

Subtype Distribution of *Blastocystis* in Pregnant Women and Analysis of Possible Risk Factors

Gebelerde *Blastocystis* Alt Tiplerinin Araştırılması ve Potansiyel Risk Faktörlerinin Değerlendirilmesi

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ABSTRACT

Objective: Since the identification of *Blastocystis* subtypes (ST) in the last decade, much has been learned about the genetic diversity of *Blastocystis* isolates in different populations, except pregnant women. The objective of this study is to investigate the genetic diversity of *Blastocystis* in pregnant women and analyse some demographic factors.

Methods: The faecal samples from 100 pregnant women were collected at an Obstetrics and Gynecology Department in Muğla, Turkey. Thereafter, *Blastocystis* positivity was detected by direct microscopy and culture. The positive cultures were subjected to DNA isolation, and the *Blastocystis* barcode region was amplified with polymerase chain reaction. Next, the sequences were queried against GenBank nucleotide and *Blastocystis* STs (18S) databases.

Results: *Blastocystis* was detected in 14% (14 out of 100) of the faecal samples by culture and 10% (10 out of 100) of the samples by direct microscopy. Nine of *Blastocystis* isolates (64.4%) were ST3, three (21.4%) were ST1 and two (14.2%) were ST2. Neither the demographic features nor the gastrointestinal symptoms were statistically related to *Blastocystis* infection.

Conclusion: The findings in this study agreed with the most of the previous human studies that found ST3 as the most abundant genotype. This study reported the frequency of *Blastocystis* in pregnant women and highlighted the importance of comprehensive studies with more cases of *Blastocystis* during pregnancy.

Keywords: *Blastocystis*, pregnancy, subtypes, symptoms

ÖZ

Amaç: *Blastocystis* alt tiplerinin (ST) yakın tarihte tanımlanmasından bu yana, farklı popülasyonlarda *Blastocystis* izolatlarının genetik çeşitliliği hakkında yeni bilgiler elde edilmiştir. Bu çalışmada, gebe kadınlarda *Blastocystis*'nin genetik çeşitliliğinin araştırılması ve bazı demografik faktörlerin değerlendirilmesi amaçlanmıştır.

Yöntemler: Çalışma kapsamında, Muğla Kadın Hastalıkları ve Doğum Bölümü'nden toplam 100 hamile kadından toplanan dışkı örnekleri incelenmiştir. *Blastocystis* pozitifliği, direkt mikroskopi ve kültür ile tespit edilmiştir. Pozitif kültürlerden DNA izolasyonu yapılmış ve *Blastocystis* genomunda barkod bölgesi polimeraz zincir reaksiyonu ile amplifiye edilmiştir. Elde edilen diziler, Genbank ve *Blastocystis* alt tür (18S) veri tabanlarına girilerek alt tipler belirlenmiştir.

Bulgular: Kültür yöntemi ile 14 dışkı örneğinde (%14), direkt mikroskopi yöntemiyle ise 10 örnekte (%10) *Blastocystis* saptanmıştır. *Blastocystis* izolatlarının dokuzu (%64,4) ST3, üçü (%21,4) ST1 ve ikisi (%14,2) ST2 alt tipi olarak tanımlanmıştır. Demografik özellikler ve gastrointestinal semptomlar değerlendirildiğinde, herhangi biri *Blastocystis* enfeksiyonu ile istatistiksel olarak ilişkili bulunmamıştır.

Sonuç: Bu çalışmada ST3'ün en yaygın genotip olarak bulunması, önceki çalışmaların birçoğu ile uyumlu görülmektedir. Ayrıca, çalışmamız gebelerde *Blastocystis* görülme sıklığını ortaya koymakta ve bu konuda örnek sayısının fazla olduğu kapsamlı çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: *Blastocystis*, hamilelik, alt tip, semptomlar



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INTRODUCTION

Blastocystis is a highly prevalent enteric protozoon in humans and a variety of animals (1). Despite of the controversy about *Blastocystis* pathogenicity, recent researches has revealed that it may be considered as an opportunistic pathogen in immunocompromised cases (2). Even though there is not a complete immunosuppression, pregnancy is a period with immunomodulation of mother (3). Additionally, iron deficiency anaemia (IDA), protein, zinc, folate deficiency may develop due to the impairment of absorption caused by intestinal parasitic infections in pregnancy. These deficiencies may result in some fatal consequences: increased perinatal mortality rates, low birth weight and intrauterine tension. It was reported that pregnant women infected with *Entamoeba histolytica* were more susceptible to penetration of intestinal mucosa and the infection increased the risk of preterm delivery two fold (4). Heavy infection with *Schistosoma mansoni* increased risk of anemia in a cross-sectional survey in Tanzania (5). Soil transmitted helminth infections including *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms were soil transmitted helminth infections were significantly associated with anemia in pregnant women (6).

The complex, collective and highly active population of living microorganisms in gastrointestinal tract of humans is defined as gut microbiota and it exerts a marked influence on the host both in homeostasis and diseases. A number of host-related and environmental factors contribute to the formation and change of microbiota from infancy to adolescence. Gut microbiota establish some dramatic changes during pregnancy, primarily as a result of increased energy requirements of the developing foetus. Additionally, recent evidence suggests that the colonisation and immune system of foetus is highly influenced by maternal gut microbiota (7). The position and role of *Blastocystis* in gut microbiota is a growing area of interest, some authors have asserted that it can be an indicator of a healthy gut microbiota (8). The genetic heterogeneity of *Blastocystis* became a major area of interest, recently. *Blastocystis* isolates have been classified into 17 different genotypes/or subtypes, with small subunit ribosomal RNA (*SSU rRNA*) gene sequence analysis. The nine of them were isolated from human faecal samples; ST3 is the predominant subtype which is also known as human subtype (9). Subtype distribution of *Blastocystis* was investigated in a variety of study groups and some factors factors have been found to be related to the *Blastocystis* infection. However, to the best of our knowledge, no previous study has investigated *Blastocystis* subtypes during pregnancy. The aims of this study were to determine the genetic diversity of *Blastocystis* in pregnant women and also analyse gastrointestinal symptoms and demographic factors for *Blastocystis* infection.

METHODS

Samples

Faecal samples were collected from 100 pregnant women who admitted to Muğla Sıtkı Kocman University, Training and Research Hospital, Clinic of Obstetrics and Gynaecology in-between November 2014 and April 2015. A semi-constructed questionnaire was used to collect data which included the demographic characteristics: residence, livestock farming, having pet animal, drinking water supply, home ownership, occupation

and monthly income. The presence of common gastrointestinal symptoms (abdominal pain, diarrhoea, constipation, flatulence, urticaria and lack of appetite) was also asked to the women in the questionnaire. A single faecal sample was taken from each woman and brought to the laboratory with a plastic container without fixatives.

The study was approved by Muğla Sıtkı Koçman University Clinical Research Ethics Committee (07.11.2014). The informed consents were obtained from pregnant women in the present study.

Direct Microscopy, Culture and Polymerase Chain Reaction

All samples were examined with both direct microscopy and Jones medium for the presence of *Blastocystis* forms. Positive cultures were centrifuged at 12,000 g for one minute and pellets were used for DNA isolation with a commercial kit (DNAzol, Invitrogen). The barcode region was amplified with a set of conserved polymerase chain reaction primers (RD5 and BhRDr) for the analysis of approximately 600 bp of *SSU rRNA* coding gene (10). The reaction was set in 30 µL volume: 1-2 µL of template DNA, 0.3 U of Taq DNA polymerase, 0.2 mM of each dNTP, 0.4 pmol of each of the primers and 1× Taq buffer with (NH₄)₂SO₄. The amplicons were purified and sequenced by a commercial facility with 377 DNA Sequencer (Applied Biosystems).

Determination of Subtypes and Phylogenetic Analysis

The barcode sequences of isolates were queried against both Genbank and *Blastocystis* Subtype (18S) databases. Sequences were aligned with references by using ClustalW algorithm in Molecular Evolutionary Genetics Analysis version 6.0 (MEGA) and a phylogenetic tree was constructed with the Neighbor-Joining method in 1.000 replicates bootstrap test. Additionally, the evolutionary distances of sequences were calculated with the maximum composite likelihood (11-13).

Statistical Analyses

The study did not have defined exclusion criteria. All of the pregnant women who volunteered to participate were included in the study. The qualitative variables including residence, livestock farming, having pet animal, drinking water supply, home ownership, occupation and monthly income were analysed with chi-square test in Statistical Package for Social Sciences (SPSS 19.0) software. The analysis of gastrointestinal symptoms (abdominal pain, diarrhoea, constipation, flatulence, urticaria and lack of appetite) was also performed in the same way. The significance level was set at $\alpha=0.05$.

RESULTS

The mean age of pregnant women was 27.4±5.3 and the ages were varying from 16 to 44. *Blastocystis* was found in 14 of 100 (14%) pregnant women by culture; however, the rate was 8% by direct microscopy. The subtype distribution of isolates were as follows: ST3 nine isolates (64.4%), ST1 three isolates (21.4%) and ST2 two isolates (14.2%) (Figure 1). *Blastocystis SSU rRNA* gene partial sequences were deposited to Genbank with accession numbers: MH349731, MH349744 and MH349753.

Among the *Blastocystis* positive 14 cases, the most common gastrointestinal symptom was flatulence 57.1% (n=8), followed by abdominal pain (n=6, 42.5%) and constipation (n=4, 28.5%). *Blastocystis* negative pregnant women (n=86) were defined as control group and compared to *Blastocystis* positive pregnant women (n=14). Statistical analysis of demographic factors and symptoms with the presence of *Blastocystis* was presented in

Table 1. None of these factors were statistically related with *Blastocystis* positivity.

Additionally, the comparison *Blastocystis* positivity according to the presence and absence of symptoms was given in Table 2. It was found that *Blastocystis* positivity was not statistically different between the cases with and without symptoms.

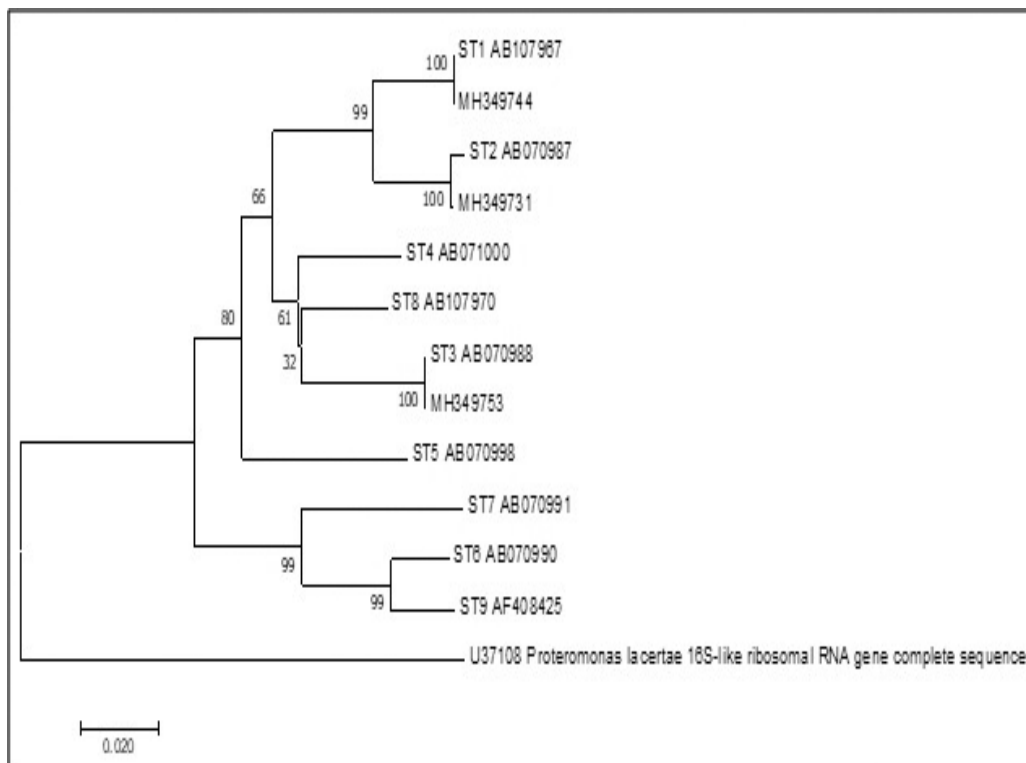


Figure 1. Evolutionary distances of *Blastocystis* isolates according to barcode region (Genbank accession numbers: U37108: outgroup; MH349731, MH349744 and MH349753: sequences from this study; STs are reference sequences)

Table 1. Descriptive and statistical analysis of possible *Blastocystis* related factors

		<i>Blastocystis</i>		Total	χ^2	p
		Positive (%)	Control (%)			
Residence	Urban	6 (42.9)	8 (57.1)	14	0.09	0.581
	Rural	38 (44.2)	48 (55.8)	86	-	-
Livestock farming	Yes	6 (43)	8 (57.1)	14	0.05	0.583
	No	36 (42.9)	50 (58.1)	86	-	-
Having pet animal	Yes	6 (42.9)	8 (57.1)	14	1.070	0.231
	No	25 (29.1)	61 (70.9)	86		
Drinking water supply	Tap water	4 (28.6)	10 (71.4)	14	1.383	0.189
	Bottled	39 (45.3)	47 (54.7)	86	-	-
Home ownership	Owner	5 (35.7)	9 (64.4)	14	1.329	0.388
	Renter	45 (52.3)	41 (47.7)	86	-	-
Occupation	Working	2 (14.3)	12 (85.7)	14	0.698	0.512
	Housewife	21 (24.4)	65 (75.6)	86	-	-
Monthly income of family	Low (<500 \$)	5 (35.7)	9 (66.3)	14	0.188	0.774
	High (≥500 \$)	36 (41.9)	50 (58.1)	86	-	-

Table 2. *Blastocystis* positivity and presence of symptoms^a

Symptoms	<i>Blastocystis</i> + n ^b (%)	Control n ^c (%)	χ^2	p
Abdominal pain	3 (13)	11 (14.3)	0.023	0.880
Diarrhoea	1 (11.1)	13 (14.2)	1.610	0.204
Constipation	4 (16.7)	10 (13.2)	0.187	0.666
Flatulence	5 (20.8)	9 (11.8)	1.225	0.268
Urticaria	2 (11.1)	12 (14.6)	0.152	0.696
Lack of appetite	3 (13.6)	11 (14.1)	0.03	0.956

^a: All women had more than one symptoms, ^b: Number and % of *Blastocystis* positive cases in symptomatic cases, ^c: Number and % of *Blastocystis* positive cases in non-symptomatic cases

DISCUSSION

Blastocystis is one of the most common protozoa in human faecal samples. Despite the recent development in the understanding of *Blastocystis* genetic diversity and symptomatology, the role of *Blastocystis* infection during pregnancy is not a well-studied area. The present study was designed to investigate *Blastocystis* frequency and subtype distribution in pregnant women. The *Blastocystis* frequency was 14% in our study, there were limited number of studies in the literature that investigated *Blastocystis* in pregnant women. It was reported that *Blastocystis* infection contributed to the development of IDA during pregnancy. They found that *Blastocystis* frequency was significantly higher in IDA patients (40%) than non-anemic pregnant controls (6.3%). Additionally, the overall frequency was 26.5% among all studied subjects (14). Another study investigated intestinal parasitism among pregnant women who lived in low socio economic residential areas in Bogotá, they reported that *Blastocystis* spp. frequency was 25% (15). Our study investigated the subtype distribution of *Blastocystis* in a pregnant population for the first time in the literature. As pathogenicity has been a controversial and much disputed area in *Blastocystis* researches, a variety of study populations such as cancer, ulcerative colitis, and Irritable Bowel syndrome patients were subjected in similar studies (16-18). Our findings were in accordance with the reported *Blastocystis* subtype distribution in these studies, all have found *Blastocystis* ST3 as the predominant genotype.

There were a few studies that investigated the frequency of *Blastocystis* in Muğla and surrounding cities. The city is located in the south-western of Turkey and the centrum is about 30 km from the seacoast in the Gulf of Gokova. It was reported that 7.4% of school aged children were infected with *Blastocystis* in Mugla (19). In addition, subtype distribution was found as follows: ST3 (34.2%), ST1 (31.4), ST2 (25.7) and ST7 (2.8%). In a study, *Blastocystis* subtypes from the culture positive 61 samples were investigated. They found a comparable result in that study: ST3 (38.6%), ST2 (29.5%), ST1 (20.5%), ST1+ST3 (9.1%), and ST1+ST2 (2.3%) (20). Moreover, a study reported that most common *Blastocystis* subtype was ST3 with a frequency of 52.6, followed by ST2 (22.1%), ST1 (17.9%), ST7 (4.2%), ST2+ST3 (2.1%) and ST1+ST3 (1.1%) in Aydin (21). In the present study we detected three different subtypes (ST1, 2, and 3) that is comparable to the other studies.

In the present study we have compared *Blastocystis* frequency with respect to residence, livestock farming, having pet animal,

drinking water supply, home ownership, occupation and income. None of these parameters were significantly related with *Blastocystis* presence. In the literature some have reported that *Blastocystis* infection has been related to the consumption of untreated water, close contact with animals, intra-family infections and mother's education (22-24).

In the present study, none of the gastrointestinal symptoms were found to be related with presence of *Blastocystis*. Flatulence, abdominal pain and constipation were the most common clinical findings in *Blastocystis* infected pregnant women. *Blastocystis* infections are highly asymptomatic and gastrointestinal symptoms are seen in a small proportion of infected cases (25). It has also been suggested that *Blastocystis* may be a marker of a healthy intestinal flora (26). However, in a number of studies, non-specific gastrointestinal symptoms such as diarrhea, abdominal pain and bloating, as well as dermatological symptoms have been linked to *Blastocystis* infection (27). In addition a study reported that fever was common in symptomatic *Blastocystis* infected individuals (28). The main limitation of such studies, however, was the lack of elimination of other possible factors such as pathogens, underlying diseases and immune status of cases. In brief, the current data are rather controversial, and there is no general agreement about clinical importance of *Blastocystis* infection. However, recently *Blastocystis* pathogenesis is reasonable thought to be a multi-factor phenomenon that is highly depended on both *Blastocystis* and host related factors (29).

CONCLUSION

Although the current study was based on relatively small number of participants, it highlighted the importance of *Blastocystis* in pregnancy because of the high infection rate. Therefore, parasitological examinations are recommended in gynaecological clinics. Further studies regarding the role of *Blastocystis* in extended series of pregnant women would be worthwhile.

* Ethics

Ethics Committee Approval: The study was approved by Muğla Sıtkı Koçman University Clinical Research Ethics Committee (07.11.2014).

Informed Consent: The informed consents were obtained from pregnant women in the present study.

Peer-review: Internally peer-reviewed.

*** Authorship Contributions**

Concept: E.M., F.S., H.E., Design: E.M., F.S., M.N.A., S.E., Data Collection or Processing: E.M., F.S., M.N.A., Analysis or Interpretation: E.M., F.S., M.N.A., H.E., Literature Search: E.M., S.E., Writing: E.M., H.E.

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