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Peer Review Report for "The First Report of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* Relative Abundance in Obese Iranian Population"

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Submit Date:	16 Dec 2017
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Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) — 10 Mar 2018

Dear AE,
Comments for the authors:
This information should be added to the manuscript
- The statistical analysis for Bacteroides populations among the log CFU of two groups
- Faecal sample collection
- Quantitative plating test
- The Firmicutes-to-Bacteroidetes ratio
- Dietary assessment of patients
- Biochemical markers of patients
Thanks.

REFEREE: Reviewer | **Revision** (0) — 5 Nov 2018

Dear AE,
The manuscript “The First Report of Bacteroides fragilis and Bacteroides thetaiotaomicron Relative Abundance in Obese Iranian Population” was reviewed, I call your kind attention to the following:
The worldwide obesity epidemic is triggering efforts to pinpoint host and environmental factors that disturb energy balance. Although the role of microbiota is still unknown, bacteria of the gut flora do contribute enzymes that are absent in humans and play an essential role in the catabolism of dietary fibers. in addition, recent reports of microbial changes in the human gut were proposed to be a possible cause of obesity.
In this study, original research was undertaken to study the abundance of two important bacterial species e.i., Bacteroides fragilis and Bacteroides thetaiotaomicron, in Iranian obese and normal weight subjects for the first time.
Although a significant difference was not observed between the two groups, the data obtained is valuable and significant to be used as a reference yardstick in future studies.
The manuscript is very well presented and merits consideration for publication in the journal.
Thanks.

REFEREE: Reviewer | **Revision** (0) 7 Nov 2018

Dear AE,
Comments for the authors:
1. English language is well, however, a final checking is needed for better performance.
2. The results are presented in brief, and I think it is better to mention all of the data, disregarding their role in conclusion.
3. It is suggeted that the authors utilize more figures and graphs to mention the importance of the results.
4. The discussio is well constructed, but the conclusion is better to appear under a separate title.
Thanks.

REFEREE: Associate Editor | **Revision** (0) 10 Nov 2018

Dear EIC,
This article needs minor revisions.
Thanks.

REFEREE: EIC | **Revision** (0) 10 Nov 2018

Dear Author,
This article needs major revisions.
Thanks.

OPEN PEER REVIEW

Revision (1)

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You can read the authors' responses to the reviewers' comments in the next page.

The First Report of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* Relative Abundance in Obese Iranian Population

Abstract

Background and Objective: Obesity is a multifactorial disorder that guts microbiota has a fundamental role in its pathophysiology. *Bacteroides* spp. has significant roles in gut microbiota-host interactions that determine health and disease development. Since, pattern of gut microbiota changes based on different criteria in each population, we have studied abundance of two important *Bacteroides* strains, *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*, in Iranian obese and normal weight subjects for the first time.

Materials and methods: One hundred participants recruited and classified based on their body mass index (BMI). All subjects were divided into normal (average BMI, 22.37 Kg/m^2) and obese (average BMI, 29.10Kg/m^2) groups. Bacterial DNA was extracted from the samples and quantitative PCR was conducted based on 16s rDNA universal primers. Finally, the correlation between bacterial abundance and obesity were analyzed.

Results: Obtained qPCR results showed that the relative abundance mean of *B. fragilis* in normal weight and obese subjects were 8.68 □ 10^{12} and 9.27 □ 10^{12} cfu/mL, respectively. In addition, 2.32 □ 10^{12} and 5.39 □ 10^{12} cfu/mL as the relative abundance mean of *B.thetaiotaomicron* in normal weight and obese subjects were reported. Although the obese Iranian subjects had more *B. fragilis* and *B. thetaiotaomicron* abundance compared to normal weight but any significant differences did not identified between *B. fragilis* (p = 0.79) and B. *thetaiotaomicron* (p = 0.18) relative abundance in two groups.

Conclusion: Although the obese Iranian subjects have more *B. fragilis* and *B. thetaiotaomicron* abundance compared to normal weights but any significant differences were not identified between two groups. Since *Bacteroides* spp. have significant role in gut microbiota- host interaction, determination of their abundance in obesity development, determination and targeting restoration of gut microbiota pattern could be valuable in controlling of obesity**.** In this regard dietary intervention could be based on determination of gut microbiota pattern in certain population.

Keywords: Gut microbiota, *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, Obesity Metabolic syndrome

Introduction

The gastrointestinal tract (GIT) is colonized with a divers, complex and dynamic microbial community that is known as gut microbiota[1]. The gut microbiota plays significant roles in human health and diseases[2]. It has important effects on host functions including homeostasis of the gastrointestinal tract, metabolism, immune and nervous system[3]. This complex community composed of diverse microorganism which is dominated by bacteria. Most of these bacterial species belong to phyla *Firmicutes* and *Bacteroidetes*. Further, another phyla such as *Verrucomicrobia* and *Actinobacteria* are present in gut microbiota[4]. The composition of gut microbiota is established during 2-3 first years of life under control of many factors including mode of delivery, genetic back ground, geography, nutrition, physical activity and gender[5].

In normal condition, symbiosis relationship between gut microbiota and host, the gut micobiota-host interaction is balanced. Conversely, in dysbiosis, alternation of gut microbiota pattern, putative interaction is disturbed. Dysbiosis resulted from high fat diet (HFD) induces low grade inflammation which leads to insulin resistance (IR). This condition is considered as turning point of various disorders and diseases such as obesity, metabolic syndrome, type 2 diabetes, inflammatory diseases (inflammatory bowel disease) and colorectal cancer[6, 7].

Recent studies consider the gut microbiota as an environmental factor that has important role in pathophysiology of obesity[8]. Energy harvest increased changes in microbial components and metabolites are attributed to the alternation of gut microbiota composition in obesity. These events are parallel with increase *Firmicutes* to *Bacteroidetes* ratio in obese subjects[9, 10]. Therefore, study of gut microbiota composition has become undeniable object in research.
It seems *Bacteroides* spp. among the gut microbiota members has an important role in pathophysiology of obesity. *Bacteroidetes* are Gram-negative rods, anaerobic and non-spore-forming bacteria. *Bacteroides* spp. such as *B. fragilis* and *B. thetaiotaomicron* are colonized in colon where dietary remained and indigestible carbohydrates reach. *Bacteroidetes* spp. extract energy from protein and carbohydrates by fermentation[11]. High enzymatic potentials of *B. fragilis* and *B. thetaiotaomicron* contribute in maintenance of homeostasis[12]. Also these bacteria have immonomudulatory effect that induces tolerance to gut microbiota[13]. Hence, due to metabolic and immune potentials of *B. fragilis* and *B. thetaiotaomicron*, their frequency could be important in obesity treatment. Population has different criteria including genetic background, ethnic, diet, life style, geography distribution that could affect the gut microbiota pattern; we focused on *B. fragilis* and *B. thetaiotaomicron* relative abundance in Iranian population for the first time.

Materials & methods

Study population

One hundred healthy adult individuals (aged 20 and 60 years) recruited. These subjects were equally grouped into two normal and obese, based on Body Mass Index (BMI). Subjects with

BMI between 18.5 and 24.9 kg/m2 were considered as normal group. Participants with significant infection or chronic diseases, and the use of antibiotics and corticosteroids were excluded. This study was performed in accordance with the ethical rules of the Helsinki Declaration**.**

DNA extraction from stool samples

The fresh stool samples were collected from subjects and stored immediately at -20 °C. DNA was extracted using a QIAamp DNA stool mini kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The quality of extracted DNA was assayed by agarose gel electrophoresis and spectrophotometric analysis. All DNA samples were stored at -20 °C.

Quantitative PCR (qPCR) analysis

The abundance of bacteria was estimated by universal primers which amplified a conserved region of the 16S rRNA gene. SYBR Green qPCR was conducted using Light Cycler® 96 SW 1.1 (Rocsh, Germany). Each reaction mixture of 20 µL was composed of SYBR Premix Ex Tag II (RR820L-Takara), 0.5µL of each of the specific primers, and 5 µL of template DNA. The amplification program were designed according to appropriate annealing temperature so that consisted of 1 cycle of 95 °C for 60 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 55 °C for 30 s and extension at 72 °C for 30s[14].

Standard curve: The abundance of bacteria calculated through DNA concentration. For this purpose, serial dilutions of DNA from standard strain Escherichia coli were prepared. The standard curve, allows to calculate DNA concentration of each bacteria in stool samples[15].

Statistical Analyses

In this study, categorical variables are presented as number (percent) and continuous variables as mean±SD. Independent t-test was used to assess mean differences between normal and obese groups. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). All statistical tests were 2-tailed and a $P \leq 0.05$ was considered statistically significant.

Results

Characteristics of the subjects are shown in Table 1. BMI classification consisted of two groups; normal weight (50%) and obese (50%) (Table 1). The results of qPCR showed that the relative abundance mean of *B. fragilis* in normal weight and obese subjects were $8.68\square10^{12}$ and $9.27\square10^{12}$ CFU ml^{-1}, respectively. Additionally, $2.32\square10^{12}$ and $5.39\square10^{12}$ CFU ml^{-1}as the relative abundance mean of *B.thetaiotaomicron* in normal weight and obese subjects were reported (Table 2). In order to find a correlation between *B. fragilis* and *B.thetaiotaomicron* abundance and obesity, the bacterial concentrations were analyzed between normal and obese Iranian subjects. Although the obese Iranian subjects have more *B. fragilis* and *B. thetaiotaomicron* abundance compared to

normal weights but any significant differences were not identified between *B. fragilis* (p = 0.79) and *B. thetaiotaomicron* (p = 0.18) relative to abundance in two groups. . (Figure 1**)**

Discussion

The gut microbiota is diverse and complex microbial community which affects metabolism and energy homeostasis. The gut microbiota composition is associated with health and diseases[3]. The pattern of gut microbiota has been established during the first 2-3 years of life and affects to many aspects in the host including immune cell functions, glucose and lipid metabolism, energy homeostasis and susceptibility to disease [16, 17]. The alteration of gut microbiota could be spotted as a biomarker in several disorders and diseases such as obesity [18-21]. *Bacteroidets* has significant roles in gut microbiota- host interaction. Therefore, determination of their abundance could be valuable in controlling of obesity**.** In regard to, relative abundance of *B. fragilis* and *B. thetaiotaomicron* were determined in Iranian population for the first time.

Recently, a correlation between gut microbiota composition and obesity is identified [22-24]. Various studies have reported that obese and non-obese subjects have different gut microbial compositions[14]. *Bacteroidets* and *Frimicutes* are two important phylum that are constituted gut microbial community. Bervoets et al.in 2013 showed an elevated *Firmicutes* to *Bacteroidetes* ratio in obese children[14]. *B. fragilis* and *B.thetaiotaomicron* are anaerobic intestinal commensal that belong to *Bacteroidetes* phylum. They have significant metabolic and immune potentials in gut microbiota- host interaction[13]. Hence their relative abundance could be influence in host metabolism.

There are various reports about abundance of *B. fragilis* and *B. Thetaiotaomicron.* For example Vael et al. in 2011 proved high intestinal *B. fragilis* abundance in infants between age of 3 weeks and 1 year were associated with a higher risk of obesity in their adulthood[25]. Karlsson et al. in 2012 did not observe a significant difference between *B. fragilis* abundance in obese children compared to normal weight subjects[26].

In turn Kasai et al. in 2015 demonstrated greater bacterial diversity and different gut microbial composition in obese compared with non-obese subjects. Also their results showed that some bacterial species including *B.thetaiotaomicron* had significantly greater concentration in non-obese group[27].

The establishment of gut microbiota composition is influenced by various factors including, mode of delivery, genetic back grand, life style, geography, ethnic, diet etc. Therefore these factors affect study of gut microbiota derived results. Determination of intestinal bacteria abundance as gut microbiota is considered in obesity etiology.

Conclusion

In this study data showed that the obese Iranian subjects had more fecal *B. fragilis* and *B. thetaiotaomicron* compared with normal weight, but no significant differences identified between *B. fragilis* and *B. thetaiotaomicron* relative abundance in obese and non- obese Iranian population. In conclusion, since, *B. fragilis* and *B. Thetaiotaomicron* have important role in metabolism and energy homeostasis, determination of relative abundance bacterial could be valuable in controlling of obesity. Regarding different of the gut microbiota pattern between a various populations which its results were different especially in ethnic, diet, and geography, the restoration of gut microbiota composition should be done by targeting in a certain population.

Acknowledgements

We would like to thank our colleagues at Department of Mycobacteriology Pulmonary Research and Microbiology Research Center of Pasteur Institute of Iran and the Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences for technical assistance.

Conflict of Interest

All contributing authors declare no conflicts of interest.

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List of tables

Table 1 Characteristics of obese and normal-weight adult in this study

	Obese	Normal-weight
Subjects (male/female)	26(12/14)	28(12/16)
Age (years)	43.56±1.92	30.55±1.42
Weight, kg	81.38±2.74	63±1.32
Height, m	1.68±0.025	1.68±0.010
BMI, kg/m2	28.42±0.59	22±0.36

BMI s.d. score	3.01	1.89

Table 2 The relative abundance of *B. thetaiotaomicron* and *B. fragilis*

	relative abundance of *B. thetaiotaomicron*	relative abundance of *B. fragilis*
Mean	3.8611×10^{12}	8.9777×10^{12}
Std. Error of Mean	1.16127×10^{12}	1.12594×10^{12}
Std. Deviation	1.16127×10^{13}	1.12594×10^{13}
Minimum	5.80×10^{5}	9.22×10^{7}
Maximum	7.75×10^{13}	5.20×10^{13}

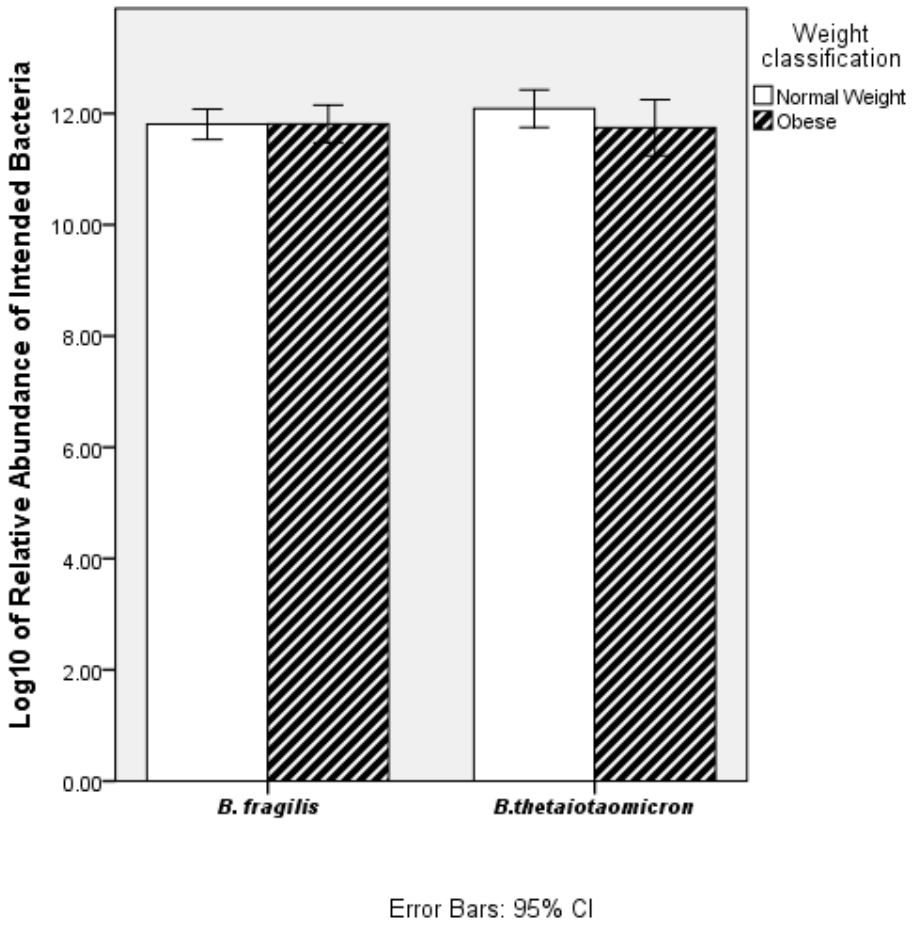


Figure.1 Comparison of *A B. thetaiotaomicron* and *B. fragilis* relative abundance in normal and obese Iranian subjects

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Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) — 19 Nov 2018

Dear AE,
This article is accepted.
Thanks.

REFEREE: Associate Editor | **Revision** (1) — 19 Nov 2018

Dear EIC,
This article is accepted.
Thanks.

REFEREE: EIC | **Revision** (1) — 19 Nov 2018

Dear Authors,
This article is finally accepted.
Thanks.