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RESEARCH ARTICLE

Association between *Blastocystis* and body mass index in healthy subjects; a theoretical pilot study

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Abstract

Background *Blastocystis* is a prevalent protozoan of the gut lumen with worldwide distribution. Recent studies showed that this microorganism may alter both richness and diversity of the gut microbiota. In the current study we assessed the dietary intakes in two *Blastocystis*-positive and *Blastocystis*-negative groups to evaluate the impact of this protozoan on the body mass index (BMI) and the dietary intakes.

Methods In total, ninety-three participants consisted of 17 and 76 *Blastocystis*-positive and *Blastocystis*-negative subjects, respectively, were included in this study. Positive cases of *Blastocystis* had been confirmed by microscopy and culture medium. Moreover, a standard FFQ was filled out for all participants and independent t-test was employed to evaluate the correlation between *Blastocystis* and the dietary intakes.

Results From participants, 21/93 (22.58%) and 72/93 (77.42%) were male and female, respectively. The mean age ± SD among *Blastocystis*-positive and *Blastocystis*-negative individuals was 39.41 ± 14.60 years and 37.37 ± 13.16 years, respectively. Furthermore, *Blastocystis*-positive participants had lower weight (67.84 ± 12.29 kg) in comparison to *Blastocystis*-negative individuals (69.80 ± 14.99 kg). Indeed, BMI of *Blastocystis*-positive subjects was 25.46 ± 4.66 and lower than *Blastocystis*-negative subjects 25.89 ± 5.01; (*P* value = 0.745).

Conclusions Our findings showed that BMI in *Blastocystis*-positive subjects was lower than *Blastocystis*-negative persons. *Blastocystis* probably affects the dietary intakes and energy metabolism.

Keywords *Blastocystis* · FFQ · Body mass index · Dietary intakes · Gut microbiota

Introduction

From past to present, parasites have been majorly conceived as harmful microorganisms that habitually use the host nutritional resources and hurt them. However, recent experimental studies showed that some parasites are able to act over the traditional expectations via manipulation of either immune system [1] or microbial community of their niches [2, 3].

Gut microbiota" is a term applied for a complex of microbial inhabitants including both prokaryotes and eukaryotes that are colonized in the gastrointestinal tract. This microbial community plays an important role in improving the metabolic and physiological conditions of the gut [4]. However, the central role of the gut microbiota has been discussed and highlighted in broad spectrum of human-life aspects from the psychological features to obesity [5–10].

Blastocystis is a unicellular non-flagellated Stramenopile, living in the gastrointestinal tract of a wide range of non-mammalian and mammalian hosts, including humans [11–13]. This protozoan is mostly transmitted via the fecal-oral route [14], and have been reported to be the most common reported intestinal protozoan in stool samples [12]. Indeed, the

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prevalence rate of *Blastocystis* in humans varies from 0.5% to 76% in developed and developing countries [13, 15–17]. In 2014, the prevalence of *Blastocystis* was reported up to 100% among Senegalese children which is the highest reported prevalence rate for this protozoan in the world [18]. Taken with the high prevalence and unknown pathogenicity of *Blastocystis* [19–22] two probable hypotheses may explain the correlation between *Blastocystis* and the gut microbiota. The first (theory of positive role): evidence show that *Blastocystis* could be related to the microbiota diversity in healthy individuals; therefore, it should be known as the indicator of a healthy gut [2, 23]. The second (theory of negative role): *Blastocystis* has been suggested to be able to modify the gut microbiota toward an unpleasant condition in some gastrointestinal disorders such as IBS [24, 25]. For example, decreased abundance of Bifidobacteria, and *Faecalibacterium prausnitzii*, two important bacteria contributing to the sustainability and maintenance of homeostasis of the gut, in *Blastocystis*-positive individuals suggesting the negative role of *Blastocystis* in gastrointestinal disorders [26–28].

However, regarding the high prevalence of *Blastocystis* and its role in the manipulation of the gut microbiota composition, this hypothesis can arise that this protozoan may affect the nutritional conditions in human subjects. Therefore, in the present pilot study, the dietary intakes were evaluated between two groups of *Blastocystis*-positive and negative subjects, without any gastrointestinal symptoms.

Material and methods

Study population

In the first step, objectives and protocol of the study were explained to all the enrolled participants and an oral consent was taken. The presence of any gastrointestinal disorder, and consumption of anti-parasitic and antibiotic drugs during 4 weeks prior to sampling were considered as exclusion criteria. A total of 93 participants met our criteria and agreed to enroll in the current study. Stool samples were collected and were immediately transferred to the parasitology lab of the Foodborne and Waterborne Diseases Research Center located in the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, for further investigations.

Microscopic examination and stool cultivation

All stool samples were examined by Lugol's iodine staining. Furthermore, the samples were concentrated with routine formalin-ethyl acetate and checked by light microscopy to detect enteric parasites. A portion of the samples was immediately cultivated in Dulbecco's modified Eagle's medium (DMEM) with 20% inactivated fetal bovine serum (FBS). All cultivated samples were incubated at 37 °C for 72 h, and 10 μL of the sediment was examined by light microscopy with magnification X400 to check the growth of *Blastocystis* every 48 h. Samples without any growth after 10 days were considered negative.

Data gathering and food intake analysis

A well-trained nutritionist filled a semi-quantitative 168-item food frequency questionnaire (FFQ) designed based on Willett format which was previously validate for the nutrients, food intake and the dietary pattern using face-to-face interview for each participant [29]. This FFQ was consisted of demographic data together with the value of food items modified based on the Iranian foods. Although standard portion size for each food item was designed based on the United States Department of Agriculture (USDA) serving sizes (e.g. bread, one slice; dairy, one cup), household measures were applied for the items that were difficult to report according the USDA standards. Therefore, regarding the presence of some differences in food items of Iranians with other countries, food items were grouped based on the similarity of nutrition profiles in a nineteen-items-food-groups table which was previously validated [29]. The frequency and portion sizes for each food item were converted to daily intake and grams, respectively.

Statistical analysis

Statistical analysis using independent t-test was applied to test the correlation between *Blastocystis* and the dietary intakes. Statistical analysis was performed using IBM SPSS statistics for Windows, v22 (Chicago, IL, USA). A probability (P) value <0.05 was considered statistically significant.

Results

In this study, *Blastocystis* was detected in 17/93 (18.27%) of samples by microscopical examinations and culture method. The mean age ± SD was reported among *Blastocystis*-positive and *Blastocystis*-negative individuals 39.41 ± 14.60 years and 37.37 ± 13.16 years, respectively. The mean weight ± SD among *Blastocystis*-positive and *Blastocystis*-negative individuals was 67.84 ± 12.29 kg and 69.80 ± 14.99 kg, respectively. The percentage of male and female among participants was 22.58% (21/93) and 77.42% (72/93), respectively (Table 1).

BMI and dietary intakes, total kilocalories (Kcal), total protein, total cholesterol, total fat, total saturated fat, polyunsaturated fatty acids (PUFAs), total trans, total folate, total

Table 1 Demographic data and BMI of participants among two *Blastocystis*-positive and *Blastocystis*-negative groups

		Blastocystis-positive	*Blastocystis*-negative
Gender	Male	2	19
	Female	15	57
Age ± SD (Years)		39.41 ± 14.60	37.37 ± 13.16
Weight ± SD (Kg)		67.84 ± 12.29	69.80 ± 14.99
BMI*		25.46 ± 4.66	25.89 ± 5.01

*P value = 0.745

Fe^{2+}, and total Ca^{2+} were calculated and are summarized in Table 2.

Accordingly, energy intake ± SD among *Blastocystis*-positive subjects was 2300.74 ± 717.95 while it was 2756.60 ± 1489.17 among *Blastocystis*-negative (P value = 0.223). BMI among two groups was close to each other, 25.46 ± 4.66 and 25.89 ± 5.01 for *Blastocystis*-positive and *Blastocystis*-negative, respectively and statistically significant differences were not seen (P value = 0.745). Besides, energy and nutrient intakes were lower in *Blastocystis*-positive subjects, although they were not statistically significant.

Discussion

Blastocystis is a prevalent protozoan among human subjects that its correlation with the gut microbiota in healthy and non-healthy conditions has been a research interest during the last decade [30]. Although the epidemiological-based studies have not established a correlation between the presence of *Blastocystis* and specific gastrointestinal disorders, it was suggested that *Blastocystis* can remain colonized in the gut for a long time [31]. Therefore, a body of evidence proposed this protozoan as an "old fiend" of human and healthy indicator of the gut [30]. In the study conducted by Mirjalali et al. [32], the prevalence of *Blastocystis* among patients who suffered from inflammatory bowel diseases (IBD) was lower than that observed among healthy individuals; therefore, they suggested that unpleasant conditions of the gut in IBD patients may lead to the lower prevalence of this parasite in this group of patients. Earlier on, other studies also showed the lower prevalence of *Blastocystis* in IBD patients [33–35].

Interestingly, in this study one of the important discontent among *Blastocystis*-positive participants was the lack of weight gain despite normal nutrition. As well, based on the results of the current study, BMI had an inverse correlation with the presence of *Blastocystis*. Although this invers correlation was not statistically significant, this finding was in line with previous studies claiming lower BMI in *Blastocystis*-positive subjects in comparison to *Blastocystis*-negative individuals [31, 36]. However, the lack of statistical significance correlation was probably due to the low number of sample size.

The current findings came from a pilot study and did not provide data on the gut microbiota diversity; however, the results of numerous previously published studies showed a strong correlation of the higher microbiota diversity and *Blastocystis* [2, 3, 37, 38]. However, concerning the crucial role of this protozoan in the diversity of the gut microbiota composition, it seems that *Blastocystis* might be associated with obesity via alteration in the either diversity of the microbiota community or abundance of a specific group of bacteria. In this regard, in the studies by Andersen et al. [36, 39], although no statistically significant, the higher number of *Blastocystis* carriers among lean individuals was reported and it was suggested that either 1) *Blastocystis* altered the gut microbiota composition toward the microbial pattern in lean individuals, or 2) the gut microbiota composition in lean individuals provides conditions favor for the *Blastocystis* colonization.

Recent studies suggested that *Blastocystis* could be related to BMI via increased abundance of *Akkermansia muciniphila*

Table 2 Nutritional variables evaluated based on the analysis of the standard FFQ in two *Blastocystis*-positive and *Blastocystis*-negative groups

Variables	*Blastocystis*-positive (n = 17)	*Blastocystis*-negative (n = 76)	P value
Total Kcal	2300.74 ± 717.95	2756.60 ± 1489.17	0.223
Total protein	98.08 ± 27.57	116.36 ± 69.68	0.292
Total cholesterol	295.55 ± 129.92	351.33 ± 224.72	0.327
Total fat	86.17 ± 27.21	111.04 ± 68.78	0.148
Total saturated fat	25.56 ± 9.64	30.97 ± 18.75	0.253
PUFAs	19.52 ± 7.87	26.49 ± 17.37	0.110
Total trans fat	2.51 ± 2.17	3.46 ± 3.23	0.256
Total folate	601.37 ± 186.81	657.11 ± 335.99	0.258
Total Fe^{2+}	17.19 ± 5.14	21.22 ± 12.91	0.122
Total Ca^{2+}	987.95 ± 319.29	1104.12 ± 543.75	0.375

PUFAs polyunsaturated fatty acid

[3]. Growing evidence resulted from the gut microbiota studies showed a strong invers correlation between the abundance of *A. muciniphila* and obesity. Recent studies demonstrated that *A. muciniphila* can decrease the weight gain and adiposity development via protecting the hemostasis of the gut barrier and preventing from the adipose tissue inflammation [40, 41]. Therefore, it is proposed that *Blastocystis* can behave like a watchtower that plays an important role in the maintenance of the gut microbiota composition.

From the biochemical point of view butyrate plays crucial role in the maintenance of colonic homeostasis and providing needed energy of coelomocytes via β-oxidation pathway [42–44]. Therefore, during the β-oxidation pathway the concentrations of oxygen decrease in a healthy gut and provide a favor niche for obligate anaerobes. Shifting away, concerning this fact that *Blastocystis* is considered as an obligate anaerobic microorganism [45], it seems that this protozoan preferably alters the gut microbiota composition toward keeping hemostasis [30]. Interestingly, it was experimentally showed that butyrate improved the energy metabolism and insulin sensitivity in obese mice [46] and protect them against diet-induced obesity [47]. Taken these evidence together, *Blastocystis* as an "old friend" [30, 36] probably plays indispensable role in keeping healthy conditions of the gut in spite of controversial evidence for pathogenicity of some subtypes.

There were some limitations to this study. For example, stool samples were collected only once; it would have been better if three specimens were collected to increase the sensitivity and accuracy of detection. As well, due to our limited facilities and funds, we did not investigate changes (increase or decrease) in the normal intestinal microflora regarding the presence/absence of *Blastocystis*.

Conclusion

The results of the current study showed that although it was not statistically significant, weight and BMI in *Blastocystis* carriers were lower than that in *Blastocystis*-negative subjects. In addition, intake energy in *Blastocystis*-negative subjects was higher than that in *Blastocystis* carriers. However, more comprehensive studies consisted of both metagenomics approaches together with assessment of the dietary intakes using standard FFQ and blood indicators can provide new insights into the dialogue between a prevalent protozoan such as *Blastocystis* and the gut microbiota.

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Author contributions Conceived and designed the experiments: HM. Performed the experiments: HM AL BH. Analyzed the data: HM AL AY. Clinical Experiments: AL BH AS MJE. Contributed reagents/materials/analysis tools/positive samples: HM MRZ. Wrote the paper: HM AT. All authors read and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The present study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences no. IR.SBMU.RIGLD.REC.1395.142.

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