Gut Microbiota Characteristics in Children After the Use of Proton Pump Inhibitors

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ABSTRACT

Background/Aims: *Prolonged acid suppression from proton pump inhibitor (PPI) has been shown to cause gut microbiota alteration, which may increase the risk of various infections in adults. We aimed to characterize gut microbiota profiles in children after a short-term use of PPI.*

Materials and Methods: *Children aged 1-18 years who underwent PPI therapy were included during April-December 2017. We excluded children who previously used antibiotics or acid suppressants and who had a history of acute gastroenteritis or specific food avoidance one month prior to the enrolment. The stool samples before and after the PPI use were collected for gut microbiota composition. The 16S ribosomal RNA gene sequencing was performed by using Illumina MiSeq. The differences in the gut microbiota profile after the use of PPI were compared to pre-PPI period.*

Results: *We completed stool collection in 20 children (median age of 5.8 years and 60% were female). No significant changes in the overall number of species-level taxonomy categories or predominant bacteria belonging to the phylum (Bacteroidetes) were noted. We found a trend increase in the proportion of the phylum Firmicutes among children living in the designated metropolitan/suburban area (P = .07) and among males (P = .11). In four children with infection-related adverse effects, we noted a nonsignificant increase in the proportion of the phylum Firmicutes after the PPI use (from 35% to 52%, P = .14).*

Conclusions: *Even the total number of and predominant gut microbiota did not significantly change after a four- to eight-week course of PPI therapy; we found a trend of increase in the proportion of the phylum Firmicutes in certain groups of children.*

Keywords: *Acid blocker, diarrhea, dysbiosis, infection*

INTRODUCTION

Proton pump inhibitors (PPIs) are the commonly used acid blocker among children. The medications have been shown to be overused globally in the past decade.[1,2] While the use of PPIs has been considered safe with a low incidence of serious adverse effects,[3] less acidic luminal environment has been associated with an increased risk of enteric infections in children, for example, *Clostridium difficile*.[4,5] From a recent meta-analysis in adults, PPIs also have been shown to increase the risk of small intestinal bacterial overgrowth.[6] Studies in children also demonstrated an evidence of bacterial overgrowth among PPI users.[7-9] An increase in the gastric pH level has been shown to affect the balance and regulation in the microbiota environment.[10,11] Gut microbiota refers to the microorganisms within the human intestinal lumen, which is important in resisting or promoting the microbial colonization and growth.[11,12] Many factors affect gut microbiota such as age,[13] gender,[14] body mass index,[15,16] and living area.[17] Acid suppression from PPIs also leads to altered profiles and diversity. However, almost all studies on PPIs affecting gut microbiota have been performed in adults, revealing discrepant results.[5,10-12,18,19] Studies in the pediatric population are very limited. Children can also have many different factors affecting the gut microbiota (such as type of food, gut immaturity, and immunity) when compared to adults.[13] Therefore, we aimed to study the change of microbiota characteristics in children after PPI therapy.

MATERIALS AND METHODS

We conducted a prospective, observational study enrolling children and adolescents aged 1-18 years, who underwent a four- to eight-week course of oral PPI therapy at a teaching and referral hospital between April and December 2017. We excluded children who previously used antibiotics or acid blockers and had a history of acute gastroenteritis and/or specific food avoidance one

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month prior to the enrollment. The institutional review board approved the study (IRB No. 01-60-12, decision date March 3, 2017). Informed consent was gathered from all participants beforehand.

Demographic data including anthropometric measurement, living area, underlying disease(s), a condition indicated for the PPI use, medication, and nutritional history that possibly affect gut microbiota were collected. PPI data including type, dosage, duration, and infection-related adverse effects were recorded in the structured case record form. We defined the country's capital (Bangkok) and five provinces surrounding the capital as urban/metropolitan area, and we used hospital records registration to track the children's area of residence. Before the first dose of PPI therapy, the fecal sample was collected by fresh stool collection, kept in a sterile plastic container, and stored at –80°C within 24 hours for further DNA extraction and analysis prior to the DNA processing. The second stool sample was collected 4-8 weeks after the first collection with the same method.

Sample Preparation

Fecal samples were then processed under the protocol for amplification and sequencing of the variable regions of 16S ribosomal subunit. Microbial DNA extraction from samples was performed using the QIAamp® Fast DNA stool mini kit (Qiagen, Duesseldorf, Germany). To determine the bacterial profile of stool samples, sequencing of the variable region V3-V4 of the 16S ribosomal RNA gene was performed using Illumina MiSeq (Illumina Inc., California, San Diego, USA) with the 16S metagenomic sequencing library preparation, following the manufacturer manual.

Gut Microbiota and Statistical Analysis

The analysis of the bacterial taxonomy profile was performed by 16S metagenomics (Illumina Inc.) software version 1.0.1.0 on Basespace application. The demographic data and within-individual differences in the number of bacterial species before and after PPI therapy were tested using the paired *t* test for normally distributed data or the Wilcoxon signed rank test. The proportions of bacterial phylum were calculated. Data were analyzed using STATA version 14 (StataCorp, Texas, College Station, USA). The microbiota profiles and diversity were analyzed using Quantitative Insights Into Microbial Ecology software (QIIME v 1.9.1; Denver, Colorado, USA) (software (v 1.9.1) on a Basespace application, while alpha and beta diversity of gut microbiota and relative abundances were compared between pre- and post-PPI therapy.

Table 1. Baseline Patient Characteristics of the Studied Children

Characteristics	Value
Age (years), median (IQR)	5.8 (3.7-10.4)
Male, n (%)	8 (40%)
Overweight and obesity, n (%)	5 (25%)
Living in the metropolitan and suburban area, n (%)	9 (45%)
Indications for starting proton pump inhibitor, n (%)	
Gastritis/erosion	8 (40%)
Clinical gastroesophageal reflux disease	5 (25%)
Dyspepsia	4 (20%)
Chronic abdominal pain	3 (15%)
Proton pump inhibitor use, n (%)	
Omeprazole	11 (55%)
Lansoprazole	8 (40%)
Esomeprazole	1 (5%)
Proton pump inhibitor dosage (mg/kg/day), mean (SD)	0.9 (0.3)
Duration of proton pump inhibitor (days), mean (SD)	35.8 (6.5)
Parent-reported adverse effects, n (%)	
Upper respiratory tract infection	2 (10%)
Acute diarrhea	2 (10%)

RESULTS

We initially enrolled 33 children for the study, but 12 children were lost to follow-up due to inconvenient timing. The stool collections (pre- and post-PPI therapy) were completed in 21 children, but an error in DNA extraction occurred in one child. Thus, the final analysis of gut microbiota profiles was performed in 20 children. Patient characteristics are shown in Table 1. Median age was 5.8 years (interquartile range: 3.7, 10.4), with 40% children being male. We noted 25% of the children were overweight or obese according to the WHO criteria. Forty-five percent resided in the metropolitan or suburban area. None used medications that were previously reported to interfere with the PPI metabolism. Indications for PPIs were mainly macroscopic gastritis/mucosal erosion and clinical gastroesophageal reflux disease. Most used omeprazole (55%) or lansoprazole (40%) as a once-daily dose with an average dosage of 0.9 mg/kg/day (SD 0.3) for 36 days (SD 6). There were four parent-reported infections (two with upper respiratory tract infection and the other two children had diarrhea). These children never had fever and

did not require antibiotics for these episodes. They all recovered at the follow-up visit.

Comparing Gut Microbiota Before vs. After the Proton Pump Inhibitor Use

Before the PPI therapy, the predominantbacteria belonged to the phylum Bacteroidetes. This also held similar even when analyzing various subgroups of childrenby age (< 3 vs. ≥ 3 years old), gender, body mass index,and living area (metropolitan/suburban vs. rural area). We observed a nonsignificant change in the overall number of species-level taxonomy categories (451 and 453 species, respectively; $P = .94$). The alpha diversity at the genus and species levels showed no difference between the pre- and the post-PPI period. The chaos1, observed OTUs, and whole tree phylogenetic diversity represented the relative abundance of gut microbiota, as shown in Figure 1. Furthermore, the pattern of gut microbiota of both groups showed no difference according to principal coordinate analysis using the weighted and unweighted UniFrac distance metrics as demonstrated in Figure 2. The predominant organisms belonged to the phylum Bacteroidetes both before and after the PPI therapy.

However, we noted a trend of increase in the proportion of bacteria in the phylum Firmicutes in some subgroups.

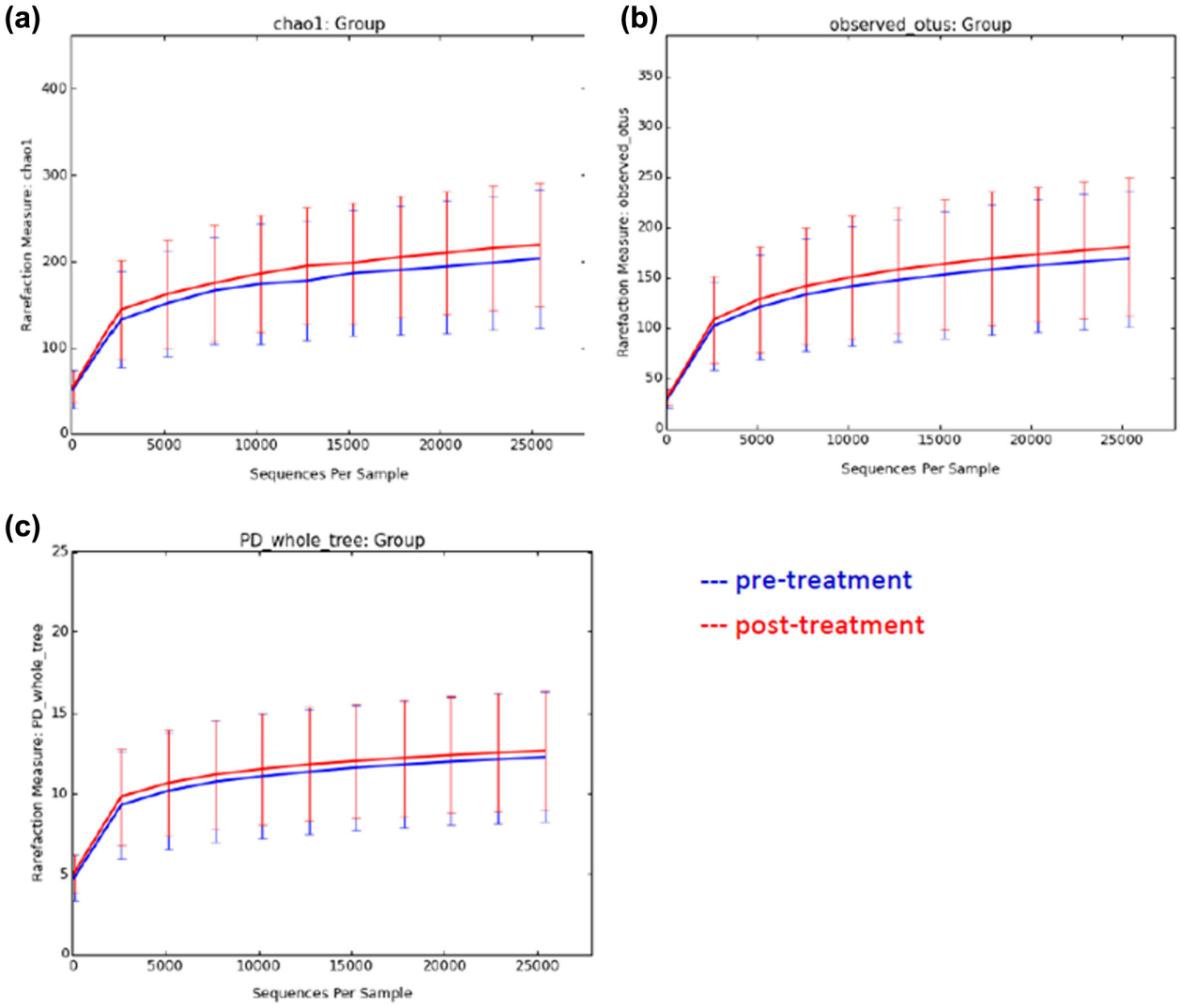

Figure 1. The chaos1, observed OTUs, and whole tree phylogenetic diversity between pre- and post-PPI treatment groups, which represented the relative abundance of gut microbiota.

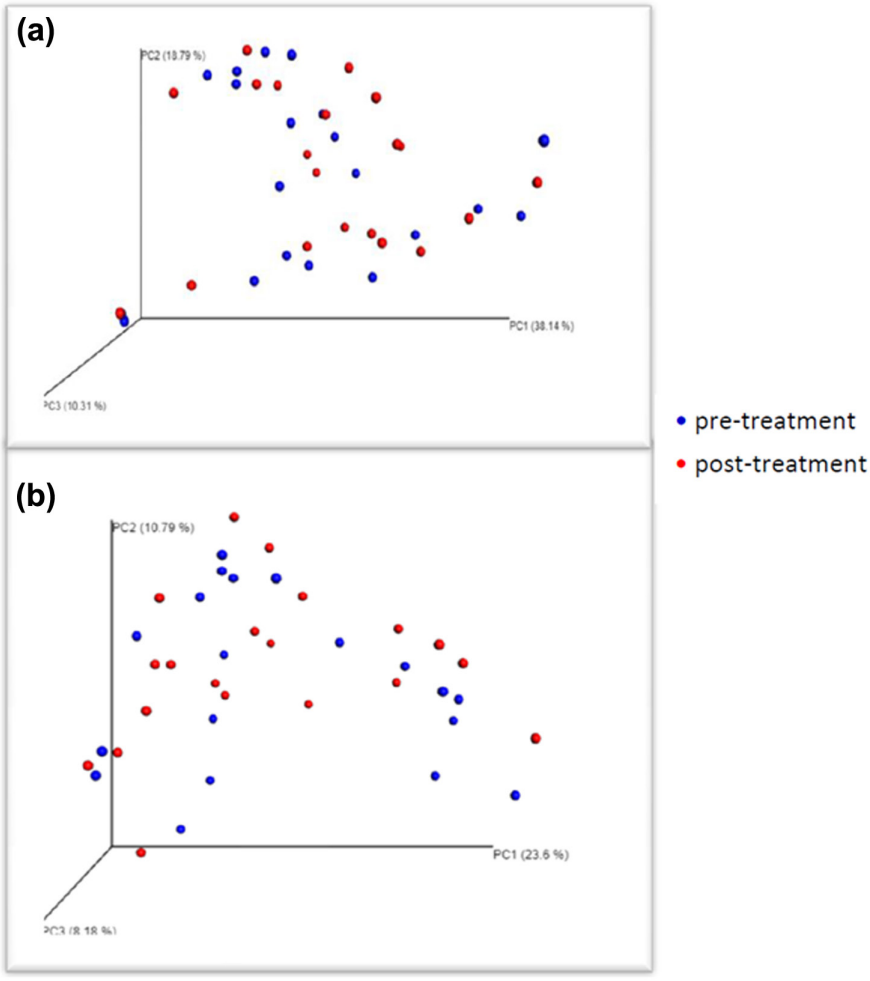

Figure 2. Pattern of gut microbiota in pre- and post-PPI treatment groups using (a) weighted and (b) unweighted UniFrac distance.

In children living in the metropolitan or suburban area, the proportion of bacteria in the phylum Firmicutes revealed an increased proportion from 34% to 45%, pre- and post-PPI therapy, respectively ($P = .07$). Furthermore, we observed this trend among males ($P = .11$). We could not find a substantial increase in the proportion of bacterial populations belonging to the phylum Firmicutes in children due to factors such as the rural area, gender, overweight/obesity, or duration of PPI therapy. In four children whose parents reported infection-related adverse effects, we also found a nonsignificant increase in Firmicutes from 35% pre-PPI to 52% post-PPI ($P = .14$).

DISCUSSION

In this study, we sought to determine the changes in gut microbiota profiles in children after the use of PPI. Before initiating PPI, we found that the baseline predominant gut microbiota was bacteria belonging to the phylum Bacteroidetes, a finding that was similar to studies in adults.[5,13,18] After the course of four to eight weeks of PPI therapy, we could not demonstrate a significant change in the total number of species-level taxonomy categories. Our results were similar to the two previous adult studies with regard to the nonsignificant changes in the total number.[5,19] The microbiota diversity analyses also showed no significant change in the alpha and beta diversity, which represented a relatively similar abundance of gut microbiota in both pre- and post-PPI groups.

In the subgroup analyses of potential associated factors, when analyzing at the phylum level, we found a trend of increase in the proportion of the phylum Firmicutes

among children living in the metropolitan/suburban area and among males. A similar finding on the increased proportion of Firmicutes was also noted in a study among adults aged older than 50 years.[18] Studies have shown that adults living in the urban area had a lower diversity of gut microbiome that is unhealthier than that in people living in rural regions.[17,20,21] We hypothesized that children living in the urbanized area might be vulnerable to an alteration in the gut microbiota owing to the modification of the gastrointestinal luminal environment (i.e., less acidic). The trend of microbiota change among males is difficult to explain with reasonable biologic plausibility. Previous studies demonstrated that hormonal differences between genders may affect gut microbiota profiles,[14] but most of our studied patients were pre-pubertal children. In children with infection-related adverse effects, the result showed no statistically significant change in the proportion of Firmicutes among a very small sample size.

We did not formally document the compliance of PPI use, but we asked the caregivers to bring the PPI package back during the follow-up visit or gave them a telephone call to check the leftover PPI. Infection-related adverse effects were noted on the basis of the parental report during the follow-up visit. The study was performed in a small group of children at a single tertiary care teaching hospital—a factor that may limit generalizability and robust analyses on the changes of complex gut microbiota profile—but we hope that this data would provide a platform for further studies with a larger group of pediatric patients with regard to the effect of PPIs on gut microbiota.

CONCLUSION

Although a four- to eight-week course of PPI therapy in children did not significantly change the total number, alpha and beta diversity, and predominant phylum of gut microbiota, we noted a nonsignificant trend of increase in the proportion of Firmicutes in children living in the metropolitan/suburban area and among males. Future pediatric studies are required before advising health-care personnel on the use of PPI-affecting gut microbiota, which may lead to various clinically significant adverse effects.

Ethics Committee Approval: The Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, approved the study (IRB No. 01-60-12, decision date 3 March 2017) with the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net).

Informed Consent: Written informed consent was obtained from the patients' parents who participated in this study.

Peer-review: Externally peer-reviewed.

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