home freezers until brought to the experimental laboratory. Stool samples (10g) were collected and held in adomestic refrigerator at 4C for no more than 2 hours before transportation to the laboratory. Weighed samples of feces were serially diluted (10^{-2}-10^{-9}) in pre-reduced 0.9% NaCl in the anaerobic glove box with a gas mixture of 85% N_2, 10% CO_2, and 5% H_2. The quantitative analysis of the feces was performed by seeding serial dilutions on nine freshly prepared media.

Yeast extract agar was applied for total aerobes count; Staphylococcus agar (Difco, USA)for staphylococci; Endo Agar for enterobacteria (Serva, USA); de Man-Rogosa-Sharpe agar (MRS; HiMedia, India) for microaerobes, such as lactobacilli and streptococci; Columbia Agar Base (BBL, USA) with kantomycin (100 mg/L), vitamin K_1 (1,5 mg/L), 5% blood for asporogenous obligate anaerobes (bacteroides, eubacteria, peptostreptococci); Blaurock Agar (HiMedia, India) for bifidobacteria; Trypton Sulfite Neomycin Agar (TSN; Oxoid, UK) with emulsion of egg yolk (50 ml/L) for clostridia; Enterococcus Agar (Serva, USA) for enterococci; Sabouraud dextrose agar with ampioxi (500 mg/L) was applied for fungi. Microorganisms incubated at 37°C after 48 hours.

The colony counts of the different dilutions were recorded, and from the highest dilutions with growth all of the colonies with different morphologies were identified by standard methods, mostly on the genus and species level. After identification of microorganisms, which grew as single colonies in the dilutions, the quantitative composition of fecal microbiota was determined. The number of various species or genus was given as colony forming unit per ml (CFU/ml) expressed in log10. The detection level was >3log CFU/ml. For each child, the counts of different bacterial groups were calculated and summarized to obtain the total count of cultivable intestinal bacteria. The relative share (%) of each bacterial group was calculated from total counts. Venous blood (5 mL) samples were collected from children. Blood samples were centrifuged and serum was separated on the day of collection and stored at -25°C until assayed for IgE. The ELISA (Vector-Best, Russia) was used to measure the serum total IgE (tIgE).

Statistics

The statistical analysis was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) statistical software package. According to data, the descriptive statistics, and the X^2-test or Fisher exact test and Student's t-test were applied to compare the prevalence and composition of gut bacteria in children. The correlation Spearman test was used to test the association between the microbiological data and body weight or BMI and total IgE. P-values less than 0.05 were considered statistically significant.

Results and Discussion

Characteristics of the study population are shown in Table 1. In total, 8 overweight with mean of body weight was 58.90 kg (range 42.00-75.20 kg) and BMI 23.10 kg/m^2 (range 22.00-27.05 kg/m^2), 15 obese with mean of body weight was 84.05 kg (range 29.20-126.00 kg) and BMI 31.55 kg/m^2 (range 27.30-40.28 kg/m^2), 20 normal-weight with mean of body weight was 30.11 kg (range 12.00-64.00 kg) and BMI 16.81 kg/m^2 (range 13.08-22.68 kg/m^2) (AC) children and 6 normal-weight with mean of body weight was 31.67 kg (range 14.00-50.00 kg) and BMI 16.83 kg/m^2 (range 14.27-19.56 kg/m^2), 1 overweight with mean of body weight was 40.00 kg and BMI 22.30 kg/m^2 and 17 obese with mean of body weight was 96.83 kg (range 65.00-142.00 kg) and BMI 34.28 kg/m^2 (range 27.40-49.00 kg/m^2) (NAC) children.

Children with allergic diseases we observed atopic dermatitis - 41.46%, asthma - 17.07%, allergic rhinitis -21.95%, food allergies - 9.76%. The prevalence and the counts in the intestinal microflora were shown in Table 2. Differences in the concentrations of bacterial genera between AC and NAC children are presented in Figure 1 and illustrate differences between gut microbiota detected by quantitative plating. Concentration of Enterococcus and Staphylococcus of children AC group were higher than in NAC group (56% vs. 33%, 44% vc. 17%, p=0.05 respectively). In allergic children we observed *S.aureus* with mean 0.51 ± 1.44 log CFU/g and Bacillus with mean 1.11 ± 2.01 log CFU/g, which were characteristic only for this group. The proportion of Bacteroides correlated with the number of Bifidobacteria in AC group of children (r=0.64, p=0.011) and in NAC group of children (r=0.67, p=0.006); proportion of Anaerobic grampositive cocci correlated

Characteristics	Children with allergic diseases (AC) (n=43) Mean ± standard deviation	Non-allergic children (NAC) (n=24) Mean ± standard deviation
Gender (F/M), n	16/27	12/12
Age, y	10,5 ± 4,85	12,08 ± 3,65
Height, m	1,92 ± 0,29	1,57 ± 0,24
Weight, kg	53,49 ± 30,05	78,17 ± 37,15
BMI, kg/m^2	23,16 ± 7,56	29,42 ± 9,13
Mode of delivery (V/C or CD),n	27/16	12/12

Table 1: Characteristics of subjects enrolled in this study.

Microorganisms	AC (n=43)		NAC (n=24)		All children (n=67)	
	Prevalence number (%)	Counts (log CFU/g); median range	Prevalence number (%)	Counts (log CFU/g); median range	Prevalence number (%)	Counts (log CFU/g); median range
Bifidobacteria	13 (30)	7,20 (5,74-9,00)	7(29)	7,09 (5,74-9,00)	20(30)	7,15 (5,74-9,00)
Bacteroides	29 (67)	6,87(5,74-9,00)	18(75)	7,09 (5,74-9,00)	47(70)	6,95 (5,74-9,00)
Anaerobic grampositive cocci	29(68)	6,85 (5,74-8,00)	18(75)	7,08 (6,00-8,00)	47(77)	6,94 (5,74-8,00)
Clostridia	32(74)	3,61(0-4,74)	20(83)	3,96 (0-5,00)	52(78)	3,77 (0-5,00)
Lactobacilli	12(28)	4,91 (3,74-7,00)	6(25)	5,00 (3,74-7,00)	18(27)	4,92 (3,74-7,00)
Enterococci	24(56)	4,26 (0-9,00)	8(33)	2,75 (0-7,00)	32(48)	3,71 (0-9,00)
Other Staphylococcus	19(44)	2,44 (0-8)	4(17)	3,5 (0-7,00)	23(34)	2,82 (0-8,00)
Fungi	5(16)	0,74 (0-5,00)	3(12)	0,54 (0-5,00)	8(12)	0,67 (0-5,00)
Staphylococcus aureus	5(12)	0,51 (0-5)	0	0	5(7)	0,33 (0-5,00)
E.coli	32(74)	6,69(3,74-8,00)	17(71)	6,54 (5,00-8,00)	49(73)	6,64 (3,74-8,00)
Enterobacter	5(12)	0,70 (0-6,00)	3(12)	0,71 (0-7,00)	8(12)	0,70 (0-7,00)
Bacillus	11(26)	1,11 (0-8,00)	0	0	11(16)	0,72 (0-8,00)
Klebsiella	4(9)	0,49 (0-6,00)	1(4)	0,21 (0-5,00)	5(7)	0,39 (0-6,00)

*p<0.05 indicates a significant difference in concentration

Table 2: The prevalence (%), the counts (log10; CFU/g) of gut microorganisms in children.

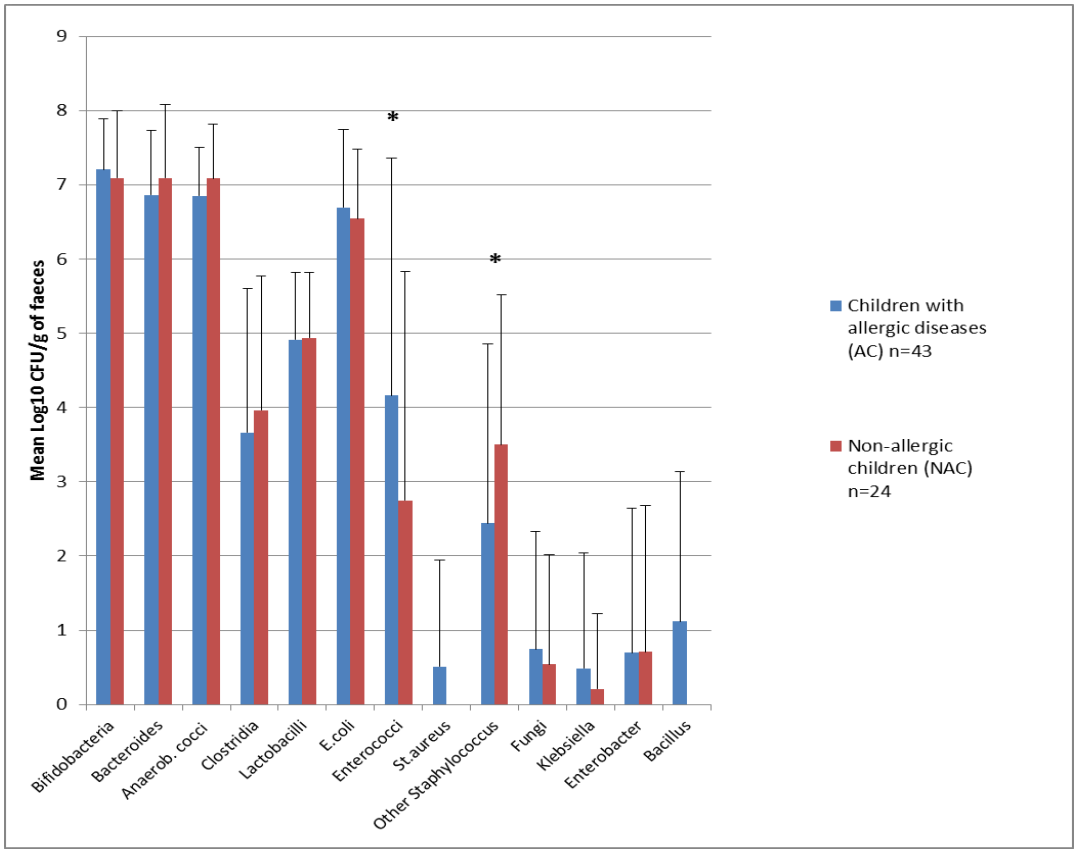

Figure 1: Differences in bacterial genera between AC and NAC group. Differences in bacterial genera between AC and NAC detected by quantitative plating. Error bars 95% CI. *p = 0.05.

with proportion of Fungi (r=0.62, p=0,014) in AC group. In addition, the proportion of *S.aureus* negatively correlated with BMI (r=-0.39, p=0.047) in AC children, while in NAC group of children number of Bacteroides and Bifidobacteria negatively correlated with BMI (r=-0.53, p=0.010; r=-0.42, p=0.046 respectively), but the proportion of Clostridia positively correlated with BMI (r=0.56, p=0.006). The level of total IgE in allergic patients averaged 152.86 ± 51.42 IU/ml and not significantly different from BMI.

Recently, it has been shown that being overweight and growth of allergy cannot be explained by genetic factors alone. According to the 16S RNA studies, a higher proportion of grampositive Firmicutes phyla with low DNA G+C content (Bergey Manual) has been found in obese

children, adults, and experimental animals in comparison to a low proportion of gram-negative Bacteroidetes phyla [21-24]. Although it is known that obesity is associated with changes in composition as well as function of gut microbiota, the mechanism behind this alteration remains to be elucidated. The influence of gut microbiota on nutrient absorption and metabolism has been suggested as a possible mechanism to explain their possible relation to obesity [23]. Alternatively, altered gut microbiota may alter the exposure to obesogenic and diabetogenic environmental chemicals [25].

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