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Detection of *Blastocystis* spp. in patients with urticaria and identification of subtypes using sequencing techniques

Detección de *Blastocystis* spp. en pacientes con urticaria e identificación de subtipos mediante técnicas de secuenciación

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ABSTRACT

Blastocystis species are zoonotic protist commonly found in animals and humans. To date, 17 subtypes of *Blastocystis* have been identified, nine of which have been isolated from humans. This study aimed to determine the frequency of *Blastocystis* subtypes in patients diagnosed with urticaria and to explore the relationship between patient symptoms and *Blastocystis* subtypes. Stool samples from 100 urticaria patients and 100 healthy volunteers were analyzed for the presence of *Blastocystis* spp. using direct microscopic examination with the native–Lugol method and the subtypes were identified through PCR and sequencing techniques. A questionnaire was administered to the patient group to gather information on symptoms, socio–economic status, and hygiene practices. *Blastocystis* spp. was detected in 9% (9/100) of the urticaria patients and 5% (5/100) of the control group. The distribution of *Blastocystis* subtypes in the patient group was as follows: ST2 (n = 4, 44.4%), ST3 (n = 3, 33.3%), ST1 (n = 1, 11.1%), and ST4 (n = 1, 11.1%). In the control group, the distribution was ST3 (n = 2, 40%), ST1 (n = 2, 40%), and ST2 (n = 1, 20%). Regarding the relationship between symptoms and *Blastocystis* subtypes, 8 of 9 (88.9%) *Blastocystis*–positive patients reported rash, 7 (77.8%) experienced itching, 6 (66.7%) had fever, 3 (33.3%) experienced swelling, and 1 (11.1%) reported abdominal pain. Notably, bloating and abdominal pain were observed exclusively in patients with ST2. It is crucial to highlight the elevated prevalence of *Blastocystis* in areas where livestock farming is prevalent and the zoonotic cycle in the transmission of the parasite. While limited studies have suggested a correlation between *Blastocystis* subtypes and urticaria, the high prevalence of ST2 in urticaria patients may indicate its significant role in pathogenicity. The data derived from the patient questionnaire highlight a notable association between ST2 and symptoms such as bloating and abdominal pain, warranting further investigation.

Key words: *Blastocystis* spp., urticaria, sequencing, subtypes, zoonosis

RESUMEN

Las especies de *Blastocystis* son protistas zoonóticos comúnmente encontrados en animales y humanos. Hasta la fecha, se han identificado 17 subtipos de *Blastocystis*, de los cuales nueve han sido aislados de humanos. Este estudio tuvo como objetivo determinar la frecuencia de los subtipos de *Blastocystis* en pacientes diagnosticados con urticaria y explorar la relación entre los síntomas de los pacientes y los subtipos de *Blastocystis*. Se analizaron muestras fecales de 100 pacientes con urticaria y 100 voluntarios sanos en busca de la presencia de *Blastocystis spp.* mediante examen microscópico directo con el método nativo–Lugol, y los subtipos fueron identificados a través de técnicas de PCR y secuenciación. Se administró un cuestionario al grupo de pacientes para recopilar información sobre síntomas, estado socioeconómico y prácticas de higiene. *Blastocystis spp.* se detectó en el 9 % (9/100) de los pacientes con urticaria y en el 5 % (5/100) del grupo de control. La distribución de los subtipos de *Blastocystis* en el grupo de pacientes fue la siguiente: ST2 (n = 4, 44,4 %), ST3 (n = 3, 33,3 %), ST1 (n = 1, 11,1 %) y ST4 (n = 1, 11,1 %). En el grupo de control, la distribución fue ST3 (n = 2, 40 %), ST1 (n = 2, 40 %) y ST2 (n = 1, 20 %). En cuanto a la relación entre los síntomas y los subtipos de *Blastocystis*, 8 de 9 (88,9 %) pacientes positivos para *Blastocystis* informaron erupciones cutáneas, 7 (77,8 %) experimentaron picazón, 6 (66,7 %) tuvieron fiebre, 3 (33,3 %) presentaron hinchazón y 1 (11,1 %) reportó dolor abdominal. Es notable que la distensión abdominal y el dolor abdominal se observaron exclusivamente en pacientes con ST2. Es crucial destacar la elevada prevalencia de *Blastocystis* en áreas donde la cría de ganado es prevalente y el ciclo zoonótico en la transmisión del parásito. Aunque estudios limitados han sugerido una correlación entre los subtipos de *Blastocystis* y la urticaria, la alta prevalencia de ST2 en pacientes con urticaria puede indicar su papel significativo en la patogenicidad. Los datos derivados del cuestionario de los pacientes resaltan una notable asociación entre ST2 y síntomas como distensión abdominal y dolor abdominal, lo que justifica una investigación adicional.

Palabras clave: *Blastocystis spp.*, urticaria, secuenciación, subtipos, zoonosis

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INTRODUCTION

Blastocystis is a zoonotic protist that is commonly found in gastrointestinal tract of humans and other mammals, as well as birds, reptiles, fish and insects. Transmission occurs via the fecal–oral route or through direct contact with the microorganism's reservoir [1]. Although *Blastocystis* was identified long ago, its pathogenicity remains a subject of debate [2].

The distribution of *Blastocystis* varies not only between countries but also across different regions within the same country. The incidence and prevalence of this parasite is increasing due to factors such as low socio–economic status, inadequente infrastructure, consumption of contaminated water and food, and poor hygiene. This parasite has been found both in the human intestine and in vertebrate animals such as mice, rats, chickens, cattle, and pigs [3].

The subtypes of *Blastocystis* differ based on their reservoirs, geographical distribution. To date, nine subtypes (ST1-ST9) have been identified in humans with ST3 being the most common. Forty two different subtypes have been identified in total, and they may have varying effects on the host, ranging from harmful to potentially beneficial [4]. Studies have shown that different subtypes are dominant in different regions [5, 6]. Researches indicate that ST1–ST4 are more prevalent in humans compared to other hosts.

Blastocystis spp. isolated from various hosts are currently classified into 18S rRNA gene subtypes (STs) . ST1, ST2, ST3, and ST4 are frequently detected among humans. [7, 8, 9]. ST1, ST2, and ST4 have been implicated in gastrointestinal symptoms, and associated with Irritable Bowel Syndrome (IBS) [10]. Additionally, ST1 has been reported to have increasing pathogenicity [11].

This study aimed to detect *Blastocystis* spp. isolates in patients diagnosed with urticaria, determine the subtype distribution of these isolates through sequencing, evaluate the relationship between *Blastocystis* spp. and urticaria symptoms, and compare findings with those of a healthy control group.

Urticaria is a condition of unknown etiology, with protozoa and helminths recognized as potential causes [12]. The itchy lesions characteristic of urticaria result from histamine release in the skin, triggered by immune mechanisms. It has been reported that parasites can stimulate the secretion of immunological mediators such as IL-3, IL-4, IL-5, and IL-13 from Th2 cells in the intestinal lumen, leading to histamine release through the IgE response. Intestinal parasites also influence the development of tolerance to allergens by modulating the immune system [13].

MATERIALS AND METHODS

Research group and method:

This study included 100 patients who applied the Selcuk University hospital, Dermatology outpatient clinic between February 2019 and February 2020 with a diagnosis of urticaria, as well as 100 healthy volunteers. A questionnaire was administered to the patient group to gather information on complaints, socio–economic status, and hygiene practices.

Blastocystis spp. was considered positive if at least one microorganism was observed in microscope field at 400× magnification. Genomic DNA isolation was performed using a commercial kit (Norgen Biotek, Canada). *Blastocystis* isolates were analyzed using a PCR thermal cycler (Sensoquest Labcycler, Germany). PCR products were examined through 1.5% agarose gel electrophoresis followed by staining with ethidium bromide and imaging with the Gel Logic 200 Imaging System (Kodak, USA).

During the purification stage, single–band PCR products were purified using the HighPrep™ PCR Clean–up System (AC-60005) according to the manufacturer's instructions. Stool samples were processed using the ABI 3730XL Sanger sequencer and the BigDye Terminator v3.1 Cycle Sequencing Kit in the Macrogen laboratory (Applied Biosystems, Foster City, CA).

Ethical Consideration

Patients meeting the diagnostic criteria for urticaria were informed about the study's purpose, details, and procedures. Each participant signed an informed consent form. Ethical approval was obtained from the Local Ethics Committee of Selcuk University Faculty of Medicine (Date: 06.02.2019; Approval Number: 2019/04).

RESULTS AND DISCUSSION

A total of 200 stool specimens were included in the study. The patient group consisted of 36 males and 64 females, while the control group included 47 males and 53 females (TABLE I).

TABLE I
Age and gender distribution of the patient and control groups

Age	Patient Group		Control Group		Total	
	Female	Male	Female	Male	N	%
0-18	4	4	11	7	26	13
18-51	46	26	28	26	126	63
>50	14	6	16	12	48	24
Total	64	36	55	45	200	

Various forms of *Blastocystis* were observed, with the vacuolar form being the most common. Overall, *Blastocystis* spp. was detected in 14 (7%) of the 200 stool samples. Among the patient group, *Blastocystis* spp. was positive in 9 (9%) of 100 stool samples. Of these, 2 (2%) samples contained both *Entamoeba* spp. and *Blastocystis* spp., while 7 (7%) contained only *Blastocystis* spp.. No other parasites were found in the *Blastocystis* spp. negative stool samples. *Blastocystis* spp. was positive in 5 (5%) of 100 stool samples in the control group. Among these, 1 (1%) sample contained both *Entamoeba* spp. and *Blastocystis* spp., while 4 (4%) contained only *Blastocystis* spp. (TABLE II).

The ameboid form of *Blastocystis* spp. has been suggested as a potential contributor to pathogenicity [14]. A study conducted in Egypt found the ameboid form in 60.6% of *Blastocystis*–positive urticaria patients, whereas it was absent in healthy controls

TABLE II
Parasite distribution according to patient and control groups

Parasite Distribution	Patient Group N=100		Control Group N=100		Total N=200	
	N	%	N	%	N	%
Blastocystis spp.	7	7	4	4	11	5,5
Blastocystis spp. + *Entamoeba* spp.	2	2	1	1	3	1,5
Entamoeba spp.	–	–	2	2	2	1

by including 54 urticaria patients and 50 healthy controls with stool samples examined microscopically and evaluated via PCR. A significantly higher number of parasites were detected in the patient group compared to the control group (P<0.001), though no significant difference was observed between acute and chronic urticaria patients (P=0.2). Among the urticaria patients, 33 (61.1%) were *Blastocystis*–positive, compared to only 4 (8%) of the controls. The vacuolar and cyst forms were the predominant forms observed [15].

After direct microscopic examination, genomic DNA was isolated from the 14 *Blastocystis*–positive stool samples, and PCR targeting the SSU rRNA gene of *Blastocystis* was performed. Amplification was observed at the expected size (~119 bp). DNA sequencing was conducted bidirectionally, and the sequences were aligned with reference sequences. Chromatogram images were reviewed to correct any false nucleotide readings.

Four distinct *Blastocystis* subtypes were identified: ST1, ST2, ST3, and ST4. In the patient group, the subtype distribution was as follows: ST2 (n = 4, 44.4%), ST3 (n = 3, 33.3%), ST1 (n = 1, 11.1%), and ST4 (n = 1, 11.1%). In the control group, the subtype distribution was: ST3 (n = 2, 40%), ST1 (n = 2, 40%), and ST2 (n = 1, 20%) (TABLE III).

TABLE III
Distribution of *Blastocystis* subtypes

Subtype	Patient Group		Control Group	
	N	%	N	%
ST1	1	1	2	2
ST2	4	4	1	1
ST3	3	3	2	2
ST4	1	1	–	–
Total	9	9	5	5

According to the survey results, 87% (87/100) of urticaria patients reported living in the city center, while 13% (13/100) lived in rural areas. In terms of water usage, 11% (11/100) used bottled water, 28% (28/100) used tap water, and 61% (61/100) used both bottled and tap water. Antibiotic use was reported by 11% (11/100) of the patients, and 4% (4/100) mentioned owning pets. The symptoms of patients were distributed between acute and chronic urticaria groups, as detailed in TABLE IV.

TABLE IV
Distribution of patient complaints according to acute and chronic urticaria patient groups

Symptoms	Acute Urticaria N=36		Chronic Urticaria N=64		Total N=100	
	N	%	N	%	N	%
Itching	34	94,4	59	92,1	93	93
Redness	31	86,1	58	90,6	89	89
Blister	11	30,5	35	54,6	46	46
Fever	8	22,2	36	56,2	44	44
Swelling	9	11,1	31	48,4	40	40
Skin Rash	3	8,3	19	29,6	22	22
Abdominal Pain	1	2,7	8	12,5	9	9
Diarrhea	3	8,3	1	1,5	4	4
Nausea	1	2,7	3	4,6	4	4
Constipation	–	–	3	4,6	3	3
Indigestion	–	–	3	4,6	3	3
Womiting	1	2,7	1	1,5	2	2
Weight loss	–	–	–	–	–	–

In this study, the five most commonly reported symptoms in the patient group, itching, rash, swelling, fever, and bloating were analyzed. It is hypothesized that the symptoms of itching and rash may be associated with the subtype distribution of *Blastocystis*. Additionally, a notable relationship between ST2 and symptoms such as bloating and abdominal pain was observed. Several studies have investigated the clinical significance of *Blastocystis* spp. in relation to symptoms. In a study, 554 stool samples were examined to assess the prevalence of *Blastocystis* in symptomatic and asymptomatic groups. The prevalence rates were 16.08% (64/398) in asymptomatic patients and 18.58% (29/156) in symptomatic patients, with no significant association between symptoms and *Blastocystis* positivity (P=0.528). However, a statistically significant relationship was observed between urticaria and *Blastocystis* positivity (P<0.05). Among specific symptoms, the association rates were diarrhea (16.37%), constipation (16.94%), bloating (15.57%), nausea (14.28%), and urticaria (71.42%) [16].

The host specificity and pathogenic potential of *Blastocystis* isolates are influenced by sequence variations in the SSU rRNA gene [17]. The genetic heterogeneity among *Blastocystis* isolates suggests that different subtypes may have varying pathogenicity. It remains unclear whether *Blastocystis* directly contributes to allergic manifestations or is merely a common component of the gut microbiota. A study conducted in Brazil in 2019 analyzed the molecular diversity of *Blastocystis* spp. in urticaria patients. Using conventional methods and PCR, six subtypes (ST1, ST2, ST3, ST4, ST6, and mixed ST1+ST3) were identified. The most common subtypes were ST1 (a4), ST3 (a34 and a36), and ST4 (a42) [18].

The relationship between symptoms and subtypes of *Blastocystis* spp. positive patients was evaluated (TABLE V).

TABLE V
Patient group relationship between symptoms and subtypes of *Blastocystis* spp.

Symptoms	ST1	ST2	ST3	ST4	Total
	N=1	N=4	N=3	N=1	N=9
Redness	1	3	3	1	8
Itching	1	3	2	1	7
Fever	1	2	2	1	8
Blister	1	1	1	–	3
Swelling	–	2	–	–	2
Abdominal Pain	–	1	–	–	1

Although most individuals infected with *Blastocystis* are asymptomatic, recent research has proposed that *Blastocystis* may even serve as a marker of healthy gut flora [19, 20]. Clinical findings related to *Blastocystis* infection are thought to depend on various factors, including genotype, host immune response, parasite density, gut microbiota, and co–infections [21, 22].

Several studies have highlighted a strong correlation between *Blastocystis* positivity and urticaria, with some suggesting that the parasite may induce urticaria by activating specific immune mediators [23, 24, 25].

Recent studies investigating the zoonotic transmission potential of *Blastocystis* have revealed significant findings. For instance, a study conducted in Egypt reported *Blastocystis* positivity rates of 38% in 136 human fecal samples and 19% in 190 cattle fecal samples, highlighting cattle as a potential reservoir in the zoonotic cycle [26]. Similarly, research conducted in Iran detected *Blastocystis* positivity in 29.1% of fecal samples collected from 395 animals, including poultry, sheep, and cattle. Among the positive samples, cattle exhibited the highest prevalence (50.6%), followed by sheep (32.0%) and poultry (20.4%) [27]. These findings emphasize the importance of further exploring animal–to–human transmission pathways and the role of livestock in the epidemiology of *Blastocystis*.

It was observed that *Blastocystis* positivity was significantly higher among individuals living in rural areas (59.3%) compared to those in urban areas (40.7%). This discrepancy was attributed to factors such as direct contact with animals and inadequate sanitation in rural settings. In this study, the majority of patients reported living in the city center, while 13% resided in rural areas, potentially influencing the transmission dynamics of *Blastocystis*.

CONCLUSION

In this study, *Blastocystis* spp. isolates were detected in 9% (9/100) of the urticaria patient group and 5% (5/100) of the control group The relatively low prevalence compared to other studies may be attributed to the high socio–economic status and better hygiene practices in the study population. Among the nine subtypes identified in the urticaria patient group, ST2 was the most dominant (4/9), followed by ST3 (3/9), ST1 (1/9), and ST4 (1/9). In the control group, the subtypes detected were ST3 (2/5), ST1 (2/5), and ST2 (1/5). ST2 was notably more prevalent in the patient group.

Regarding the relationship between symptoms and subtypes, 8 of the 9 *Blastocystis*–positive patients exhibited rash, 7 experienced itching, 6 had fever, 3 reported swelling, and 1 had abdominal pain. Bloating and abdominal pain were observed exclusively in patients with ST2. Based on the analysis of the questionnaire data, it is concluded that there may be a significant association between ST2 and specific symptoms in urticaria patients.

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Conflict of interest statement

The authors have no conflict of interests.

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