PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 14 (11): 762-764, 2015
ISSN 1680-5194

A Comparative Study of Gut Microbiota Profiles of Children Living in Kulon Progo and West Lombok

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Abstract: The role of gut microbiota in human health is highly understandable. Recent research highlights the influence of geographical terms in gut microbiota composition. Unfortunately, baseline data of microbiota composition in children are limited. We compared the number of fecal microbiota of children ages 9-12 years, living in Kulon Progo (KP), Yogyakarta, with same age children in West Lombok (WL) and West Nusa Tenggara. The study was an observational study with comparative design. The subjects were elementary school students, 29 subjects in KP and 27 subjects in WL. Fecal microbiota was analyzed by pour plate culture technique on MRS agar for lactic acid bacteria (LAB) and TBX agar for *E. coli*. The difference of total bacteria was tested using independent sample t-test. Mean of total fecal LAB of children in KP was 7.58±0.61 CFU/g, whereas in WL was 7.19±0.83 CFU/g. There was no significant difference between the number of fecal LAB of children in KP and in WL (p = 0.255). Mean of total fecal *E. coli* of children in KP was 7.06±0.75 CFU/g. whereas in WL was 7.23±1.12 CFU/g. There was no significant difference between the number of fecal *E. coli* of children in KP and in WL (p = 0.078). Further research is needed to determine the factors that can affect the composition of the gut microbiota.

Key words: Child health, *Escherichia coli*, gut microbiota, Indonesian children, lactic acid bacteria

INTRODUCTION

The role of gut microbiota in human health is highly understandable. Hippocrates had said that “death sits in the bowels” and “bad digestion is the root of all evil” (Sekirov *et al*., 2010). It showed the importance of gut health for human health. The exhaustive description of human microbiota and their relationship with health and disease are major challenges in the twenty-first century (Turnbaugh *et al*., 2007). The human gut microbiota represents the ‘last discovered organ’ of the human body, showing functions ranging from digestion and protection against pathogen colonization to host immunity and central nervous system regulation. Its composition is influenced by genetics, the mode of delivery, diet, lifestyle, medical treatments and other factors (Tyakht, 2013). The elucidation of global microbiota diversity is important for understanding the role of the microbiota in host health and for discovering ways to modulate the microbial community for disease prevention and treatment.

The composition of gut microbiota vary between individuals (Sekirov *et al*., 2010). Delivery mode and diet can influence gut microbiota development in the first year of life (Kim *et al*., 2013). After age of one. the gut microbiota develop and influence the composition of gut microbiota in adults (Sekirov *et al*., 2010). In addition, age and geographical distribution affect the variation of the number of microbiota phylum in every part of the body (Yatsunenko *et al*., 2012).

A study had conducted to investigate the hypothetical association between gut composition and cancer. Early culture dependent studies compared populations at high-risk (western countries) and at low risk (Japan, Uganda, India) and reported different compositions of microbiota (Hill *et al*., 1971; Drasar *et al*., 1973; Finegold *et al*., 1974). The high-risk population had a microbiota composed primarily of *Bacteroidetes* and there were specific differences, including patterns of food consumption, between western countries and Asian or African populations. although multiculturalism and population exchanges have reduced these differences. The number of lactobacilli and *Escherichia coli* ranges between 10^5-10^8 CFU/g (Ishibashia and Shimamura, 1993 cited by Lourens-Hattingh and Viljoen, 2001). The role of the gut microbiota in human health is highly understandable. Recent research highlights the influence of geographical terms in explaining gut microbiota composition. Unfortunately, baseline data of microbiota composition and its variation in children are limited, especially in developing countries.

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MATERIALS AND METHODS

Study design: The study was an observational study with comparative design. This study was took place in Kulon Progo. Yogyakarta (KP) and West Lombok. West Nusa Tenggara (WL). Indonesia on June 2013-December 2014. We compared the number of fecal microbiota of children, ages 9-12 years, living in KP with same age children in WL. The subjects were elementary school students, 29 subjects in KP and 27 subjects in WL.

Data collection: Gut microbiota was collected from feces sample. For stool collection, we employed stool collectors who is local people who trained in stool collecting. Once children were going to go to toilet, they were asked to call stool collector. The stool collector then came to children's home. Stool collector collected the stool correctly and placed stool into a sterile container with a lid and stick label (child's name, school and date). Before defecation, children were asked to urinary so that stool was not mixed with urine. Stool was collected at 2-5 g. The collected stool that was placed in cool box containing ice gel then was carried to laboratory.

Materials: Fecal microbiota was analyzed by pour plate culture technique on MRS agar for lactic acid bacteria (LAB) and TBX agar for *E. coli*. Materials and equipments needed were samples (feces), disposable petri dish. laminary air flow, 37°C incubator, autoclave, water bath, Quebec Colony counter, refrigerator, vortex, glassware, blue tip, micropipette and centrifuge tube.

Data analysis: Number of gut microbiota was expressed as log CFU/g. Descriptive and comparative analyses were performed using statistical software. The difference of total bacteria was tested using independent sample t-test.

RESULTS AND DISCUSSION

There were no significant differences both LAB and *E. coli* between children in KP and WL (p>0.05). The mean value of total fecal lactic acid bacteria of children in Kulon Progo was 7.58±0.61 log CFU/g, whereas in West Lombok was 7.19±0.83 log CFU/g. There was no significant difference between the number of fecal lactic acid bacteria of children in Kulon Progo and in West Lombok (p = 0.255).

The mean value of total fecal *E. coli* of children in Kulon Progo was 7.06±0.75 log CFU/g whereas in West Lombok was 7.23±1.12 log CFU/g. There was no significant difference between the number of fecal *E. coli* bacteria of children in Kulon Progo and in West Lombok (p = 0.078).

The number of LAB and *E. coli* in both groups were normal according to the normal range of 10^5-10^8. The number of *E. coli* was not much different as mentioned by Zimmermann *et al.* (2010) as 6-8 log CFU/g.

Table 1: Characteristics of subjects

	Groups			
	Kulon Progo -- (n = 29) --		West Lombok --- (n = 27) ---	
Characteristics	n	%	N	%
Age groups by years				
8 years old	3	10.00	5	18.52
9 years old	12	40.00	4	14.81
10 years old	4	13.33	10	37.04
11 years old	8	26.67	5	18.52
12 years old	2	6.67	3	11.11
Gender				
Female	21	72.41	10	37.04
Male	8	27.59	17	62.96
Father's Job				
Unemployed	1	3.70	1	3.70
Farmer	5	18.50	1	3.70
Labour	4	14.80	19	70.37
Civil servant	4	14.80	0	0.00
Entrepreneur	11	40.70	6	22.22
Private employees	2	7.40	0	0.00
Father's income (thousand rupiahs)				
<500	15	55.60	15	55.56
500-1.000	6	22.20	8	29.63
1.000-2.000	2	7.40	1	3.70
>2.000	4	14.80	2	7.60
BMI/Age*				
Obese	2	6.70	0	0.00
Overweight	3	10.00	0	0.00
Normal	21	73.30	26	96.30
Underweight	3	10.00	1	3.70

*There was no significant differences between groups (p = 0.687)

Table 2: Nutrients intake of subjects

	Groups		
	Kulon progo	West lombok	p-value
Energy (kcal)	1020	1385.27	0.124
Protein (g)	27.5	43.65	0.991
Fat (g)	59	47.56	0.439
Carbohydrate (g)	171.2	199.55	0.105

Table 3: Comparison of gut microbiota (CFU/g) of children in kulon progo and west lombok

	Groups		
Microbiota (µ±SD)	Kulon progo	West lombok	p-value
Lactic acid bacteria	7.58±0.61	7.19±0.83	0.255
Escherichia coli	7.06±0.75	7.23±1.12	0.078

The differences in the fecal microbiota of children reflect a number of potentially important and contributing factors, such as socioeconomic status, genetics, dietary habits, sanitary and other environmental conditions. Dietary factors may be particularly important in explaining the observed differences in the composition of the fecal gut microbiota between the Kulon Progo and Lombok children.

The gut microbiota composition varies among individuals and susceptible to change. Dietary factor is known to be a major factor influencing the gut microbiota modification (Lopez-Legarrea *et al.*, 2014). Some studies had been reported the relationship between the gut microbiota and diet. For instance, *Bifidobacteria* reduced significantly with decreased carbohydrate intake as well as some *Clostridium* such as *Roseburia* and *Eubacterium rectale* (Russell *et al*., 2011), there was correlation between the decrease of *Bacteroidetes* and the carbohydrate-restricted diet (Brinkworth *et al*., 2009) and the increase of *Bacteroidetes*, *Lactobacillus* and *Bifidobacterium* because of high protein diets (Lopez-Legarrea *et al*., 2014).

Variety of food source and its intake may be the cause of no significant differences in gut microbiota in children in Kulon Progo and Lombok. According to Tyakht (2013), the similarities between the microbiota of Russian city populations and those found in Western cities are presumably associated with higher social standards and a Western lifestyle, which is particularly reflected in the diet in the form of increased consumption of meat products and processed food.

Another study stated that Bangladeshi children from low-income families have access to a smaller variety of foods compared to U.S., children from affluent families. Hence, variability in the diet probably does not contribute to the larger degree of temporal variation observed in the gut microbiota of Bangladeshi children. One potential, alternative explanation is that a high level of temporal variation may result from more frequent or intensive exposure to bacteria in the environment due to unhygienic conditions, resulting in subclinical perturbations to their gut microbiota (Lin *et al*., 2013).

There are many factors affecting the composition of gut microbiota. Further research is needed to determine the factors affecting the composition of the gut microbiota.

ACKNOWLEDGEMENT

The authors would like to thank The Nestle Foundation for funding this study. We also thank to all the volunteers (children) involved in this study.

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