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Ayurveda Treatment (Virechana and Basti) and Changes of Intestinal Microbiota at Phyla and Species Level

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Abstract

Introduction: Ayurvedic therapies and medical practices have been elaborated for some patients in Japan. The characteristic of Ayurvedic treatment is a detoxication with a large amount of oil treatment by body surface oil massage and purgation therapy with ghee or specially arranged herbal oil. Changes of intestinal microbiota during these treatments have not been well studied.

Method: Participants were recruited from Hatai Ayurveda Clinic in Tokyo. Virechana therapy, a purification therapy, or Basti therapy (decoction and oil enema) was carried out on 13 patients with various manifestations. All participants provided lifestyle, dietary habits, past, and present illness by the questionnaire, and precise condition was recorded during admission to the end of camp. Fecal samples were taken at the entry, during treatment, at the discharge, and three weeks later for analyzing intestinal microbiota by seqyebcubg 16srRNA gene.

Results: Body weight decreased by about 5% by Virechana therapy, while it did not occur by Basti, but body fat increased 4% (2.2 kg) on average in both groups. Various clinical manifestations of participants became improved, especially on a skin rash and atopic change.

The depressed patient also revived with a will of living. They are mostly vegetarians and had more Bacteroidetes (48.09±7.51%), Firmicutes (38.27±10.82%), and Actinobacteria (3.30± 3.58%) than omnivores who had more Proteobacteria (10.73±4.75%), Fusobacteria (2.40±6.25%) and Cyanobacteria (0.09± 0.24%). When the groups were divided by oil consumption, ghee users showed higher Fusobacterium and less Firmicutes and Actinobacteria. Virechana therapy caused remarkable microbiota changes after the pretreatment, such as the decrease of Firmicutes and increase of Proteobacteria. At the genus-species level, the increase of Enterobacteriaceae and loss of Akkermansia municiphila were noteworthy. Niruha Basti and Matra Basti decreased Firmicutes and increased Proteobacteria (p=0.096). Fusobacterium also increased. After the discharge, Proteobateria remained high, but Firmicutes returned to 30% on average, ranging from 25% to 50%. Three weeks later, the variety increased by Fusobacterium, Verrucomicrobia, Tenericutes, and Lentisphaerae. The variety of species also increased three weeks later.

Conclusion: Various complaints of the participants improved by the Ayurvedic treatment with a large amount of oil treatment by body surface oil massage and purgation therapy. It caused changes in intestinal microbiota, and bacterial metabolites may affect skin lesions and mental health like depressive feeling.

Keywords: Ayurveda; Virechana; Niruha Basti; Matra Basti; Intestinal Microbiota; Bacteroidetes; Firmicutes;Actinobacteria; Fusobacterium; Verrucomicrobia; Tenericutes; Lentisphaerae; Purgative; Case Study

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Introduction

Ayurvedic therapies and medical practices have been elaborated for some patients in Japan. Ayurveda means the science of life in Sanskrit, emphasizes the adoption of many healing therapies, which can purify and rejuvenate the body, mind, and soul [1-4]. The medicinal form of science is not just a healing system but also an art of appropriate, healthy, and disease-free living [5-7].

Ayurvedic medicine's central concept is the theory that health exists when there is a balance between the three fundamental bodily bio-elements, called *Dosha,* which is composed of *Vata, Pitta*, and *Kapha. Doshas* are the forces that create the physical body. They determine conditions of growth, aging, health, and disease. Typically, one or two of the three *Doshas* predominates and picks a constitution or mind-body type. By understanding individual habits, emotional responses, and body type, practitioners can adapt their practice accordingly.

A traditional Ayurvedic detoxification program referred to as *Panchakarma* (consisting of five varieties of purificatory therapy) aims to help the body re-establish a healthy metabolic system and immunity [8].

The characteristic of Ayurvedic treatment is detoxification with a large amount of oil treatment by body surface oil massage and purgation therapy (or eliminating therapy) with ghee or specially arranged herbal

oil [9]. *Virechana* therapy is an effective Ayurvedic treatment that can cure many health problems naturally. The *Virechana* therapy detox program, which may take seven to 14 days, is reported safe from any side effects.

Ayurveda treatments focused on alleviating any excesses *Dosha* (illness) via powerful herbs and/or through the improvement of general lifestyle practices such as *Dinacharya* (daily Ayurvedic rituals practiced regularly, help to support a life of optimal wellness through routine), Pranayama (the regulation of the breath through certain techniques and exercises) and Meditation [3].

Panchakarma therapy encompasses five treatments that can prevent and heal a number of illnesses. *Virechana* therapy is defined as the medicated purgation therapy, which cleanses the excess Pitta, leading to purifying blood by clearing the toxins from the body [10]. The treatment concentrates on the lipophilic toxins that are accumulated in the liver and gall bladder. The gastro-intestinal tract is also cleansed by *Virechana* therapy. *Virechana,* a purification therapy, causes severe diarrhea, but the effects on intestinal microbiota have not been well studied yet. In this study clinical products and changes of intestinal microbiota during 11 days*of Virechana therapy* and 5 or 6 days change by Basti Therapy (alteration of decoction and oil enema) are reported.

Subjects and Methods

Subjects

Participants in the current Ayurveda therapy camp were recruited from patients who had been treated in the Hatai Clinic in Tokyo. They were nine females and four males, with various complaints (Table 1). They are requested to fulfill the questionnaire that was used in the GENKI study [11,12]. It focuses on dietary habits, health status, current illness, family condition, and food preference. Food intake can be calculated from a semi-quantified food frequency questionnaire. Body composition, such as water, muscle, and fat%, was measured during the Tanita Impedance Equipment [13].

Two doctors and the several staffs were accompanied to support this program. Dr. Shiho Oikawa in Panchakarma, who was the registered Ayurveda doctor, graduated from Gujarat Ayurveda University India.

The Ethical Committee approved the study design of the Life Science Promoting Association (No. 2018-3).

Treatment

Three treatment methods were applied to the participants according to their condition [2-4]. Four patients received a full course of *Virechana* therapy for 11 days, and two received *Sastikashali pinda sweda* (milk porridge Sudation therapy), and one received daily oil. Basti and remedy were done for 11 days. Other six people received *Swedana Basti* therapy, oil massage, and treatment for 5 or 6 days. Milk porridge sudation therapy is meant to improve general strength, including muscle tone, flexibility of the joint movements, and motor impairment syndrome [14].

Two doctors were participated in the program as controls without receiving Ayurvedic therapy (Table 1).

In *Virechana* therapy, patients drank ghee for four days subsequently whole-body oil massage for three days as the pretreatment, then drank 50ml castor oil on the 9th day. It induces multiple purgations on the day, so the diet needed to change to thin rice porridge and drank two litter raisins juice on the same day to avoid dehydration. After the purgation completed from the next day onwards, they are recommended to take a soft meal avoiding wheat and bread easy to digest.

The small piece of feces was collected four times in the sample tubes at the time of admission, after castor oil drinking, just before discharge (11thday), and then after three weeks at home. The treatment schedule is shown below (Figure 1).

Virechana therapy (upper figure): Day 1. Swedana: Sweat subjects' whole body in a steam box to improve metabolism and dry their body. Day 2-5 Snephapana (internal oleation): After excretion in the morning, face washing and brushing, take 30ml of ghee warmed to human skin temperature at 5:30 am. Then eat nothing until to be hungry. If feel hungry around noon, so eat about half of the usual meal.

Table 1: Outline of participants in the Ayurveda treatment camp at Kawaguchi Lake.

Id	Age	Sex	Present illness	Drug	Profession	Dietary habit	Staple food
1	42	F	Atopy, abortion, stomatitis, fever		Ayurveda therapist	Vegetalian	Genmai+barley
2	54	F	Rheumatoid arthritis, insomnia	Immunosuppressant	Ayurveda therapist	Vegetalian	White+barley
3	63	M	Atopy, systemic alergic eruption, vulvectomy	Anticoaggulant	Retired banker	Balanced flexitarian	Germ rice
4	38	F	Prurritus, delayed type food allergy		Unemployed	Omnivor (like fatty meat)	White rice
5	67	M	Hypertension, angina, cerebral infarction, coronary a.bypass ope. 10 days before	Antihypertension, anticoaggulant	Tax counsellor	Vegetable main, beer	Genmai+barley
6	67	F	Peptic ulcer, stress	none	Wife	Omnivor (ish, meat 2-3/W)	White rice/genmai
7	53	F	Hypercholesterolmia, hypertension	none	Public health nurse	Vegetalian	White rice/genmai
8	56	F	Cervical dysplasia, hyperchoestrolemia, gallic stone	none	Wife	Vegetalian, low salt	White rice+barley
9	49	F	Insomnia, anemia, fatigue, headache	none	Care worker	Omnivor (port, fish 1-2/W)	White rice+barley
10	40	F	Asthma, post breast ca. depression	none	Wife	Vegetable main, sea weed, fish	White rice
11	53	F	Diabetes, subarachnoid bleeding	none	Yoga studio owner	Vegetable main, sea weed, fish	Germ rice/genmai
12	40	F	Depression	Antidepressant	Wife	Vegetalian	White rice
13	41	M	Fatigue, loose stool	none	Employee	Vegetalian (chicken, fish 3-4/W)	White rice+barley
14	42	F	Healthy control	none	Ayurveda doctor	Omnivor	Partially polished rice
15	46	M	Healthy control	none	Medial doctor, Integrative medicine	Ominivor (chiken, fish 3-4/W)	White rice

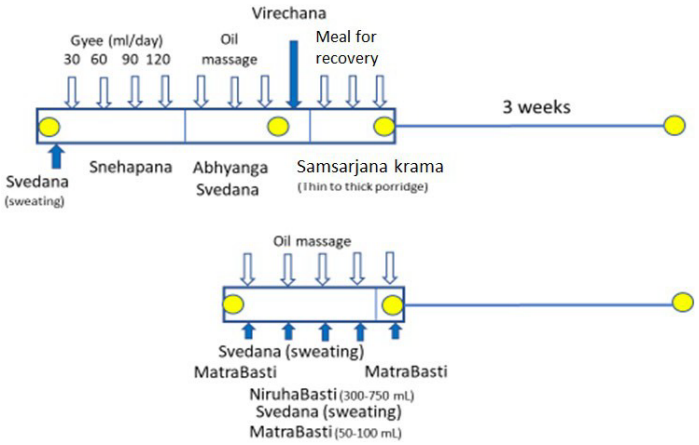

Figure 1: Scedule of Panchakarma in Kawaguchi Camp. One arrow (One day).

They have consumed oil inside the body to soften the damaged Dosha. On days 2-5, repeat the same procedure as the previous day with a daily increase of ghee from 60 ml to 120 ml. Day 6-8 Abhyanga and Swedana: Perform oil massage on the whole body, including the head and sweat from the entire body in the steam box. Infiltrate oil quality from the body surface. On Day 9 purgation was done: Drink laxatives prescribed at 9:30 am (no breakfast). To excrete Doshas collected in the digestive tract. Till the end of purgation, the patient drank two litter raisins juice little by little. Thin rice gruel could be taken at night; Day 10, 11 Samsarjana Krama: Half rice porridge, 70 % rice porridge, and gradually return to the normal diet. After that, patients returned to a normal diet by 5 to 7 days.

Basti therapy (lower figure): Day 1: *Matra Basti*: After a whole-body oil massage, including the head, steam sweating was performed, and then 50 to 100 ml of medicated oil was administered from the anus. Keep it until the next day of excretion. On Day 2 to 5: *Niruha Basti* and *Matra Basti*: *Niruha Basti* is performed in the morning empty stomach. After massage and sweating as in the previous day, a 300-750 ml decoction-based drug was administered from theanus. Excretion desire occurs in 5 to 20 minutes and evacuates the bowel contents to the toilet. After Lunch can be taken after excretion. *Matra Basti* is performed in the afternoon to apply oil massage to the abdomen, waist, thighs, and Swedana with a hot towel or steam, and administer 50 to 100 ml of medicinal oil from the anus, which was done after Lunch.

Day 6. (Last day) *Matra Basti*: After a light breakfast, whole body oil massage, steam sweat was performed, and 50 to 100 ml of medicinal oil was administered from the anus. Discharge in the afternoon when bowel movement was settled.

The amount of ghee to drink, the number of days, the combination of herbal medicines taken, the menu of meals, etc. was changed depending on the patient's physical condition and physical strength. The drugs used for the purgation therapy vitiate the Doshas and bring them into the abdomen. While performing the *Virechana therapy*, the vitiated Doshas are eliminated through the rectum.

We have arranged in Japan to split the decoction enteral in the morning and the oil enteral in the afternoon and do both types of Basti on the same day as we did on the second to fourth day. In India, only a kind of enteral is done per day.

Dietary Therapy

The menu of meals was performed in Japanese style food by the Ayurveda dietitian. During the procedure, the patient is subjected to Oleation first, then Fomentation, followed by Purgative and *Samsarjana Karma* (post-operative therapy). The internal Oleation is followed for three to seven days. After that, a medicated steam bath is performed for three days. A light and warm diet is prescribed for the patient a day before starting *Virechana therapy*. However, certain factors like body and mind constitution, age of the person, a mental condition should be considered while opting for *Virechana* therapy *karma*.

Main meals: Rice with cereals (500 g of rice), clear soup (Leek onion, winter melon, ragged kelp, rock salt, soy sauce), Sauce of *Tsurumurasaki* (vine, rock salt, black pepper, unroasted sesame oil), Purple radish with kalonji (purple radish, rock salt, kalonji, Taihaku sesame oil), *Tempura* of lotus root and carrot (lotus root, carrot, rock salt, millet, soy sauce, sesame, unroasted sesame oil), Cumin rice (rice, barley cumin, ginger, ghee), Eggplant and beans curry (onion, cherry tomato, eggplant, beans, unroasted sesame oil, cumin, mustard seed, turmeric, rock salt), Bean curry with winter melon (winter melon, mung bean, ginger, ghee, cumin, turmeric), Okra spice *sauté* (okra, turmeric, coriander seeds, rock salt, unroasted sesame oil), Boiled dried figs with cinnamon (dried figs, cinnamon), Buttermilk (yogurt, rocksalt, cumin, ginger, mustard seed, fenugreek,turmeric, ghee) Milk tea with spices (cinnamon, cardamom, clove, tea, milk, cane sugar), Ginger rice (rice, barley, ginger, kelp, rock salt, Shiso leaves, sesame), Corn soup (corn, onion, ghee, rock salt), sautéed radish with sauce (daikon, sesame oil, *kuzu*, Mitsuba leaf, ginger), soak spinach and ginseng (spinach, ginseng, ginger, kelp stock, rock salt, roasted sesame oil, sesame), Punch holon saute of yam (yam, mustard seed, cumin, fennel, karonji, fenugreek, were served during the camp (Figure 2). Black pepper, cumin, brown mustard, coriander, turmeric, garam masala, cinnamon, cardamom, clove, kalonji, ajowan were used for spice[14].

Various kinds of oils, such as ghee, olive oil, white sesame oil, roasted sesame oil, perilla oil, and coconut oil, were frequently used. Rice porridge and mung beans are a digestive meal that helps to recover weak internal organs.

Daily energy intake was adjusted between 1200-1500 kcal (about 30 kcal/kg body weight) depending on the patients' body weight.

DNA Extraction from Fecal Samples

DNA extraction and 16S rRNA was performed as previously described (Vet Microbiol 2020 Uchiyama). Briefly, the genomic DNA was extracted from the samples using Chemagic DNA Stool 200 Kit (PerkinElmer, Waltham, MA, USA). The V3-V4 regions of

Figure 2: Vegetarian meals during Ayurveda therapy. Each row from upper to lower shows morning, Lunch, and supper in 3 days, and the mid two photos show afternoon teatime snacks(*Shiratama Shiruko* and Jelly).

Table 2: Anthropometric change during Ayurveda treatment. The clinical findings decided on the outcome.

id	Treatment	Necessary calorie**	Weight pre	Weight post	Difference kg	weight change%	BMI pre	BMI post	Fat% pre	Fat% post	Fat change%	Outcome
1	Virachana	1594	49.8	47	-2.8	-5.6*	18.1	17.1	20.7	25.1	4.4*	better
2	Virachana	1699	53.1	51.4	-1.7	-3.2*	21.3	20.6	34.4	33.6	-0.8	improved
3	Virachana	1738	54.3	50	-4.3	-7.9*	19.9	18.4	10.3	16.3	6.0*	improved
4	Virachana	2054	64.2	58.4	-5.8	-9.0*	23	20.9	17.3	23.7	6.4*	improved
5	Milk porradge sedation	2371	74.1	72.2	-1.9	-2.6	24.8	24.1	27.9	28.4	0.5	improved
6	Milk porradge sedation	1581	49.4	48.9	-0.5	-1.0	20.6	20.4	32.4	33.1	0.7	better
7	Basti	1843	57.6	57.4	-0.2	-0.3	21.9	21.9	21.8	31.3	9.5*	better
8	Basti	1517	47.4	47.1	-0.3	-0.6	20.5	20.5	20.4	25.7	5.3*	better
9	Basti	1645	51.4	51.1	-0.3	-0.6	17.6	17.6	17.5	24.1	6.6*	better
10	Basti	2182	68.2	67.3	-0.9	-1.3	25.1	17	17.6	23	5.4*	better
11	Basti	1606	50.2	49.6	-0.6	-1.2	18.9	18.9	18.4	22.2	3.8*	better
12	Basti	1299	40.6	42	1.4	3.4	17	25.1	23.7	33.8	10.1*	calm
13	Basti	1846	57.7	57.6	-0.1	-0.2	19.3	19.3	19.2	14.6	-4.6	living will
14	cont	2368	74	72	-2.0	-2.7	27.5	26.7	na	na		
15	cont	2336	73	73	0.0	0	26.1	26.1	na	na		

*< 0.05
**calculated from "body weight (kg) x 0.4 x 80 kcal)"

the 16S rRNA gene were amplified by PCR and subjected to pair-end sequencing using Illumina MiSeq. The sequence data were processed using Quantitative Insights into Microbial Ecology 2 (QIIME 2) v2019.4.0. The DADA2 software package v2019.4.0 incorporated in QIIME 2 was used to correct the amplicon sequence errors and construct an amplicon sequence variant (ASV) table. The Green genes 99% reference database v13.8 was used for the taxonomic classification of each ASV. Microbial taxonomy was assigned using a Naïve Bayes classifier trained on the SILVA 138 database. The sequence data were processed using Quantitative Insights into Microbial Ecology 2 (QIIME 2) v2019.4.0. The DADA2 software package v2019.4.0 incorporated in QIIME 2 was used to correct the amplicon sequence errors and to construct an amplicon sequence variant (ASV) table. More details are described in the previous paper [15-18].

The fecal microbiota of 15 subjects was analyzed using the 16S rRNA amplicon sequencing method. A total of 2,032,860 nonchimeric reads (39,860.0±7,307.8 nonchimeric reads/sample; mean±SD) were used in this study. To rarefy the data, we used 24,000 reads from eachsample.

Statistics

Microbial taxonomy was assigned using a Naïve Bayes classifier trained on the SILVA 13299% database. First, changes of microbiota were screened at the phylum level. The more detailed analysis was done at the genus-species level, if each microbiota occupied more than 1% of the composition. The statistical analysis was performed using statistical software IBM-SPSS ver 24 [19] unless otherwise stated. In all statistical analyses, significance was set at $P<0.05$ unless otherwise stated. A paired t-test was used to detect significant changes, and Spearman's correlation analysis and chief component analysis were done to see interrelation of microbiota for proliferation and inhibition. ASV number and Shannon's index were calculated by R software [20].

Results

Anthropometric Data

All patients who participated in the Ayurveda therapy camp showed improvement in both clinical and mental condition (Table 1). The accompanying doctors checked the state of the patients. Especially diarrhea and dehydration were carefully watched. Meditation, yoga, and health lecture were done in between the therapy.

Bodyweight decreased 1.7 to 5.8 kg (3.7±1.8kg) or 3.2 to 9% (6.4±2.6%) by *Virechana* therapy ($p=0.026$), but there was no significant decrease in other methods (Table 2). On the contrary, body fat percentage increased significantly in both *Virechana* and *Basti* groups.

Clinical manifestations of participants became better (Figure 3). It

Figure 3: Improvement of skin rashes by Virchana. Itchy eruptions became flat and not itchy. Left Pro and Right Post.

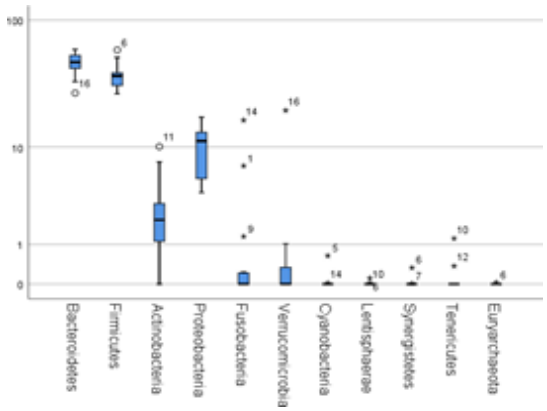

Figure 4: Frequencies of intestinal microbiota profile at the admission.

was effective in skin rashes by atopic change. The depressed patient had the will to live.

Intestinal Microbiota at the Admission of the Camp

At the admission, the most frequent bacteria phylum was *Bacteroidetes* (46.61±8.90%), *Firmicutes (*37.5±9.06%), *Actinobacteria* (2.87±3.02%), *Proteobacteria* (9.63±4.38%), *Cyanobacteria* (0.04±0.16%), *Fusobacteria* (1.68±4.49%), *Lentisphaerae (*0.01±0.03%), and *Synergistetes* (0.02±0.09%). Another phylum was not detected above 0.1% (Figure 2).

Everybody possessed *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria*, but others showed variation; *Fusobacteria* 33.3%, *Verrucomicrobia* 46.7%. *Cyanobacteria* 6.7%, *Lentisphaerae,* and *Synergistetes* 13.3% each, *Euryarchaeota* 6.7%, and *Tenericutes* 13.3%. At the discharge, *Tenericutes* increased to 40%, but other phyla returned to the profile at the admission.

Microbiota profile showed different dietary habits pattern, although there was no statistical significance (Table 3 a,b,c). Vegetarians had more *Bacteroidetes*, *Firmicutes,* and *Actinobacteria* than omnivores, who had more *Proteobacteria, Fusobacteria,* and *Cyanobacteria.*

Kind of staple foods was divided into polished white rice [5], white rice and barley [4], white rice and brown rice [3], and genmai (brown rice) [3]. Genmai eaters showed higher Firmicutes and less *Actinobacteria* and *Proteiobacteria*, but "white rice+barley" group also showed the similar trend. Significant difference among groups was not present.

When the groups were divided by oil consumption, ghee users showed higher *Fusobacterium*and less *Firmicutes* and *Actinobacteria* (Table 5).

Changes of Microbiota by*Panchakarma*

Changes by *Virechana therapy:* The order of top-six microbiota that occupied 99.8% of microbiota at the admission did not change. These were *Bacteroidetes* (46.6%), *Firmicutes* (37.5%), *Proteobacteria* (9.6%), *Actinobacteria* (2.9%), *Fusobacteria* (1.7%) and *Verrucomicrobia* (1.5%). By *Virechana, Proteobacteria* and *Fusobacteria* increased to 32.5% and 8.1% respectively. At the discharge time in both *Virechana* and *Basti* treated groups, the unassigned bacillus occupied 40.8%, and the Bacteria became 21.1%. These three occupied 93.8%. It was noteworthy that *Verrucomucrobia* became zero.

Three weeks after the discharge, the composition of the profile returned to the previous pattern. Verrucomicrobia returned to the 6th position.

Treatment-group specific change of microbiota was shown in Table 6. The remarkable changes of microbiota after the pretreatment for *Virchana* were a decrease of the *Firmicutes* and an increase of the *Proteobacteria* (p=0.012 and p=0.023, respectively) (Table 4).

The top six bacteria occupied 99.8% at the admission. These were *Bacteroidetes* (46.6%), *Firmicutes* (37.5%), *Proteobacteria* (9.6%), *Actinobacteria* (2.9%), *Fusobacteria* (1.7%) and *Verrucomicrobia* (1.5%). By Virechana therapy, almost all cases showed a median value of 50% Bacteroides, and in one person, *Proteobacteria* occupied 96.4%.

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Table 3: Intestinal microbiota by dietary habits at baseline.

Table 3a: Dietary habit and micribiota.

Life habit	Vegitarian n = 8		omnivor n = 7		Total n = 15	
	mean	sd	mean	sd	mean	sd
Bacteroidetes	48.09	7.51	44.92	10.61	46.61	8.9
Firmicutes	38.59	10.82	36.27	7.19	37.51	9.06
Actinobacteria	3.3	3.58	2.39	2.4	2.87	3.02
Proteobacteria	8.67	4.09	10.73	4.75	9.63	4.38
Cyanobateria	0	0	0.09	0.24	0.04	0.16
Fusobacteria	1.05	2.4	2.4	6.25	1.68	4.49
Lentisphaerae	0.01	0.04	0	0.01	0.01	0.03
Synergistetes	0.04	0.12	0	0	0.02	0.09

Table 3b: Staple food and microbiota.

Staple food	white n = 5		white/barley n = 4		white/genmai n - 3		genmai n = 3	
	mean	sd	mean	sd	mean	sd	mean	sd
Bacteroidetes	48.75	12.66	43.45	4.69	48.53	4.05	45.32	12.02
Firmicutes	32.99	4.84	42.38	8.59	33.08	5.93	42.98	14.47
Actinobacteria	4.34	4.06	1.04	1.22	4.29	2.97	1.47	1.21
Proteobacteria	9.57	3.7	8.07	4.46	13.91	3.18	7.56	5.28
Cyanobateria	0.09	0.12	4.47	8.09	0	0	2.3	3.98
Fusobacteria	0.13	0.29	0.01	0.01	0	0	0	0
Lentisphaerae	0	0	0.01	0.01	0	0	0.04	0.06
Synergistetes	0	0	0	0	0	0.01	0.11	0.19

Table 3c: Daily oil and microbiota.

Oil usage	Ghee n = 6		Plant oid n = 9		
	mean	sd	mean	sd	*p*
Bacteroidetes	47.67	12.38	45.9	6.43	0.76
Firmicutes	33.1	4.1	40.45	10.43	0.08
Actinobacteria	1.55	0.84	3.75	3.64	0.11
Proteobacteria	10.01	4.5	9.38	4.55	0.8
Cyanobateria	0	0.01	0.07	0.21	0.37
Fusobacteria	4.16	6.63	0.03	0.08	0.08
Lentisphaerae	0	0	0.01	0.04	
Synergistetes	0	0	0.04	0.01	

Table 4: Changes of microbiota profile by Virchana.

	Admission				Verechana					At discharge					3 weeks later				
n=15	mean	sd	median	max	mean	sd	*p*	median	max	mean	sd	*p*	median	max	mean	sd	p	median	max
Bacteroidetes	46.61	8.90	47.48	59.48	41.02	22.19	*0.886*	50.92	54.44	1.89	1.86	*0.324*	1.33	5.99	49.58	8.66	*0.233*	46.57	69.49
Firmicutes	37.51	9.06	37.11	58.96	18.05	12.14	*0.012*	17.64	30.88	0.02	0.05	*0.137*	0	0.20	32.69	10.14	*0.007*	31.31	53.21
Proteobacteria	9.63	4.38	11.2	17.50	32.49	35.78	*0.023*	17.36	96.42	0.01	0.03	*0.027*	0	0.13	11.51	8.50	*0.376*	8.71	28.99
Actinobacteria	2.87	3.02	2.07	10.07	0.24	0.36	*0.184*	0.08	0.84	1.95	3.64	*0.221*	0.02	10.34	2.79	5.06	*0.941*	1.31	20.42
Fusobacteria	1.68	4.49	0	16.57	8.13	11.19	*0.181*	0.2	22.90	0.01	0.05	*0.795*	0	0.21	2.37	5.87	*0.512*	0	20.97
Verrucomicrobia	1.49	5.09	0	19.86	0	0.00		0	0.00	0	0.00	*0.420*	0	0.00	0.87	1.60	*0.552*	0	5.09
Tenericutes	0.11	0.32	0	1.22	0	0.00		0	0.00	0	0.00		0	0.00	0.06	0.22		0	0.84
Cyanobacteria	0.04	0.17	0	0.64	0	0.00		0	0.00	0.05	0.13		0	0.47	0.12	0.33		0	0.99
Synergistetes	0.02	0.09	0	0.33	0.01	0.03		0	0.07	0.00	0.01		0	0.02	0.00	0.01		0	0.04
Bacteriap__TM7	0.01	0.02	0	0.04	0.02	0.04		0	0.09	0	0.00		0	0.00	0.00	0.01		0	0.02
Lentisphaerae	0.01	0.03	0	0.11	0	0.00		0	0.00	0.01	0.05		0	0.19	0.01	0.02		0	0.06
Bacteria	0.01	0.01	0	0.04	0.03	0.03		0.03	0.08	21.07	14.76	*0.000*	17.66	55.43	0.00	0.01		0	0.02
Euryarchaeota	0	0.01	0	0.03	0	0		0	0.00	31.95	8.62	*0.000*	29.88	50.09	0	0		0	0.00
Unassigned	0	0	0	0.01	0	0		0	0.00	40.77	18.59	*0.000*	48.72	60.00	0	0		0	0.00
Bacteriap	0	0.01	0	0.02	0	0		0	0.00	2.26	4.43	*0.000*	0	13.33	0	0		0	0.00
Spirochaetes	0	0	0	0.00	0	0		0	0.00	0.00	0.01		0	0.03	0	0		0	0.00

Citation: Watanabe S, Oikawa S, Mibayashi H (2021) Ayurveda Treatment (Virechana and Basti) and Changes of Intestinal Microbiota at Phyla and Species Level. Prensa Med Argent, Volume 107:3. 319. DOI: https://doi.org/10.47275/0032-745X-319.

Table 5: Changes of microbiota prolife by Basti and other treatment.

Treatment	phyla	Baseline				At discharge				3 weeks later			
		mean	sd	median	max	mean	sd	median	max	mean	sd	median	max
Basti	Bacteroidetes	47.27	5.65	48.38	53.92	48.42	8.8	49.92	60	48.48	7.70	46.57	61.71
	Firmicutes	36.02	5.94	33.29	47.56	27.69	5.8	28.99	34.17	30.45	11.01	27.04	53.21
	Proteobacteria	9.79	3.74	11.47	13.78	18.13	6.4	17.66	31.24	16.26	10.75	17.06	28.99
	Actinobacteria	3.82	3.56	2.46	10.07	2.33	1.8	1.64	5.99	1.86	1.60	1.76	5.08
	Fusobacteria	2.59	6.19	0	16.57	1.79	3.7	0	10.12	1.84	4.11	0	11.08
	Verrucomicrobia	0.26	0.41	0.07	1.02	1.56	4.0	0	10.68	0.71	1.28	0	3.38
	Tenericutes	0.23	0.46	0	1.22	0.0	0.1	0	0.22	0.13	0.32	0	0.84
	Bacteriap__TM7	0.01	0.02	0	0.03	0.0	0.0	0	0.02	0.00	0.01	0	0.02
	Bacteria	0.01	0.02	0	0.04	0.0	0.1	0	0.19	0.26	0.45	0	0.99
	Cyanobacteria	0.00	0.01	0	0.02	0.0	0.0	0	0.03	0.00	0.01	0	0.02
	Lentisphaerae	0.00	0.01	0	0.02	0	0	0	0	0.00	0.01	0	0.02
	Synergistetes	0	0	0	0	0	0	0	0	0	0	0	0
milkporridge	Bacteroidetes	40.5	9.9	40.5	47.48	35.69	11.78	35.69	44.02	42.33	0.113	42.33	42.41
	Firmicutes	43.2	22.3	43.2	58.96	38.91	5.30	38.91	42.65	37.85	11.519	37.845	45.99
	Proteobacteria	10.9	9.3	10.91	17.5	13.90	11.63	13.90	22.12	7.2	0.368	7.2	7.46
	Actinobacteria	4.8	3.8	4.77	7.47	3.44	2.14	3.44	4.95	11.12	13.152	11.12	20.42
	Fusobacteria	0	0	0	0	2.73	3.85	2.73	5.45	0	0	0	0
	Verrucomicrobia	0.36	0.339	0.36	0.6	4.81	3.55	4.81	7.32	1.455	1.874	1.455	2.78
	Tenericutes	0	0	0	0	0.24	0.33	0.24	0.47	0	0	0	0
	Bacteriap__TM7	0.01	0.014	0.01	0.02	0.13	0.10	0.13	0.20	0	0	0	0
	Bacteria	0	0	0	0	0	0	0	0	0	0	0	0
	Cyanobacteria	0.025	0.021	0.025	0.04	0	0	0	0	0	0	0	0
	Lentisphaerae	0.055	0.078	0.055	0.11	0.065	0.092	0.065	0.13	0.03	0.04	0.03	0.06
	Synergistetes	0.17	0.226	0.17	0.33	0.105	0.148	0.105	0.21	0.02	0.028	0.02	0.04
Control	Bacteroidetes	43.36	22.80	43.36	59.48	49.58	0.25	49.58	49.75	60.48	12.75	60.48	69.49
	Firmicutes	32.38	7.84	32.38	37.92	30.74	6.85	30.74	35.58	27.41	7.33	27.41	32.59
	Proteobacteria	12.43	1.74	12.43	13.66	7.25	5.28	7.25	10.98	7.75	3.12	7.75	9.95
	Actinobacteria	1.78	0.64	1.79	2.24	0.59	0.80	0.59	1.15	1.00	0.20	1.00	1.14
	Fusobacteria	0.10	0.14	0.1	0.2	5.17	7.31	5.17	10.34	0.81	1.14	0.81	1.61
	Verrucomicrobia	9.93	14.04	9.93	19.86	6.67	9.43	6.67	13.33	2.55	3.60	2.55	5.09
	Tenericutes	0	0	0	0	0	0	0	0	0	0	0	0
	Bacteriap__TM7	0.005	0.007	0.005	0.01	0.01	0.01	0.01	0.02	0	0	0	0
	Bacteria	0	0	0	0	0	0	0	0	0	0	0	0
	Cyanobacteria	0.015	0.021	0.015	0.03	0.01	0.01	0.01	0.01	0	0	0	0
	Lentisphaerae	0	0	0	0	0	0	0	0	0.03	0.04	0.03	0.05
	Synergistetes	0	0	0	0	0	0	0	0	0	0	0	0

Four out of 5 cases had *Fusobacteria,* with 8.1% on average. It was noteworthy that *Verrucomucrobia* became zero by *Virchana.*

By Virechana therapy, almost all cases showed a median value of 50% Bacteroides, and in one person, *Proteobacteria* occupied 96.4%. Four out of 5 patients had *Fusobacteria,* with 8.1% on average. It was noteworthy that *Verrucomucrobia* became zero by *Virchana.*A decrease in *Bacteroides* and increased *Fusobacterium* were also present. *Firmicutes* returned to 36% at the time of discharge and three weeks later at home (p=0.022 and p=0.007, respectively). *Proteobacteria* returned to baseline level at a range of 4.4% to 8.7%. *Fusobacterium* dropped at the discharge but increased more than that of baseline three weeks later.

Changes of microbiota by *Basti* and other treatment

Basti treatment did not change the predominance of *Bacteroides* throughout treatment (47-48%). The remarkable change of microbiota by *Basti* and *Svedana* for one week was the decrease of *Firmicutes* (p=0.09) and increase of *Proteobacteria (p=0.096). Fusobacterium* also increased but no statistical significance. After the discharge, *Proteobateria* remained high, but *Firmicutes* returned to 30% on average, ranging from 25% to 50%. Three weeks later, the variety was increased by *Fusobacterium*, *Verrucomicrobia*, *Tenericutes,* and *Lentisphaerae.*

By milk porridge therapy, a slight decrease of *Bacteroides* and *Firmicutes* was observed at the discharge. No significant changes at the phylum level occurred. Recovery of *Bacteroides* to 42% was observed three weeks later. In this group increase of *Actinobacteria* was noticeable, but no statistical significance (p=0.2). In controls, *Bacteroides* predominated but no other remarkable change.

Correlation between the Phyla

Correlation between the phyla at the admission showed that *Firmicutes* showed a negative correlation with *Bacteroides* and *Verrucomicrobia. Verrucomicrobia* was not present in Virchana group at all and half of Basti therapy group (Table 7).

At the time of *Virechana*, a strong correlation was present between *Proteobacteria* and *Actinobacteria* (CC 0.999, p=0.001), and *Cyanobacteria* and *Proteobacteria* (CC -0.980, p=0.02).

Table 6: Correlation analyses between microbiota at the baseline (lower left) and 3 weeks later (upper right).

3 Ws later Admission		Bacteria	Actino bacteria	Bacte-roidetes	Cyanobac-teria	Fir-micutes	Fusobac-teria	Lenti-sphaerae	Proteobac-teria	Syner-gistetes	Bacteriap__ TM7	Teneri-cutes	Verrucomi-crobia
Bacteria	P's CC	0.29	0.313	-0.04	-0.1	0.07	-0.2	0.355	-0.01	0.37	.663**	-0	-0.1
	probability	0.29	0.256	0.88	0.67	0.82	0.59	0.194	0.97	0.17	0	0.6	0.8
Actinobacteria	P's CC	0.15	0.444	0.17	-0.2	-0.4	-0.1	-0.12	0.22	-0.1	0.2	-0	-0.1
	probability	0.6	0.097	0.55	0.52	0.1	0.61	0.669	0.44	0.83	0.6	0.4	0.6
Bacteroidetes	P's CC	-0.2	-0.02	0.45	0.25	-.629*	-0.1	-0.43	-0.02	-0.4	0	-0	-.630*
	probability	0.48	0.957	0.09	0.38	0.01	0.77	0.107	0.95	0.13	0.9	0.6	0
Cyanobacteria	P's CC	-0.1	-0.12	0.21	-0.1	0.01	-0.1	-0.09	-0.26	-0.1	-0	-0	-0.1
	probability	0.8	0.664	0.46	0.77	0.97	0.79	0.758	0.36	0.79	0.5	0.7	0.8
Firmicutes	P's CC	-0.1	-0.23	-0.5	0.2	.817**	-0.1	.708**	-0.44	.647**	0.1	0.3	0
	probability	0.75	0.403	0.06	0.48	0	0.7	0.003	0.1	0.01	0.8	0.3	0.9
Fusobacteria	P's CC	.805**	-0.2	-0.14	.649**	-0.1	.734**	-0.12	-0.44	-0.1	-0	-0	-0.1
	probability	0	0.477	0.61	0.01	0.79	0	0.665	0.1	0.7	0.3	0.6	0.8
Lentisphaerae	P's CC	-0.1	-0.03	-0.29	0.01	0.46	-0.1	.700**	-0.34	.983**	0.5	0.1	-0.1
	probability	0.68	0.916	0.29	0.98	0.08	0.64	0.004	0.22	0	0.1	0.8	0.8
Proteobacteria	P's CC	-0.3	.538*	0.09	-0.3	-0.4	-0.4	-0.03	0.38	-0.3	0.1	0	0.2
	probability	0.33	0.039	0.74	0.29	0.14	0.11	0.919	0.16	0.24	0.7	1	0.4
Synergistetes	P's CC	-0.1	-0.02	-0.24	-0.1	0.36	-0.1	.722**	-0.15	-0	.546*	-0	-0.1
	probability	0.72	0.934	0.4	0.7	0.19	0.68	0.002	0.59	0.88	0	0.7	0.9
Bacteriap__ TM7	P's CC	-0	-0.01	-0.04	-0.3	-0.2	-0.3	0.249	0.44	-0	-0	-0	-0.2
	probability	0.95	0.985	0.9	0.29	0.5	0.34	0.372	0.1	0.97	0.3	0.6	0.5
Tenericutes	P's CC	-0.1	0.107	-0.37	.579*	0.47	-0.1	-0.16	-0.14	-0.1	.634*	0.3	-0.1
	probability	0.66	0.704	0.17	0.02	0.08	0.61	0.579	0.62	0.75	0	0.3	0.7
Verrucomicro-bia	P's CC	-0	-0.11	0.04	-0.1	-0	-0.1	.599*	-0.05	-0.1	-0	-0	.771**
	probability	0.88	0.695	0.88	0.8	0.99	0.73	0.018	0.87	0.79	0.7	0.8	L

At the time of discharge, *Cyanobacteria* showed a positive association between *Firmicutes* (CC 0.574, p=0.025) and *Proteobacteria* (CC 0.534, p=0.04) and a negative association with *Bacteroides* (CC -0.608, p=0.016). Firmicutes also showed a positive association with Bacteria (CC 0.608, p=0.016), and with *Tenericutes* (CC 0.647 p=0.009). The strong association among *Lentisphaerae* with *Bacteria* (CC 0.904 p=0.000) and *Tenericutes* (CC0.889 p=0.000) was observed in the couple who have been treated with the milk porridge method.

Changes of Variety of Microbiota by *Panchakarma*

At the genus-specimen level, changes of microbiota profile corresponded well to the changes of phylum level. Both *Virchana* and *Basti* therapy caused increased γ- *Proteobacteria* (*Enterobacteriaceae*) (10-20%), *Prevotalla copri, and Lachnospiraceae (3-5%)*. Akkermancia muciniphila increased at discharge in the milk porriage and *Basti* group.

The number of species more than 1.0% at baseline was 26, while it decreased to 18 during *Virchana* and 24 after *Basti,* then it became 22 at the discharge, and increased to 27 after three weeks. It may correspond to the diversity of microbiota.

ASV number and Shannon's index showed corresponding change. One patient showed a marked predominance of *Proteobacteria* up to 96% during Virchana.

Discussion

Ayurveda is a system of medicine with historical roots in the Indian subcontinent [1-4]. Globalized practices derived from Ayurveda traditions are included in a type of alternativemedicine [3,21]. It is said that Ayurveda medicine does not have a scientific basis, so it is often deemed pseudoscientific or trans-science system. Ayurvedic medicine's central concept is the theory that health exists when there is a balance between the three fundamental bodily bio-elements (*doshas*) called *Vata*, *Pitta*, and *Kapha*.

Panchakarma therapy aims to eliminate excessive *Doshas* from the body to maintain the state of health for a longer duration. *Virechana therapy* is one of the *Panchakarma* therapies wherein detoxication is done by drugs and oil to exclude liposoluble toxins. It specifically aims to eliminate excessive *Pitta Dosha* from the body. It is said to be beneficial for detoxication in skin disorders, abscesses, and liver disorders because *Pitta* is considered to control metabolism. Rais S, et al. (2013) [10], conducted the study to evaluate *Virechana* therapy's effect on serum electrolytes in 15 people and ascertain the safety of therapeutic purgation.

The current study focused on the changes in intestinal microbiota before and after the *Panchakarma*, and the results were satisfactory to show clinical improvement. Each participant received *Virechana, Basti*, or milk porridge sudation therapy according to their condition. A personalized plan has carefully scheduled the right selection of oil, sweating, massage, and laxatives or meditation and mindfulness. All symptoms became lighter, especially with skin eruptions. Participants who took part in the trial also got mental settlement to develop a calm mind. All 13 patients showed improvement of skin rash, depression, and other symptoms.

The diet in the present camp was the modified Japanese style. They preferred vegetarian food basically, and brown rice eaters showed healthy bowel movement in conjunction with intestinal microbiota [22]. Bodyweight loss seemed to occur only by *Virechana* therapy, but as the body fat increased in all, the non-fatty mass had also decreased by *Basti* therapy.

Citation: Watanabe S, Oikawa S, Mibayashi H (2021) Ayurveda Treatment (Virechana and Basti) and Changes of Intestinal Microbiota at Phyla and Species Level. Prensa Med Argent, Volume 107:3. 319. DOI: https://doi.org/10.47275/0032-745X-319.

Table 7: Microbiota profile by genus-species level according to the therapy.

Baseline n=15				**Verchana n=5**				**Bast n=7**				**at discharge n=15**				**3 weeks later n=14**			
	mean	**sd**	**median**		**mean**	**sd**	**median**		**mean**	**sd**	**median**		**mean**	**sd**	**median**		**mean**	**sd**	**median**
Bacteroides	21.21	8.08	23.26	Bacteroides	19.93	15.58	17.96	Bacteroides	25.14	6.70	37.48	Bacteroides	19.20	10.85	22.19	Bacteroides	22.34	12.19	21.54
Bacteroides plebeius	5.35	11.42	0.01	Klebsiella	18.96	40.79	0.56	Enterobacteriaceae	9.56	9.57	25.79	Enterobacteriaceae	13.15	16.94	9.89	Bacteroides plebeius	5.43	11.48	0.32
Faecalibacterium prausnitzii	4.37	2.78	3.67	Enterobacteriaceae	8.57	6.06	12.04	Bacteroides uniformis	6.72	3.85	11.07	Prevotella copri	4.61	10.56	0.09	Lachnospiraceae	4.57	4.91	2.85
Lachnospira	4.18	3.01	3.46	Prevotella copri	7.64	12.69	0.89	Lachnospiraceae	4.54	2.80	8.19	Clostridiaceae	3.97	12.88	0.06	Bacteroides fragilis	4.39	14.77	0.01
Bacteroides uniformis	3.73	2.74	3.36	Lachnospiraceae	5.00	3.83	6.14	Lachnospira	3.08	2.97	8.75	Bacteroides uniformis	3.92	3.82	2.73	Faecalibacterium prausnitzii	3.91	2.76	3.41
Ruminococcaceae	3.43	2.60	2.37	Fusobacterium	3.55	7.81	0.05	Bacteroides ovatus	3.04	2.72	7.40	Bacteroides plebeius	3.29	8.53	0.02	Bacteroides uniformis	3.67	2.41	3.36
Rikenellaceae	3.21	2.91	2.55	Bacteroides caccae	2.79	5.34	0.05	Faecalibacterium prausnitzii	2.93	3.58	10.35	Lachnospiraceae	3.18	2.76	2.76	Lachnospira	3.25	2.81	2.52
Enterobacteriaceae	3.19	4.49	0.60	Roseburia	2.70	2.72	2.41	Sutterella	2.77	1.31	4.92	Akkermansia muciniphila	2.47	4.39	0.10	Rikenellaceae	3.14	3.56	2.30
Lachnospiraceae	3.16	1.34	2.74	Phascolarctobacterium	2.66	2.43	2.35	Rikenellaceae	2.65	2.46	6.87	Phascolarctobacterium	2.44	2.95	0.98	Ruminococcaceae	2.49	2.59	1.52
Phascolarctobacterium	2.75	2.64	1.64	[Prevotella]	2.64	5.83	0.06	Bacteroides plebeius	2.41	4.63	12.09	Sutterella	2.43	1.38	2.83	Citrobacter	2.35	6.22	0.06
Sutterella	2.51	1.65	2.43	Fusobacteriaceae	2.35	5.25	2.35	Akkermansia muciniphila	2.02	4.01	10.68	Faecalibacterium prausnitzii	2.35	2.92	1.28	Oscillospira	2.14	1.71	1.91
Oscillospira	2.43	1.99	1.97	Fusobacteriaceae	2.23	5.00	2.23	Phascolarctobacterium	1.86	2.42	6.45	Lachnospira	2.17	2.52	1.82	Bifidobacterium adolescentis	2.07	5.34	0.55
Prevotella copri	2.38	6.97	0.01	Citrobacter	1.75	3.13	0.21	Bacteroides caccae	1.72	1.99	5.32	Peptostreptococcaceae	2.16	8.22	0.02	Sutterella	2.03	1.45	1.95
Ruminococcus	1.99	3.48	0.36	Dialister	1.26	2.56	0.16	Klebsiella	1.51	3.03	8.27	Bacteroides ovatus	1.74	2.23	1.13	Phascolarctobacterium	1.96	2.09	1.63
Bacteroides ovatus	1.96	1.57	1.28	Sutterella	1.16	1.49	0.77	Oscillospira	1.50	1.07	3.52	Klebsiella	1.74	3.10	0.47	Bacteroides ovatus	1.95	1.92	1.58
[Barnesiellaceae]	1.65	1.69	1.12	Roseburia	1.09	1.51	1.18	Ruminococcaceae	1.48	1.04	2.64	Megasphaera	1.65	3.54	0.06	Enterobacteriaceae	1.94	4.07	0.29
Parabacteroides	1.58	1.59	1.44	Bacteroides fragilis	1.03	2.01	0.14	Parabacteroides	1.46	1.46	4.37	Rikenellaceae	1.63	2.00	1.20	Parabacteroides	1.74	1.73	1.37
Bacteroides caccae	1.57	2.27	0.12	Bacteroides ovatus	1.02	0.81	1.49	[Barnesiellaceae]	1.44	1.15	2.88	Megamonas	1.62	5.52	0.00	αproteobacteria	1.67	3.30	0.39
Akkermansia muciniphila	1.49	5.09	0.05					αproteobacteria	1.43	3.20	8.62	Ruminococcaceae	1.45	1.47	0.80	Klebsiella	1.48	2.47	0.23
Bifidobacterium adolescentis	1.35	2.32	0.13					Acidaminococcus	1.35	1.87	4.72	Bacteroides caccae	1.27	1.84	0.80	Parabacteroidesdistasonis	1.44	2.10	0.87
Roseburia faecis	1.22	2.58	0.18					Prevotella	1.26	2.47	6.59	Parabacteroides	1.21	1.43	0.59	Ruminococcus	1.41	2.35	0.39
Parabacteroides distasonis	1.08	1.27	0.83					Morganella morganii	1.17	3.01	7.99	Oscillospira	1.01	1.02	0.63	Fusobacteriaceae	1.27	3.28	1.11
Streptococcus	1.08	2.92	0.04					Veillonella	1.15	3.04	8.04					Acidaminococcus	1.24	3.08	0.08
αproteobacteria	1.03	1.74	0.87					Bifidobacterium	1.11	1.83	4.63					Coprococcus	1.22	1.76	0.42
Blautia	1.02	1.53	0.54													Bacteroides caccae	1.18	2.22	0.08
Roseburia	1.01	1.31	0.45													[Barnesiellaceae]	1.15	1.03	1.28
																Fusobacterium	1.14	2.89	0.40

Changes in Microbiota and Clinical Manifestation

The present participants showed dominancy of *Bacteroidetes* than *Firmicutes*. Most Japanese showed dominancy of *Firmicutes* [22]. It was reflected in their dietary habits, mostly vegetarian way. Vegetarians seemed to have more *Bacteroides*, *Firmicutes,* and *Actinobacteria* than omnivores who had more *Proteobacteria, Fusobacteria,* and *Cyanobacteria*. David LA, et al. (2014) [23], analyzed 18 healthy people and found different bacterial profiles by Prakriti (constitution of a person), by Vata, Pitta, and kappa. In this series, Bacteroides, Desulfovibrio, slackia, and succinivibrio were common.

Kind of staple foods were five polished white rice, four white rice and barley, three mixtures of white rice and brown rice, and three genmai (brown rice). Genmai eaters showed fewer *Actinobacteria* and *Proteobacteria*, but the "white rice+barley" group also showed a similar trend. Significant difference among groups was not present because of the small number of cases. When the groups were divided by oil consumption, ghee users showed higher *Fusobacterium* and less *Firmicutes* and *Actinobacteria.*

Panchakarma observed the remarkable changes of the microbiota. After the pretreatment for *Virechana* therapy, a decrease of *Firmicutes*

and increase of *Proteobacteria* were noticed. *Bacteroides* predominated between 47-48% throughout the treatment.

The change of microbiota by *Basti* and *Swedana* for one week was also the decrease of *Firmicutes* and increase of *Proteobacteria*. After the discharge, *Firmicutes* returned to 30% on average, ranging from 25% to 50%. *Proteobacteria* returned to baseline level at a range of 4.4% to 8.7% three weeks later. *Fusobacterium* also increased but no statistical significance. Three weeks after discharge, the variety was increased by *Fusobacterium*, *Verrucomicrobia*, *Tenericutes,* and *Lentisphaerae*.

Hirakawa A, et al. (2019) [12], reported microbiota of 109 healthy people and noted that standard bacterial profiles at the phylum level were 44.3±9.9% *Firmicutes*, 20.7±8.8% *Bacteroides*,8.3±6.3% *Actinobacteria*, 1.7±2.7% *Proteobacteria,* and 1.2±4.2% (max 39.4%) *Verrucobacteria.*

Their series's dietary habit was mostly brown rice eaters, so they showed a certain characteristic profile. At the genus level, 12.7% *Bacteroides*, 8.3% *Blautia*, 7.9% *Faecalibacterium*, 6.3% *Bifidobacterium,* 5.3%*Prevotella,* 4.9% *Eubacterium,* 3.8% *Ruminococcus,* 2.6% *Fusicatenibacter,* 1.9% *Collinsella,* 2.4% *Streptococcus,* 2.1% *Subdoligranulum,* 1.7% *Anaerostipes,* 1.2% *Akkermansia* and 1.7% *Roseburia* occupied more than 1%. The difference between the brown rice and white rice eaters by microbiota profile was high butyrate-producing bacteria and low fusobacterium.

The distribution of butyrate-producing bacteria among *Firmicutes* phylum seemed to be uneven [25]. Butyrate was not only produced from dietary fiber but lactate. Barley dietary fiber may have a similar effect on microbiota because white rice+Barley showed a similar profile with *genmai.*

The subjective feelings of well having complex determinants and butyrate-producing bacteria could be added among them [12]. We have identified a possible correlation between a high personal sense of health and the presence of butyric acid-producing bacteria in the gut. Besides, increased *Enterobacteriaceae* in γ-*Proteobacteria* may produce β-hydroxybutyrate, which yield happy feeling in the brain is a common phenomenon in fasting [26,27].

A weakness of the present study: This was a pre and post therapy study on the relationship between Ayurveda *Panchakarma* and intestinal microbiota changes. Ayurveda therapy is based upon the tailor-made approach, so it does not fit the ordinary RCT. Accumulation of cases would make new evidence of patient-centered-therapy or narrative medicine. Pre and post-tests would be useful on such occasions [28]. Four significant phyla, *Firmicutes, Bacteroidetes, Proteobacteria,* and *Actinobacteria,* were present in all, but other phyla were less current, so the statistical power was insufficient. Decreased *Firmicutes* and increased *Proteobacteria*, and loss of *Verrucomicrobia* were commonly present in all participants, but these changes were usually considered to be worse for health, so more accumulation of clinical cases and long observation is necessary [29]. Changes in bacterial profile would occur by repeated irrigation, which changed the enteroenvironment and many metabolic changes. Laboratory tests werenot done at this time, so we could not measure the concentration of short-chain fatty acids and β- hydroxybutyrate, which has a mental effect. A larger number of cases and integrated studies should be done in the future.

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Conflict of Interest

All authors do not have any COI for this work. The research has done the researchfund from the Lifescience Promoting Association.

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