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Original paper

Investigation of the relationship between obesity and *Blastocystis* infection in an adult population in Aydin, Turkey

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ABSTRACT. *Blastocystis* is one of the most frequent protozoa in human faecal samples, however, little is known about its relation with obesity. The present study aimed to analyse *Blastocystis* infection and subtypes in three adult populations classified according to body mass index (BMI). Faecal samples from 346 individuals were classified according to BMI: control (124 cases), overweight (110 cases), and obese (112 cases). Nucleic acid extraction from the samples was followed by amplification of partial 18S ribosomal RNA (18S rRNA) gene of *Blastocystis*. The neighbour-joining method was used to construct a phylogenetic tree from evolutionary distance data. Clinical findings were compared between *Blastocystis* infected and non-infected cases. *Blastocystis* was detected in 52 (15%) of 346 individuals with PCR assay. *Blastocystis* was less frequent in obese group (8%) than both control group (18.2%) and overweight group (18.5%). Subtype distribution was as follows: ST3 (n=21; 43.8%), ST2 (n=15; 31.3%), ST1 (n=10; 20.8%) and ST7 (n=2; 4.2%). The overall nucleotide diversity of 18S ribosomal RNA gene was 0.049. None of the gastrointestinal symptoms and gender was not significantly related with the infection. Despite the cross sectional nature of the study including a specific population, it suggests a negative association between *Blastocystis* infection and obesity. In addition, the lack of significant relation further supports asymptomatic colonization of *Blastocystis*.

Keywords: *Blastocystis*, obesity, subtype, symptoms

Introduction

Blastocystis is a unicellular stremonopile that colonize in gastrointestinal (GI) tracts of a variety of animals, including humans. The isolates of *Blastocystis* from diverse hosts are morphologically indistinguishable; however, they exhibit an extensive genetic divergence [1]. Phylogenetic analysis of *Blastocystis* 18S ribosomal RNA gene identified the presence of at least 17 subtypes (STs) or arguably species, indicating non-host-parasitic specificity [2]. The recent studies showed that ST1–9 and ST12 were isolated from humans and ST1–4 accounted the great majority of *Blastocystis* isolates in humans [3,4].

Most people infected with *Blastocystis* do not develop any symptoms; however, some nonspecific GI symptoms are linked to the infection, particularly abdominal pain and diarrhea [1]. Most clinical and prevalence data in the literature relied on the traditional parasitological techniques, especially direct microscopy of faecal samples. However, the poor sensitivity and specificity of this method cause inaccurate diagnosis of *Blastocystis* and contradictory findings [5]. Recently, the association between *Blastocystis* and intestinal flora has been interested in a growing body of literature. It was reported that *Blastocystis* was an important component of the intestinal microbiota and directly related to certain microbial enterotypes [6].

Obesity represents a major public health challenge in a global scale since it considerably increases the risk of many life-threating diseases, thus causing a decrease in both life quality and expectancy [7]. The prevalence of obesity has reached to pandemic levels within the past 50 years; the mean prevalence of obesity in Organisation for

Table 1. The frequency of *Blastocystis* in three BMI groups

			Blastocystis		
			Positive	Negative	Total
BMI	Control (18 to 24.9 kg/m^2)	Count	23	101	124
		%	18.5%	81.5%	
	Overweight (25 to 29.9 kg/m^2)	Count	20	90	110
		%	18.2%	81.8%	
	Obese (≥30 kg/m^2)	Count	9	103	112
		%	8%	92%	
Total		Count	52	294	346
		%	15%	85%	

Economic Co-operation and Development (OECD) countries is 19.5% in adults [8]. Intestinal flora is an important regulator of obesity among the non-genetic factors and a correlation between intestinal dysbiosis and obesity has been suggested [9].

In the present study, we aimed to investigate the association between *Blastocystis* colonisation and body mass index in adult population. Genetic variability of *Blastocystis* isolates and clinical features of the infection were further analysed.

Materials and Methods

Study groups and collection of samples

In this cross sectional study, three groups were created based on body mass index (BMI) of individuals: control (18 to 24.9 kg/m^2), overweight (25 to 29.9 kg/m^2), and obese (≥30 kg/m^2), according to World Health Organisation (WHO) criteria. The individuals under 18 were not included in the study.

Faecal samples were collected in Aydin Adnan Menderes University, Research and Training Hospital, Parasitology Laboratory. Clinical properties of individuals were acquired from the hospital information system. The city is located in the south western of Turkey on the coastal zone of the Mediterranean Sea.

The study was reviewed and approved by the Local Ethical Committee for Human Studies in Aydin Adnan Menderes University (2018/1383).

Molecular detection of Blastocystis

Faecal samples were subjected to DNA isolation with a commercial DNA extraction kit (Qiagen DNA stool mini kit, Germany). A single PCR reaction was designed for partial amplification of *Blastocystis* 18S ribosomal RNA gene (barcode region) with the primers RD5 and BhRDr, as previously described [10]. The isolate ADUBI201 (GenBank: KU361300) was used as positive control in the assays. The positive samples were sequenced by a commercial facility with using 377 Applied Biosystems DNA Sequencer. An online available software (DNASP version 6) was used to determine the nucleotide and haplotype diversity [11].

The subtypes of *Blastocystis* isolates were determined according to closest or exact match at 18S rRNA database for *Blastocystis* (http://pubmlst.org/*Blastocystis*) [12]. The sequences from the present study and the references were constructed a phylogenetic tree with neighbour-joining method, the evolutionary distances were computed using the Maximum Composite Likelihood [13,14]. The partial 18S rRNA gene of *Proteromonas lacertae* (AY224080) was included in the phylogenetic analysis as the out-group.

Statistical analysis

All statistical analyses in the present study was carried out using Statistical Package for the Social Sciences (SPSS) program, version 19.0 (PASW Inc, USA). Chi-square ($\chi 2$) tests were used to compare *Blastocystis* frequencies infection between groups and in the evaluation of symptoms. Student's t-test was used in comparison of mean BMI values of *Blastocystis* positive and negative individuals. Significance levels were set at 0.05 in the analyses.

Results

Blastocystis positivity and BMI

Faecal samples from 346 cases were screened for *Blastocystis* positivity with SSU rDNA-PCR and *Blastocystis* was detected in 52 (15%) of the samples. The mean age of participants was 41.2±14.1. Control, overweight and obese groups were compared for the presence of *Blastocystis*. The

Table 2. The genetic analysis of partial 18S rRNA gene of *Blastocystis* with DNAsp

The number of polymorphic (segregating) sites, (S)	84
Total number of mutations, (η)	98
Number of haplotypes, (h)	10
Haplotype (gene) diversity, (Hd)	0.982±0.046
Nucleotide diversity, (π)	0.049±0.014

frequency of *Blastocystis* was not significantly different between the control and overweight group (χ2=0.641; p=0.800). However, a significant difference was found when obese and control group was compared (χ2=4.522; p=0.039). In addition, *Blastocystis* was not statistically more frequent in obese group than in overweight group (χ2=3.402; p=0.075). Table 1 represents the distribution of *Blastocystis* according to BMI group of cases. In addition, the average BMI of *Blastocystis* positive cases (26±5.7) was significantly lower than *Blastocystis* negative cases (27.8±5.9) according independent to the t-test (p=0.047).

Blastocystis subtypes and genetic variability

Blastocystis subtypes could be detected in 48 of 52 PCR positive samples. The sequenced were submitted to Genbank with accession numbers: MW036232-42. The query of *Blastocystis* 18S rRNA partial gene sequences to the database revealed four different subtypes of *Blastocystis* in our study population. The most common subtype was ST3 (n=21; 43.8%), followed by ST2 (n=15; 31.3%), ST1 (n=10; 20.8%), and ST7 (n=2; 4.2%). In addition, intra-subtype variation of subtypes were as follows: ST3 had single allele (MW036241), ST7 had single allele (MW036242), ST1 had three

Table 3. Comparison of symptoms and gender between *Blastocystis* infected and non-infected cases

	Blastocystis		
	Infected	Non-infected	Statistical analysis
Gender	n (%)	n (%)	
Male	27 (51.9)	121 (41.2)	
Female	25 (48.1)	173 (58.8)	χ2=2.09 p=0.148
Symptoms			
Constipation			
Yes	6 (11.5)	29 (9.9)	
No	46 (88.5)	265 (90.1)	χ2=0.136 p=0.712
Diarrhoea			
Yes	10 (19.2)	55 (18.7)	
No	42 (80.8)	239 (81.3)	χ2=0.08 p=0.929
Abdominal pain			
Yes	15 (28.8)	75 (25.5,9)	
No	37 (71.2)	219 (74.5)	χ2=0.255 p=0.613
Nausea/vomitting			
Yes	7 (13.5)	31 (10.5)	
No	45 (86.5)	263 (89.5)	χ2=0.385 p=0.535
Lack of appetite			
Yes	10 (19.2)	38 (12.9)	
No	42 (80.8)	256 (87.1)	χ2=1.475 p=0.225

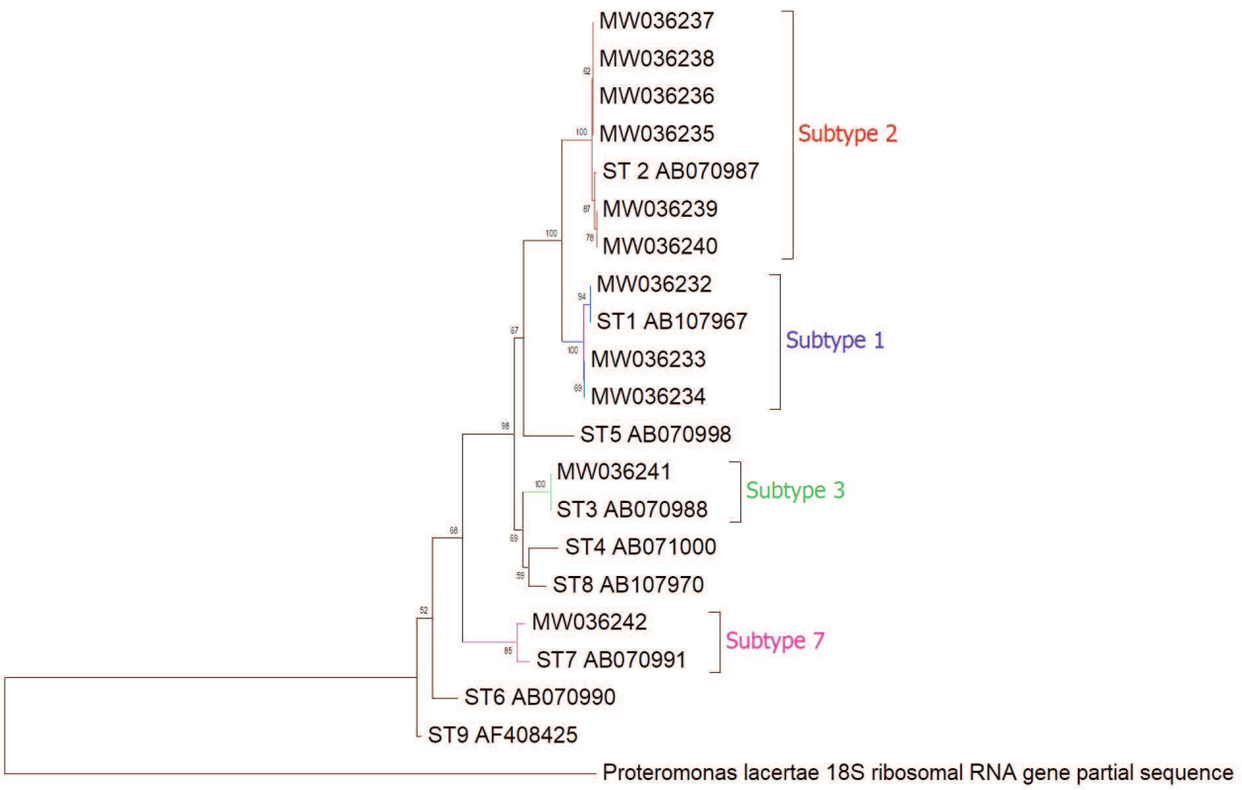

Figure 1. The phylogenetic tree constructed with the neighbour-joining method, based on the partial 18S rRNA gene sequences of *Blastocystis* from the present study and the references. The bootstrap test percentages (1000 replicates) are presented over the branches

alleles (MW036232-34), and ST2 had the highest number, six alleles (MW036235-40).

Several measures of DNA sequence variation in *Blastocystis* population were calculated with DNAsp software and presented in Table 2. The estimation of evolutionary distances between *Blastocystis* 18S rRNA partial gene sequences and common reference sequences was presented in Figure 1. This phylogenetic analysis also showed similar ST findings as in the 18S rRNA database.

Analysis of symptoms and gender

The statistical analysis of particular gastro-intestinal symptoms and gender showed that none of them was significantly different between *Blasto-cystis* infected and non-infected group (Table 3).

Discussion

Blastocystis is one of the most common eukaryotic microorganisms in human stool samples [4]. Its role in human health is a controversial topic and a question of great interest, recently. The present study compared the frequency of *Blastocystis* in normal weight, overweight and obese individuals from a research hospital in Aydin, Turkey. We found a lower infection rate (8%) in obese population as compared to normal weight and overweight population. This finding was consistent with a previous research that included data of 316 individuals (110 obese, 62 overweight, and 143 lean) and reported an increasing tendency of *Blastocystis* in lean individuals, when they included only Danish individuals in the analysis [15]. Similar to our findings, another study found a negative correlation between *Blastocystis* and BMI of individuals. *Blastocystis* frequency was 39.4% in normal weight group and it was 15.4% in obese group [16]. The average BMI of *Blastocystis* infected individuals (26±5.7) was significantly lower than non-infected cases (27.8±5.9) in our study. However, a recent study reported that average BMI of *Blastocystis*-positive cases was 25.5±4.7 and it was 25.9±5 in *Blastocystis*-negative cases, with no significant difference [17]. Nevertheless, their findings might be somewhat limited by the small sample size, because of only 17 *Blastocystis* positive cases in the study.

Overall, it seems that the observed decrease of *Blastocystis* colonisation in obese population may be attributed to the changed intestinal flora or dysbiosis in the progress of obesity. A number of study found lower diversities and significant differences in bacterial composition of intestinal flora when they compared obese and normal weight population [9]. In addition, obese individuals had an increased *Firmicutes/Bacteroidetes* ratio in their intestinal microbiota [18]. Recent studies showed that microbial richness and diversity were also linked to *Blastocystis* colonisation. The individuals with *Bacteroides* dominant intestinal flora were less infected with *Blastocystis* as compared to those with the *Ruminococcus* or *Prevotella* dominant flora [19,20]. In addition, a recent study reported that *Blastocystis* had a positive correlation with *Prevotella* enterotype in children because of the selective enrichment of the bacterium [21]. A recent study suggested that certain isolates of *Blastocystis* showed pathogenic effects by disrupting the gut microbiota [22]. They reported that *Blastocystis* ST7 changed the bacterial diversity of microbiota and potentially caused to an imbalance of the gut microbiota. In addition to obesity, some other microbiota related diseases also effect the colonization of *Blastocystis* negatively, including, ulcerative colitis and irritable bowel syndrome [23,24].

Blastocystis frequencies have been widely reported in the literature; however, the results were highly influenced by the diagnostic methods [5]. In the present study, *Blastocystis* was detected in 52 (15%) of 346 cases from Aydin, Turkey. The finding was in accordance with the previous reports in Turkey, the prevalence varied from 1.4% to 23% in those studies [25]. In general, the prevalence of *Blastocystis* in developing and undeveloped countries (up to 86.6% and 100%) was higher than in developed countries (0.5–24%) [1,2,26].

The majority of *Blastocystis* isolates were ST3 (43.8%) in the present study and followed by ST2 (31.3%), ST1 (20.8%), and ST7 (4.2%). The finding was consistent with the literature, it reported that ST1–4 constitutes 90% of human *Blastocystis* infections worldwide, and ST3 was the most common subtype in most of the human studies [2,27]. *Blastocystis* subtypes were investigated in different provinces of Turkey, distribution of subtypes was mostly similar, but the number of detected subtypes differed in some of them. Some other subtypes, including ST4, ST5 and ST6 were reported in previous studies in Turkey [25,28].

In the present study, we found that 18S rRNA partial sequences of *Blastocystis* ST3 isolates were identical (21 isolates, one allele), however, ST2 isolates were more polymorphic (15 isolates, six alleles). A previous study in Iran also reported a high similarity among ST3 isolates and lowest similarity among ST2 isolates [29]. In addition, multilocus sequence typing of *Blastocystis* showed that ST3 was restricted to one of four clades, suggesting that ST3 had relatively high host specificity, the predominance of human-to-human transmission, and had early history of human colonisation than ST4 [30].

Blastocystis infections are mostly asymptomatic; however, nonspecific GI and dermatological symptoms may be observed in some cases [31]. In our study, when *Blastocystis* positive and negative individuals were evaluated in terms of both gender and clinical findings, no significant relationship was found. Many studies reported that no potential link was found between gender and *Blastocystis* positivity [32]. The studies dealing with clinical role of *Blastocystis* were mostly designed as comparison symptomatic patient group and control groups for the presence of *Blastocystis* and the results supported our findings [31,33]. However, it has been reported that *Blastocystis* causes symptoms in some studies, especially in the paediatric age group [34]. In addition, *Blastocystis* was less frequent in the diarrheal patients than the non-diarrheal group in a Korean population [35]. *Blastocystis* pathogenicity and clinical features have long been one of the most controversial issues for researchers. The lack of current findings and conflicting results reveal the need for experimental studies and animal models [1,36]. *Blastocystis* pathogenesis is thought to be a multi-factor phenomenon that changes depending on the genetic and biological characteristics of *Blastocystis* isolates [37].

Although the present study was relatively limited to a specific study population, the findings suggested a possible negative association between obesity and *Blastocystis* in Turkish adult individuals. Therefore, further in vivo and in vitro researches regarding the role of *Blastocystis* in obesity will be worthwhile. In addition, non-existence of a significant relationship in clinical characteristics of cases and *Blastocystis* further supported the idea of *Blastocystis* could be an indicator of healthy gut flora.

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