Associations between Dietary Intakes and the Gut Microbiome in Children with Solid Tumors after Chemotherapy and Healthy Controls

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Abstract

Background: Malnutrition is a common complication in children with cancer. Cancer treatment and malnutrition can disrupt gut microbiome diversity and composition. This study aims to compare the dietary intakes between children with solid tumors post-chemotherapy and healthy controls, and investigate associations between the dietary intakes and the gut microbiome. Procedure: Children (7-18 years) with solid tumors were recruited during year 1 after the completion of chemotherapy from Children's Healthcare of Atlanta, Atlanta, Georgia. Healthy controls were recruited via flyers. Children completed the Block Kids Food Screener for dietary intakes in the past week. Fecal specimens were collected and processed for the gut microbiome. QIIME2 and Mann-Whitney U tests were conducted to answer the research questions. Results: Forty-nine children (25 cancers vs 24 controls) were analyzed. Two groups had no differences in age, race, sex, and body mass index. Children with solid tumors reported significantly higher mean daily intakes of macronutrients: calories, protein, fat, carbohydrate, and fiber, and antioxidant nutrients (vitamin E, vitamin C, and selenium) than controls. Children with adequate vitamin B6 had a higher Chao1 diversity index than children with inadequate or excessive intake (P = 0.0004). Children with excessive selenium intake had a trend of higher Pielou's_e index than children with inadequate intake (P = 0.091). Conclusion: Children with cancer reported significantly higher intakes of macronutrients and antioxidant nutrients than healthy children, but no differences in major energy ratios. Macronutrients, particularly antioxidant nutrients, were associated with disruptions of the gut microbiome in children with solid tumors.

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Running Head: Diet and Gut Microbiome in Children with Cancer

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Abbreviations:

ALL	acute lymphoblastic leukemia
ANCOM	analysis of composition of microbiomes
ASV	exact sequence variant
BKFS	Block Kids Food Screener
CHOA	Children's Healthcare of Atlanta
CNS	central nervous system
ESPEN	European Society for Clinical Nutrition and Metabolism
Faith's PD	Faith's phylogenic diversity
FFQ	Food Frequency Questionnaire
GI	gastrointestinal
HMP	Human Microbiome Project
OTU	operational taxonomic unit
PermANOVA	permutational multivariate analysis of variance
Pielou's_e	Pielou's evenness
QIIME 2	Quantitative Insight Into Microbial Ecology 2
RDA	recommended dietary allowance
U.S.	the United States

Abstract

Background: Malnutrition is a common complication in children with cancer. Cancer treatment and malnutrition can disrupt gut microbiome diversity and composition. This study aims to compare the dietary intakes between children with solid tumors post-chemotherapy and healthy controls, and investigate associations between the dietary intakes and the gut microbiome.

Procedure: Children (7-18 years) with solid tumors were recruited during year 1 after the completion of chemotherapy from Children's Healthcare of Atlanta, Atlanta, Georgia. Healthy controls were recruited

via flyers. Children completed the Block Kids Food Screener for dietary intakes in the past week. Fecal specimens were collected and processed for the gut microbiome. QIIME2 and Mann-Whitney U tests were conducted to answer the research questions.

Results: Forty-nine children (25 cancers vs 24 controls) were analyzed. Two groups had no differences in age, race, sex, and body mass index. Children with solid tumors reported significantly higher mean daily intakes of macronutrients: calories, protein, fat, carbohydrate, and fiber, and antioxidant nutrients (vitamin E, vitamin C, and selenium) than controls. Children with adequate vitamin B6 had a higher Chao1 diversity index than children with inadequate or excessive intake ($P = 0.0004$). Children with excessive selenium intake had a trend of higher Pielou's_e index than children with inadequate intake ($P = 0.091$).

Conclusion: Children with cancer reported significantly higher intakes of macronutrients and antioxidant nutrients than healthy children, but no differences in major energy ratios. Macronutrients, particularly antioxidant nutrients, were associated with disruptions of the gut microbiome in children with solid tumors.

1. INTRODUCTION

Approximately 16 000 children and adolescents are diagnosed with cancer in the United States (U.S.) each year [1]. Among children with cancer, about 30% of them are diagnosed with extracranial solid tumors [2]. Malnutrition is a common complication in children with cancer [3]. Current literature has reported that both malnutrition and undernutrition are highly prevalent from diagnosis until the completion of therapy, particularly among children with solid tumors [4,5].

The gut microbiome, defined as the collection of microbes and their genomes in the gastrointestinal (GI) tract [6], plays a critical role in human health and disease [7]. Accumulating evidence has demonstrated that long-term diet is a primary driver of the diversity and composition of the gut microbiome[8,9], accounting for 44% of the total variation in average microbiome composition. Previous study showed that there was significant longitudinal pairing of diet with the microbiome for 78% of the subjects [10]. Intake of specific dietary components further indicated how certain bacteria respond to specific nutrients[11]. Nutrients such as protein, fat, digestible and non-digestible carbohydrate, prebiotics, and polyphenols could individually induce shifts in the gut microbiome with secondary effects on host immunologic and metabolic markers [10,11]. Thus, it is important to build a healthy gut microbiome through modulating diet [9].

Maintaining a healthy gut microbiome is critical among children with cancer as dysbiosis in the gut microbial composition has been widely reported across the continuum of cancer treatment[12-15] and even survivorship [16]. Dysbiotic gut microbiome (i.e., loss of keystone taxa, loss of diversity, shifts in metabolic capacity, or blooms of pathogens)[17,18] not only interferes with cancer chemotherapeutic metabolism, but also serves as a potential biomarker of GI toxicity in children with cancer, including mucositis, diarrhea, constipation, and infections [19]. Based on the microbiome-gut-brain axis[20,21], disrupted gut microbiome was associated with psychoneurological toxicities such as inflammatory pain, fatigue, anxiety, depression, and cognitive dysfunction[12,21-23]. Currently, dysbiotic gut microbiome profiles have been reported in children with cancer receiving treatments (e.g., chemotherapy) as well as cancer survivors [13,24]. Specifically, children and adolescents with acute lymphoblastic leukemia (ALL) reported a lower diversity of the gut microbiome than healthy controls [13,25]; compared with the day before chemotherapy, the number of bacteria dramatically decreased after chemotherapy started [26]. Additionally, Cozen et al. found that cancer survivors of adolescent and young adult Hodgkin lymphoma showed a significantly lower value of unique operational taxonomic units (OTUs) of the gut microbiome than healthy controls[16].

There is a lack of research focusing on the gut microbiome in children with solid tumors [12] and relationships between diet and changes in the gut microbiome have yet to be studied[8]. As children with cancer experience both malnutrition and alterations in the gut microbiome across cancer treatments, understanding associations between diet and the gut microbiome could provide new insights into biological mechanisms of cancer treatment-related symptoms and toxicities. Finding out the relationship between the gut microbiome and diet in children with solid tumors could help clinicians better understand how to use diet to modulate the gut microbiome, therefore relieving treatment-related GI toxicities (e.g., stomatitis, constipation, and diarrhea)

and central nervous system (CNS)-related toxicities (e.g., anxiety and cognitive dysfunction). Thus, the purposes of this study were to: 1) compare the intake of macronutrients and antioxidant nutrients between children with solid tumors post-chemotherapy (within 1 year) and those of healthy controls; and 2) examine the association between macronutrients, antioxidant nutrients and the gut microbiome in this population.

2. METHODS

2.1 Design and Setting

This study used a cross-sectional design. We enrolled 7-18-year-old children with solid tumors after they were consented to participate in this study from the AFLAC Cancer and Blood Disorder Center in Children's Healthcare of Atlanta (CHOA) in Atlanta, Georgia. Age, sex, and race-matched healthy controls were recruited via flyers, online e-news blast, and ResearchMatch (a disease-neutral web-based recruitment registry).

2.2 Participants

This study included two groups of children: one group with solid tumors (case group) and one group of healthy children (control group). For the case group, eligible children had to meet the following criteria: 1) were diagnosed with solid tumors (e.g., sarcomas, germ-cell tumors, and neuroblastoma); 2) received at least one cycle of chemotherapy; 3) completed chemotherapy within one year; and 4) agreed to participate. Children were excluded if they did not receive any chemotherapy, could not understand and answer the questionnaires, or had a cognitive impairment, such as Down's syndrome. Regarding the control group, healthy children who had not received antibiotics within the past 4 weeks and who had not been diagnosed with chronic or autoimmune diseases or conditions that can influence the gut microbiome profiles were included. These two groups were matched by age, sex, and race during recruitment.

2.3 Measures

2.3.1 Gut microbiome. The gut microbiome was assessed using fecal specimens. According to the Human Microbiome Project (HMP) protocol[27], parents and children were taught to collect fecal specimens using the stool collection kit and store them in freezer at home before shipping to the laboratory. During the hospital visit, the trained research staff provided the parent with the fecal sample collection kit. The samples were frozen before being shipped to the Biobehavioral Laboratory at School of Nursing, Emory University. Once received by the research staff, the stool samples were stored in a -80 freezer until DNA extraction and assaying.

2.3.2 Dietary intakes. The dietary intake of nutrients was measured using the Block Kids Food Screener (BKFS), which includes 41 items developed by NutritionQuest (Berkeley, CA, US). This instrument has been validated to evaluate dietary intake of nutrients and food groups among children aged 2-18 years. Parents, together with their child, completed the BKFS to estimate the child's intake of fruit, vegetables, dairy, whole grains, protein sources, saturated fat, and sources of added sugars. The frequency of food and beverage consumption ranges from "none" to "every day". Studies have proved that BKFS has good relative validity to examine the nutrients and food groups in children and adolescents. Overall correlations with 24-hour dietary recalls and with the Food Frequency Questionnaires (FFQ) were high and Bland-Altman plots showed strong agreements between BKFS and FFQ[28]. In this study, macronutrients and antioxidant nutrients were analyzed [29,30].

2.3.3 Demographic and clinical variables. Child's demographic data (e.g., age, gender, race/ethnicity, height, weight, and BMI percentile), health history (e.g., use of antibiotics and disease history), cancer diagnosis and treatment data (e.g., diagnosis and cycles of chemotherapy) were obtained from the electronic medical record.

2.4 Procedures

Participants in the case group were recruited during their routine outpatient clinic visits. Clinical collaborators from CHOA identified eligible patients and asked them whether they were willing to discuss the study

with our research team. After they agreed, one trained research staff described the study, consented parents, and assented age-eligible patients. Questionnaires were distributed to the children to complete during clinic visits, and parents were instructed on the stool specimen collection at home. The electronic medical records of the pediatric patients with solid tumor were used to collect the demographic information, health history, cancer diagnosis and treatment-related information. For the control group, all the procedures were the same, excluding the use of the electronic medical records.

2.5 DNA extraction and sequencing

Based on the HMP standard operating protocol, the microbial DNA was extracted from fecal samples using the PowerSoil isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) at Environmental Microbial Genomics Laboratory in Georgia Institute of Technology. The 16S rRNA amplicon libraries were prepared for the 16S rRNA V4 region[31,32]. These 16S rRNA amplicons were generated using KAPA HiFi HotStart ReadyMix (KAPA Biosystems, KK2600) and primers specific to 16S V4 region of Bacteria and indices were attached using the Nextera XT Index kit (Illumina, FC-131-1001). Clean-up was performed on the indexed libraries using AMPure XP beads. The 16S libraries were pooled in equal amounts based on fluorescence quantification. Each run included a control template to test for PCR accuracy and possible contamination. Final library pools were quantitated via qPCR (Kapa Biosystems, catalog KK4824). The pooled library was sequenced on an Illumina miSeq using miSeq v3 600 cycle chemistry (Illumina, catalog MS-102-3003) at a loading density of 8 pM with 20% PhiX, at PE300 reads. The microbial sequencing led to paired-end sequences.

2.6 Bioinformatics and statistical analysis

The macronutrients and micronutrients intake from the BKFS were calculated by the NutritionQuest. Based on the recommended daily nutritional intake from 2015-2020 dietary guideline, the child's nutritional intake was categorized into three levels: inadequate level (less than 90% of the recommended dietary allowance [RDA]); adequate level (within the range of 90%-110% of the RDA); and excessive level (more than 110% of the RDA).

Quantitative Insight Into Microbial Ecology 2 (QIIME 2) was used to analyze the taxonomic composition and diversity of the gut microbiome[33,34]. QIIME 2 default parameters were used for sequencing data, and sequence quality was filtered with DADA2[35] to infer exact sequence variants (ASVs). By removing the length of primers, the raw sequences were trimmed at 0 and 0 base pairs, and then truncated at 250 and 215 base pairs based on the Phred Quality score >30. Taxonomies were assigned by a Naive Bayes classifier trained on the Greengenes database with sequences adapted to the 16S rRNA V4 gene region. The α-diversity (richness and evenness of the gut microbiome within samples) was calculated using four different parameters: Shannon's index, Chao1, Faith's phylogenetic diversity (Faith's_PD), and Pielou's evenness (Pielou's_e). Mann-Whitney U tests were used to compare dietary status between children with cancer and healthy controls. Spearman's correlation was used to explore correlations between the relative abundance of gut microbiome taxa and the alpha diversity index with diet. Pairwise permutational multivariate analysis of variance (PermANOVA) [36] was used to test taxa dissimilarities between nutritional intake levels. The analysis of composition of microbiomes (ANCOM) [37] was used to analyze associations between diet and the abundance of the gut microbiome.

3. RESULTS

3.1 Characteristics of participants

Forty-nine children were enrolled including 27 cancer cases and 22 healthy controls **(Table 1)** . The cancer group included 13 boys and 14 girls, with a mean age of 14.4 years. The control group consisted of 9 boys and 13 girls, with a mean age of 12.1 years. No significant differences were found between the two study groups in age ($P = 0.053$), gender ($P = 0.774$), race ($P = 0.172$), and BMI ($P = 0.346$).

3.2 Dietary intakes in children with cancer and healthy controls

Table 2 shows comparisons of dietary intake between the cancer and healthy control groups. Macronutrients were analyzed both on the net weight of intake and the percentage they took up in total intake based on calories. The cancer group had a significantly higher total daily calories intake ($P = 0.047$) while there were no significant differences of percentage intake of macronutrients between the two groups. As for micronutrients and trace elements, the cancer group had a significantly higher intake in vitamin E ($P = 0.026$), vitamin C ($P = 0.022$), and selenium ($P = 0.027$).

3.3 Profiles of the gut microbiome

The raw sequence count per sample ranged from 3 456 to 234 172 for the gut microbiome samples, with an average sequence count of 58 613 per sample. After the DADA2 process, 1 138 features were reported, with a total frequency of 1 926 036. Frequencies per feature ranged from 2 to 249 452, with a median frequency of 72; feature frequencies per sample ranged from 1 857 to 165 900, with a median frequency of 33 888. By using the trained classifiers based on Greengenes 13_8 99% OTUs (taxonomic assignment based on a 99% similarity), the bacterial taxonomy of the fecal specimens included 11 bacterial phyla and 133 genera. The top dominant bacterial phyla **(Figure 1A)** were*Bacteroidetes* , *Firmicutes* , *Proteobacteria* ,*Verrucomicrobia* , and *Actinobacteria* , whereas the dominant bacterial genera **(Figure 1B)** included *Bacteroides* ,*Faecalibacterium* , *Prevotella* , *Roseburia* , and*Ruminococcus* .

3.4 Associations between dietary intakes and the gut microbiome diversity

Table 3 shows correlations between diet and the alpha-diversity indices of the gut microbiome. Total protein intake showed negative associations with Shannon's index ($P = 0.02$) and Pielou's_e index ($P = 0.03$); based on the calorie's percentage, intake of carbohydrate ($P = 0.07$) and fiber ($P = 0.07$) showed trends of positive associations with Chao1. Regarding the micronutrients, the amount of beta-carotene intake had a positive correlation with Faith's_PD ($P = 0.02$) and a trend of positive association with Chao1 ($P = 0.08$); however, the amount of selenium intake was negatively correlated with Shannon's index ($P = 0.05$) and Pielou's_e ($P = 0.03$), and vitamin A showed a trend of negative association with Pielou's_e ($P = 0.06$).

The alpha-diversity was compared between three nutritional intake levels (inadequate, adequate, and excessive) **(Table 4)** . Compared with the group with inadequate carbohydrates intake, the adequate intake group had a significantly higher Chao1 ($P = 0.005$) and Faith's_PD ($P = 0.008$), as well as a trend of higher Shannon's index ($P = 0.083$). Children with adequate vitamin B6 had a higher Chao1 diversity index than children with inadequate or excessive vitamin B6 ($P = 0.0004$). Children with excessive selenium intake had a trend of higher Pielou's_e index than children with inadequate selenium intake ($P = 0.091$).

3.5 Associations between levels of dietary intakes and the gut microbial abundance

ANCOM was used to analyze associations between gut microbiome abundance and levels of nutritional intake. At the phylum level, children with adequate fiber showed a higher abundance of *Cyanobacteria*($W = 8$). At the genus level, children with excessive total calories intake had a higher abundance of *Catenibacterium*($W = 32$). Children with inadequate fat intake based on calories percentage had higher abundances in family *S24-7* ($W = 26$) and in genus *Megasphaera* ($W = 9$) while children with adequate fat intake had higher abundances in bacterial family*Erysipelotrichaceae* and *Peptostreptococcaceae* . Children with adequate fiber intake had a higher abundance of bacterial order *YS2* ($W = 99$).

4. DISCUSSION

This study compared dietary intakes between children with solid tumors and healthy children and examined associations between dietary intakes and the gut microbiome in these children. We found that children with cancer reported significantly higher intakes of macronutrients and antioxidant nutrients than healthy children, but no differences in major energy ratios. Additionally, we found significant associations between macronutrients (e.g., carbohydrates and fiber) and micronutrients (e.g., selenium intake and vitamin A) and the gut microbiome alpha-diversity.

In this study, children from the cancer group had higher intake of macronutrients and micronutrients. Specif-

ically, they showed significantly higher intakes of daily calories, and a trend of higher intakes of total protein, fat, carbohydrates, and fiber than the control group. However, there were no significant differences between the two study groups if the amount of macronutrients intake is viewed based on intake percentage. The compromised GI functions and manifestations of cancer treatment-related GI symptoms affect the absorption of nutrients among children with cancer, and therefore they need to compensate by increasing intakes to meet the required energy for daily activities and cancer recovery. Due to the importance of nutrients in cancer recovery, the European Society for Clinical Nutrition and Metabolism (ESPEN) guideline strongly recommends the energy intake of the patients ranging between 25 and 30 kcal/kg/day to meet the energy expenditure, and the protein intake above 1g/kg/day and even up to 1.5g/kg/day. Clinically, this point is also emphasized to the parents, possibly explaining the reason behind higher nutrition intake in children with cancer. The ESPEN guideline strongly recommends against any dietary provisions that restrict energy intake in patients with or at risk of malnutrition[38]. Therefore, more attention should be paid to adequate dietary intakes which may be associated with cancer-related toxicities, such as fatigue and comorbidities such as obesity.

A positive correlation was found between beta-carotene intake and α-diversity index Faith's_PD. A high diversity of the gut microbiome had more healthy effects and a low gut microbiome diversity was associated with a higher weight gain in the long-term[39]. When the intake of beta-carotene increases, there is a higher microbial richness in our sample. Beta-carotene is the most abundant vitamin A carotenoid precursor in the human diet and can only be acquired through food or supplements [40]. Both beta-carotene and vitamin A function as antioxidants and participate in the regulation of host immune responses by activating immune cells such as macrophages and natural killer cells [41]. Studies have shown that retinoic acid, converted from vitamin A, is a critical regulator for the intestinal immune response. In mice, a lack of carotenoids and vitamin A in the diet reduces commensal microbes, and thus suppresses pro-inflammatory Th17 cell generation in the gut[42]. Mechanisms of the association between the beta-carotene intake and microbial richness might be due to the fact that the supplementation of beta-carotene increases IgA production and regulates the immune responses in the GI system [41], which in turn protect the commensal microbes in the gut and help maintain a high microbial alpha-diversity.

Through the analyses of associations between the gut microbiome and the diet and nutritional intake levels, positive correlations were reported between alpha-diversity and carbohydrates and vitamin B6 intakes. These findings were consistent with previous studies. Vitamin B6 functions as an essential cofactor for enzymes involved in various metabolic activities and an increase of vitamin B6 aids in polyunsaturated fatty acid metabolism, and biosynthesis of arachidonic acid and hepatic cholesterol [43]. An increase of vitamin B6 also reduces the production of lithocholate [43], a toxic bile acid, and promotes the homeostasis of microbial communities in the distal gut[44], therefore leading to a higher diversity in the gut microbiome.

Adequate intake of carbohydrates and fiber is positively correlated with alpha-diversity, probably because children are therefore less likely to have excessive intake of fat, and the energy density of diet is reduced. Fiber plays a critical role in the diversity of healthy gut microbiome. As reported, an increased fiber intake produces more short-chain fatty acids, which in turn promote intestinal gluconeogenesis and liponeogenesis [39]. Further work suggested that transitions to the refined diet that lacks soluble fiber is the primary driver of gut microbiota alterations [45]. Therefore, adequate intake of carbohydrates and fiber promotes GI tract health and prevents infections and colonization of the gut by pathogenic microbes[46]. Until now, the exact mechanisms behind the relationship of carbohydrate intake level and gut microbial diversity are still not well studied and should be explored in future investigations.

This study has several limitations. We have a small sample size, and all children were recruited from Children's Healthcare of Atlanta, Georgia. Our findings may not be generalized into other clinical settings. In addition, we only analyzed the correlations between diet and alpha-diversity and microbiome abundance. Lastly, these analyses were conducted without controlling primary confounders, which should be considered in future work.

5. CONCLUSION

Compared with healthy children, children with cancer exhibited a higher intake of daily calories, and thus had a higher intake of both macro- and micro-nutrients, probably attempting to compensate their compromised GI function affected by cancer and treatment. Positive associations were found between the intake of certain macronutrients (carbohydrates and fiber), micronutrients (beta-carotene and vitamin B6) and alpha-diversity. Future studies should confirm our findings in a larger sample and try to better understand the impact of gut microbial alterations on cancer treatment symptoms and toxicities.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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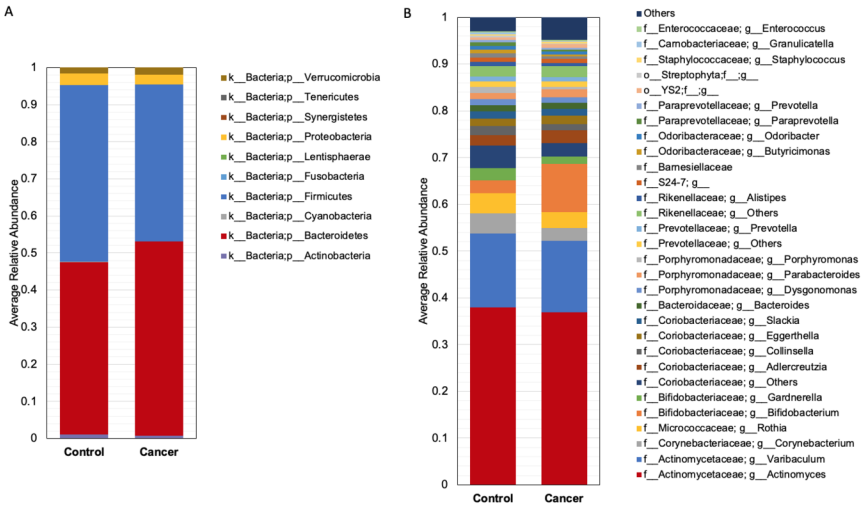
Data Availability Statement: The data that supports the findings of this study are available from the corresponding author upon reasonable request.



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