Gut microbiome pattern in adolescents with functional gastrointestinal disease

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A B S T R A C T

Background: Functional gastrointestinal disease (FGID) has a worldwide prevalence of 10–45%, and is one of the most common causes of recurrent abdominal pain in children. FGID is characterized with abdominal discomfort and changes in bowel movement. Alteration in gut microbiota is associated with FGID, but data are limited, and there are no data from Indonesia.

Methods: A case–control study was conducted in 22 FGID children and 28 healthy subjects aged 13–18 years at the junior high school and senior high school in Central Jakarta. FGID was diagnosed using Rome IV criteria. Age, sex, and level of education were recorded. Stool samples were collected and investigated for Bifidobacterium spp. and Enterobacteriaceae.

Results: Most of the FGID subjects were females (17/22), with a median age of 16 years. The median values of Bifidobacterium spp. were 138.95 (range: 0.2–22,735.8) CFU/gram for the FGID subjects and 232.5 (range: 1.9–38,985.6) CFU/gram in healthy subjects, which showed no statistically significant difference (P = .49). The median values of Enterobacteriaceae were 58.9 (range: 2.5–9577.8) CFU/gram in FGID subjects and 85 (range: 12.1–3139.4) CFU/gram in healthy subjects, which showed no statistically significant difference (P = .94). Our findings indicate that the gut microbiome of adolescents with FGIDs is characterized by a huge variability in levels of Bifidobacterium spp. and Enterobacteriaceae.

Conclusion: Because of the wide range detected in the number of Bifidobacterium spp. and Enterobacteriaceae in FGID and healthy subjects, no statistically significant difference was observed. More studies in larger groups of selected patients may be needed.

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1. Introduction

Functional gastrointestinal disease (FGID) is defined using the Rome IV criteria and must, by definition, include all of the following: i) abdominal discomfort (an uncomfortable sensation not described as pain) or pain associated with 2 or more of the following: at least 25% of the time improved with defecation and/or onset associated with a change in the form of stool and/or onset associated with a change in the appearance of stool and ii) no evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explains the subject’s symptoms [1]. Data of the prevalence of FGID in Asian children and adolescents are limited. According to Rome II criteria, the prevalence of FGID in the outpatient clinic of the Pediatric Gastrohepatology Clinic, Cipto Mangunkusumo Hospital, Indonesia, in 2001 was estimated at 4% [2]. In a study based on Rome III criteria in 2014, 14 out of 232 high school students in Jakarta were classified as suffering from FGID, a prevalence of 6.0% [3]. Different mechanisms have been suggested for the pathophysiology of FGID, including psychological disorder, visceral hypersensitivity, and gut microbiome composition [4]. Recent studies suggest that FGID is associated with changes in the gut microbiome [5–8]. The goal of this study was to analyze the Bifidobacterium and Enterobacteriaceae microbiome in children with and without FGID.

2. Materials and methods

2.1. Subjects

From May 2016 to September 2016, 22 Rome IV criteria-positive...
adolescents for FGID aged 13–18 years were recruited from the 216th Junior High School and 68th Senior High School in Central Jakarta. Clinical symptoms of recurrent abdominal pain occurring at least 1 day per week in the last 3 months are associated with two or more of the following symptoms (1) related to defecation, (2) associated with a change in the frequency of stool, and (3) associated with a change in the form (appearance) of stool. Participants were excluded if they had taken antibiotics during the 3 months before inclusion or had suffered any organic disease during 6 months before inclusion in the study. During the same period, 28 healthy age-matched volunteers without any sign or symptoms of organic disease and without intake of antibiotics were included as control. Briefly, the patient and control groups were comparable with similar characteristics, except for the presence of symptoms of an FGID. Approval of the Ethical Committee of the Medical College of Indonesia University was obtained (number 875/UN2.F1/ETIK/2015).

2.2. Study protocol

Stools were collected and transported in an icebox to the Prodia Research and Esoteric Laboratory in Central Jakarta, Indonesia within 1 h after defecation. Once arrived in the laboratory, samples were frozen at −70 °C before DNA extraction. The laboratory was blinded about the origin of the stools, i.e., patients versus controls. Real-time polymerase chain reaction (PCR) was performed using CFX96 from Bio-Rad Laboratories, Inc. The bacterial reference strains were Bifidobacterium breve (ATCC 15700) and Escherichia coli (ATCC 11775) by Remel™. The sequences for Bifidobacterium are CGCGTCTGGGTAAGG and CCCCCATCCAGCCATGCA. The sequences of Enterobacteriaceae are TCGCGAACACTTCGGGGAGAAGC and TCAAGGACACGTTCAGTGC. DNA extraction was performed using QIAamp DNA Stool Mini Kit by Qigen.

2.3. Statistical analysis

All data were collected and analyzed with SPSS version 20.0. The Mann–Whitney U test was used to analyze the data. P value less than 0.05 was considered statistically significant. Data are expressed as median (minimum to maximum value).

3. Results

A total of 50 subjects (22 subjects with FGIDs and 28 healthy controls; 37 females and 13 males) were included. The median age of the FGID group was 16 years, and the median age of the control group was 14 years (Table 1).

The median value of Bifidobacterium spp. was 138.95 (0.2–22,735.8) CFU/gram in the FGID group and 232.5 (1.9–38,985.6) CFU/gram in the control group (p = 0.49). The median value of Enterobacteriaceae is 58.9 (2.5–9577.8) CFU/gram in the FGID group and 85 (12.1–3139.4) CFU/gram in the control group (p = 0.94). No statistically significant difference was found for Bifidobacterium spp. and Enterobacteriaceae between the FGID and control groups (Table 2).

The data of Bifidobacterium spp. and Enterobacteriaceae were transformed, which produced a normal curve (Shapiro–Wilk test > 0.05). The mean value of log Bifidobacterium spp. was 1.94 (95% CI 1.29–2.59) in the FGID group and 2.31 (95% CI 1.89–2.73) in the control group (P = 0.31). The mean value of log Enterobacteriaceae was 2.09 (95% CI 1.63–2.55) in the FGID group and 2.0 (95% CI 1.72–2.28) in the control group (P = 0.73). No statistically significant difference was found for Bifidobacterium spp. and Enterobacteriaceae between the FGID and control groups (Table 3).

These data indicate that the gut microbiome of adolescents with FGID is characterized by a huge variability in levels of Bifidobacterium and Enterobacteriaceae, which result in a statistically nonsignificant trend for lower levels of Bifidobacterium spp. and higher levels of Enterobacteriaceae in adolescents with FGIDs.

4. Discussion

Risk factors for FGID include differences according to age, race, sex, diet, region, psychological insults and familial history [9]. The etiology of FGID is not fully understood. Recent studies showed that the gut microbiota pattern of patients with FGID differs significantly from that of healthy individuals, with an increase in potentially pathogenic bacteria and a decrease in beneficial bacteria [9–12]. Probiotics given during management induce significant changes in the gut microbiota pattern and reduce FGID symptoms [13,14].

This study uses PCR to analyze Bifidobacterium spp., which represents beneficial bacteria, and Enterobacteriaceae, which represents potentially pathogenic bacteria. The two types of bacteria were selected, as there are studies that show significant differences between both types [12,13,15].

This is the first study conducted on Indonesian children and adolescents with FGID and their gut microbiota pattern. This study did not compare food and drinking habits or socioeconomic status. A previous study performed in the same school showed no significant differences between gender, age, academic records, parental educational level, number of siblings, socioeconomic status, use of antibiotics during the previous two months, and consumption of bread and drinking milk/coffee/energy drinks [4]. The prevalence of FGID in this study is 6%, which is the same as that observed in the previous study. devanarayana et al. found in a meta-analysis that FGID prevalence in Asia is approximately 2.8–25.7%, with a median of 12.4% [16].

This study also did not exclude subjects who sometimes consumed commercial probiotic dairy products such as Yakult® containing the Lactobacillus casei Shirota strain. A previous study conducted in 39 subjects with FGID showed that L. casei Shirota, by consuming Yakult® twice daily for 8 weeks (6.5 × 10^8 CFU), failed to show significant improvement [17]. L. casei Shirota strain or placebo administered to 10 healthy subjects thrice daily as 100 ml (minimum 10^9 CFU/ml) for 4 consecutive weeks showed a significant increase in Lactobacillus but no increase in Bifidobacterium. To date, no trial has been carried out in patients with FGID along with a comparable amount of L. casei Shirota strain.

Generally, the gut microbiota is composed of beneficial and potentially pathogenic bacteria. In healthy individuals, there is a balance between beneficial bacteria such as Bifidobacterium and Lactobacillus and potentially pathogenic bacteria such as Enterobacteriaceae, Streptococcus, Enterococcus, Bacteroides, Clostridium, Staphylococcus, Escherichia, and Proteus [19]. The healthy balance in the gut microbiota is the fundamental for a normal gut function, while any dysbiosis may produce gut symptoms.

Table 1

| Subject characteristics                  | FGID group (n = 22) | Control group (n = 28) |
|------------------------------------------|---------------------|------------------------|
| Sex                                      | Male                | Female                 |
|                                          | 5                   | 17                     |
| School                                   | Junior High         | Senior High            |
|                                          | 9                   | 13                     |
| Age (years old)*                         | 16 (13–17)          | 14 (13–17)             |

* Data expressed as median (minimum to maximum value).
Table 2

| Bacterial count (CFU/gram) | FGID (N = 22) | Control (N = 28) | P* |
|---------------------------|---------------|-----------------|----|
| Bifidobacterium spp.      | 138.95 (0.2–22.735.8) | 232.5 (1.9–38.958.6) | .493 |
| Enterobacteriaceae        | 58.9 (2.5–9577.8) | 85 (12.1–3139.4) | .938 |

Legend: FGID: Functional Gastrointestinal Disorder; Data are expressed as median (minimum to maximum value); * Mann–Whitney U Test.

Table 3

| Log Bacterial count | FGID (N = 22) | Control (N = 28) | P* |
|---------------------|---------------|-----------------|----|
| Bifidobacterium spp. | 1.94 (1.46) | 2.31 (1.08) | .312 |
| Enterobacteriaceae  | 2.09 (1.03) | 2.00 (0.71) | .726 |

Legend: FGID: Functional Gastrointestinal Disorder; Data are expressed as mean (±SD); * Unpaired t-Test.

Bifidobacterium spp. is a dominant microorganism in the human intestine that produces essential nutrients for the mucosa, such as short-chain fatty acids (SCFAs) and lactic acid. They eliminate toxins and unnecessary substances by decreasing the intraluminal pH and inhibiting the growth of potential pathogenic organisms such as E. coli, Salmonella, and Staphylococcus aureus [20,21]. Bifidobacterium also stimulates gut epithelial cell turnover, hence correlating positively with secretory IgA, and prevents bacterial translocation, hence correlating negatively with E. coli counts [22,23]. This study found a trend for higher counts of Bifidobacterium in healthy subjects, although the difference was not statistically significant. Previous studies also showed higher counts of Bifidobacterium spp. such as Bifidobacterium catenulatum in healthy subjects. [9] The tendency of lower counts of Bifidobacterium in subjects with FGID might be a marker for the gut microbiota pattern in Indonesia. The composition and diversity of the microbiome are established on the basis of the luminal environment (physical, chemical, and biological exposure) and host surveillance. The chemical exposure derived from nutrients and other xenobiotics can influence the dynamics of the microbiome community (the stability, diversity, or resilience) [24].

Enterobacteriaceae, the main cause of endotoxin production, comprises many species such as E. coli, Klebsiella pneumoniae, Salmonella typhimurium, Shigella, Proteus, Enterobacter, Serratia, and Citrobacter. These microorganisms in Enterobacteriaceae may produce ammonia and sulfureted hydrogen and can impair gut absorption of water, glucose, and electrolytes [25]. Pimentel et al. found that 78% of the subjects with FGID had small intestinal bacterial overgrowth. Bacterial eradication revealed an improvement in symptoms in these patients; 48% of eradicated subjects did no longer meet the Rome criteria for FGID [26]. Our study found a trend for higher counts of Enterobacteriaceae in subjects with FGID, although not statistically significant. Previous studies found a statistically significant increase in subjects with FGID [12,22,27–29].

5. Conclusion

Because of the wide range observed in the number of Bifidobacterium spp. and Enterobacteriaceae in healthy adolescents and those with FGID, no statistically significant difference was observed. The wide range may be due to environment, such as dietary factors. Consequently, more data are needed in large patient groups under strict control of environmental factors.

Conflicts of interest

There is no conflict of interest. This research was funded by the researcher.

Ethical statement

Approval of the Ethical Committee of the Medical College of Indonesia University was obtained (number 875/UN2.F1/ETIK/2015).

CRediT authorship contribution statement

Andrew R. Nafarin: Data curation, Project administration, Writing - original draft, Conceptualization. Badriul Hegar: Data curation, Project administration, Writing - original draft, Conceptualization. Hikari A. Sjaktil: Data curation, Project administration, Writing - original draft. Yvan Vandenberg: Writing - original draft, Data curation, Conceptualization.

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