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The relationship between bifidobacteria and allergic asthma and/or allergic dermatitis: A prospective study of 0–3 years-old children in Turkey

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ABSTRACT

Bifid bacteria are beneficial bacteria for humans. These bacteria are particularly effective at protecting against infectious diseases and modulating the immune response. It was shown that in newborns, the fecal distribution of the colonizing *Bifidobacterium* species influences the prevalence of allergic diseases. This study aimed to compare the faecal *Bifidobacterium* species of allergic children to those of healthy children to detect species level differences in faecal distribution. Stool samples were obtained from 99 children between 0 and 3 years of age whose clinical symptoms and laboratory reports were compatible with atopic dermatitis and allergic asthma. Samples were also obtained from 102 healthy children who were similar to the case group with respect to age and sex. Bifid bacteria were isolated by culture and identified at the genus level by API 20 A. In addition, 7 unique species-specific primers were used for the molecular characterization of bifidobacteria. The McNemar test was used for statistical analyses, and $p < 0.05$ was accepted as significant. *Bifidobacterium longum* was detected in 11 (11.1%) of the allergic children and in 31 (30.3%) of the healthy children.

Statistical analysis revealed a significant difference in the prevalence of *B. longum* between these two groups (X^2: 11.2, $p < 0.001$). However, no significant differences in the prevalence of other *Bifidobacterium* species were found between faecal samples from healthy and allergic children. ($p > 0.05$). The significant difference in the isolation of *B. longum* from our study groups suggests that this species favors the host by preventing the development of asthma and allergic dermatitis. Based on these results, we propose that the production of probiotics in accordance with country-specific *Bifidobacterium* species densities would improve public health. Thus, country-specific prospective case–control studies that collect broad data sets are needed.

1. Introduction

The flora of the human gut is one of the most complex ecosystems in nature, and the number of bacteria in the lumen of the gut is approximately 10 times larger than the number of eukaryotic cells in the human body [1–3]. The human gut is sterile at birth but exhibits a rich microflora that contains more than 500 species and approximately 1×10^{14} bacteria one week after birth; the

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establishment of this microflora depends on a number of factors, such as the mode of delivery genetics, environmental contact and nutrition [4]. Within this microflora, bifidobacteria are the most extensively studied lactic acid-producing anaerobic bacteria, comprising approximately 95% of the newborn gut and 25% of the adult gut [5,6].

Bifid bacteria, facilitating the absorption of milk proteins, producing B_1, B_2, B_6, and B_{12} vitamins, and additionally nicotinic acid and folic acid, contribute to protein and vitamin metabolism [7–10]. A number of experiments demonstrated that bifidobacteria are effective not only at activating the immune system but also at enhancing lactose digestion, preventing diarrhea and constipation, and reducing the serum cholesterol level. Most importantly, these bacteria are integral to the daily diet and to therapy due to their antimicrobial and anti-carcinogenic activities [7,8,10].

As a chronic and recurrent inflammatory skin disease, atopic dermatitis is induced by various allergens and genetic factors, particularly in children between the ages of 0 and 6 years [11]. Allergic asthma is a chronic inflammatory disease in patients with bronchial hypersensitivity; a number of cells and cell products play important roles in this disease [12]. This study aimed to identify the *Bifidobacterium* species in stool samples from asthmatic and/or allergic children and in those from healthy children to detect species level differences in fecal distribution.

2. Materials and methods

2.1. Case-control group

This study was a case-controlled, cross-sectional study performed between September 2011 and October 2012. The case group included 99 children between 0 and 3 years of age. Of these children, 57 (57.5%) were boys and 42 (42.4%) were girls.

Clinical symptoms and laboratory reports (i.e., immunological, histological and biochemical reports) compatible with atopic dermatitis or allergic asthma were present in all subjects; 50.5% of the children were diagnosed with asthma, 38.3% were diagnosed with atopic dermatitis and 11.1% were diagnosed with asthma and atopic dermatitis.

In addition, the children in both the case and control groups had been maternally breastfed during their lives and had not taken any antibiotics in the two weeks prior to sample collection. The case group included randomly selected patients who applied to the Department of Pediatric Infectious Disease, the Department of Allergy and Immunology and the Department of Infant Care Service at Kanuni Sultan Suleiman Education and Research Hospital. The control group included 102 healthy children who applied to the same hospital during the same time period. The control group resembled the case group with respect to age and sex and included 58 boys (56.8%) and 44 girls (43%).

2.2. Collecting the samples

One gram of the stool sample obtained from each of the 99 children in the case group and from each of the 102 children in the control group was cultured. As discussed above, we only included children who had not taken any antibiotics in the two weeks prior to sampling and who had been breastfed during their lives.

The stool samples were collected in plastic containers and transferred to liquid Tripticase Phyton Yeast (TPY) medium, they were then kept in anaerobic jars and transported to the laboratory.

2.3. Microscopy and culture

In the laboratory, the specimens were incubated at 37 °C under anaerobic conditions provided with AnaeroGen (Oxoid and Mitsubishi Gas Company) for 48 h. After incubation, 0.5 mL were removed from the liquid TPY medium and transferred to Tripticase Phyton Yeast Agar (TPYA), Wilkins Chalgren Agar (WCA), Man Rogosa Sharpe Agar (MRSA) and Modified Columbia Agar (DP) [13–16]. These media were incubated at 37 °C under anaerobic conditions for 72 h to properly isolate bifidobacteria. At the end of the incubation period, colonies with different morphologies were collected from the media and Gram-stained. The Gram-stained smears were examined, and colonies that exhibited a branched morphology typical of bifidobacteria were selected. The anaerobic characteristics of these colonies were confirmed using the aerotolerance test [17,18]. These anaerobic non-spore-forming Gram-positive rods were identified using API 20 A (BioMérieux, Marci L'etoile, France) to ensure that they belonged to a *Bifidobacterium* species.

2.4. Molecular tests

Throughout the PCR procedure, we isolated the DNA's from PBS suspensions bearing the cultivated colonies of bifidobacteria and we also used the selective liquid mTPY medium containing the stool samples. The High Pure PCR Template DNA isolation kit (Roche Diagnostics, Switzerland) was used for the isolation of bacterial DNA according to the manufacturer guidelines. Species-specific primers for *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Bifidobacterium bifidum*, *Bifidobacterium infantis* and *Bifidobacterium catenulatum* group were selected according to the research of Matsuki T et al. [19]. Species-

Table 1
Bifidobacterium species-specific primers used in the study [19,20].

Target species	Name of primers	Sequence	Primer concentration (nM)	Product size (bp)
Bifidobacterium longum	BILON-1f	TTCCAGTTGATCGCATGGTC	200 nmol	831
	BILON-2r	GGGAAGCCGTATCTCTACGA		
Bifidobacterium adolescentis	BIADO-1f	CTCCAGTTGGATGCATGTC	200 nmol	279
	BIADO-2r	CGAAGGCTTGCTCCCAGT		
Bifidobacterium breve	BIBRE-1f	CCGGATGCTCCATCACAC	200 nmol	288
	BIBRE-2r	ACAAAGTGCCTTGCTCCCT		
Bifidobacterium bifidum	BIBIF-1f	CCACATGATCGCATGTGATTG	200 nmol	278
	BIBIF-2r	CCGAAGGCTTGCTCCCAAA		
Bifidobacterium infantis	BINF-1f	TTCCAGTTGATCGCATGGTC	200 nmol	828
	BINF–2r	GGAAACCCCATCTCTGGGAT		
Bifidobacterium catenulatum group	BICATg-1f	CGGATGCTCCGACTCCT	200 nmol	285
	BICATg-r	CGAAGGCTTGCTCCCGAT		
Bifidobacterium pseudocatenulatum	B-pcat f	AGCCATCGTCAAGGAGCTTATCGCAG	250 nmol	325
	B-pcat r	CACGACGTCCTGCTGAGAGCTCAC		

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