

# Prevalence of *Blastocystis* in Patients Referred to Bushehr Medical Centers and Its Relationship with Urticaria

## Buşehr Tıp Merkezlerine Başvuran Hastalarda *Blastocystis* Prevalansı ve Ürtiker ile İlişkisi

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### ABSTRACT

**Objective:** Recent studies determined that the amoeboid form of *Blastocystis* acts as a factor in stimulating the host's immune responses and ultimately results in urticaria and other skin disorders. The present study was conducted in order to determine the prevalence of *Blastocystis* in people referred to Bushehr city health centers and the relationship of this parasite with urticaria.

**Methods:** Fecal samples were collected from 180 males and females referred to Bushehr health centers and a questionnaire containing demographic information was completed for each person. Samples were examined by preparing direct smear (wet mount) and then formalin-detergent sedimentation techniques. Data were analyzed using SPSS 22.0 software and chi-square test.

**Results:** The results showed that 11.1% of cases infected with *Blastocystis* and 55% of patients with *Blastocystis* had various gastrointestinal symptoms. Statistical analysis showed that there was no significant relationship between infection with some demographic factors such as sex, age, literacy level and residence, but this was significant with some clinical symptoms such as itching and urticaria.

**Conclusion:** Despite the existence of conflicting information and many ambiguities about the *Blastocystis*, this emerging pathogen is very important in terms of causing allergic and skin disorders in sufferers, therefore, it is necessary that patients with urticaria be evaluated for *Blastocystis* along with other diagnostic procedures and physicians should request a test before any medical intervention. Thus, diagnosis and treatment of these people can play an important role in improving the health of society.

**Keywords:** Prevalence, *Blastocystis*, urticaria, Bushehr, Iran

### ÖZ

**Amaç:** Son araştırmalar *Blastocystis*'in ameboid formunun konağın bağışıklık yanıtlarını uyaran bir faktör olarak hareket ettiğini ve sonuçta ürtiker ve diğer deri bozukluklarına yol açtığını göstermiştir. Bu çalışma Bushehr şehir sağlık merkezlerine başvuran kişilerde *Blastocystis* prevalansını ve bu parazitin ürtiker ile ilişkisini belirlemek amacıyla yapılmıştır.

**Yöntemler:** Bushehr sağlık merkezlerine başvuran 180 erkek ve kadından dışkı örnekleri toplandı ve her kişiye demografik bilgileri içeren bir anket dolduruldu. Örnekler direkt smear (wet mount) hazırlanarak ve ardından formol eter çöktürme teknikleri kullanılarak incelendi. Veriler SPSS 22.0 programı ve ki-kare testi kullanılarak analiz edildi.

**Bulgular:** Bulgular olguların %11,1'inin *Blastocystis* ile enfekte olduğunu ve *Blastocystis* ile enfekte olan hastaların %55'inde çeşitli gastrointestinal semptomların bulunduğunu gösterdi. İstatistiksel analiz, enfeksiyon ile cinsiyet, yaş, okuryazarlık düzeyi ve yerleşim yeri gibi bazı demografik faktörler arasında anlamlı ilişki olmadığını, ancak enfeksiyon ile kaşıntı ve ürtiker gibi bazı klinik semptomlar arasında anlamlı ilişki olduğunu gösterdi.

**Sonuç:** *Blastocystis* hakkında çelişkili bilgiler ve birçok belirsizlik bulunmasına rağmen bu patojen, alerjik deri bozukluklarına neden olması açısından oldukça önemlidir, bu nedenle ürtikerli hastaların diğer tanı işlemleriyle birlikte *Blastocystis* açısından da



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değerlendirilmesi ve hekimlerin herhangi bir tıbbi müdahale öncesinde test istemesi gerekmektedir. Dolayısıyla bu kişilerin tam ve tedavisi toplum sağlığının iyileştirilmesinde önemli bir rol oynayabilir.

**Anahtar Kelimeler:** Prevalans, *Blastocystis*, ürtiker, Bushehr, İran

## INTRODUCTION

*Blastocystis* is one of the most common zoonotic protozoan parasites that can be found anaerobically in the digestive system of humans and many vertebrates, including mammals, birds, amphibians, reptiles and fish, and even in invertebrates such as insects (1).

This protozoan was first described by Alexieff in 1912 as a fungus, but years later, Zierdh placed this microorganism in the amoeba order of protozoa according to its phenotypic and morphological characteristics. Finally, in 1996, Siberman named this protozoan as a member of the highly diverse group of Stramenopiles, citing phylogenetic studies of ribosomal RNA (2).

For many years, *Blastocystis* was considered a saprophytic and non-pathogenic microorganism, so that even with the diagnosis of positive cases and laboratory reports, no action was taken to treat the infected patients by physicians. But recently, due to new studies and findings about the physiological properties and pathogenesis mechanisms of this parasite, the importance of diagnosing the infection and treating the patients has increased (3-5). In many reports, *Blastocystis* has been mentioned as an emerging pathogen or a mysterious organism (2).

This parasite is a polymorphic protozoan and has different forms such as vacuolar, granular, amoeboid, and cystic transfer form, which is directly transmitted to different hosts through contaminated water and food (6,7).

The prevalence of infection in the world is variable and this rate is different in different countries and even in different societies (0.8-61.8%). In general, countries located in tropical regions have a higher prevalence than other regions. Factors affecting the prevalence of this parasitic infection include poor health in communities, close contact with animals, and seasonal weather changes. Some studies have shown that in some seasons, the prevalence of infection increases and unlike many intestinal parasites, its prevalence is higher in adults than children (3,8,9). In a research conducted for the first time in Lorestan province, Iran, the rate of infection was reported to be about 6.5% (10). In a similar study in Mazandaran province, this amount was found to be about 14.3% (11).

The main method of diagnosing this parasite is stool exam by preparing a wet smear and direct examination under a microscope. Other complementary methods such as permanent staining, culture or molecular polymerase chain reaction test can also be used for this purpose.

The disease caused by this protozoan includes a wide range of non-specific gastrointestinal disorders which is known as *Blastocystis*. Symptoms such as diarrhea, abdominal pain, bloating, nausea, vomiting, constipation, and weight loss can appear in patients with varying severity from acute to chronic. There are even hypotheses about the relationship between this parasite and colorectal cancer (12). On the other hand, it has been found that in people with irritable bowel syndrome, the prevalence of *Blastocystis* is significantly higher than in people without this syndrome (13,14).

Also, the recent studies have introduced the amoeboid form of this protozoan as a factor in stimulating the host's immune responses through binding to the intestinal epithelium and affecting its homeostasis. Correlation between *Blastocystis* infection and urticaria has been repeatedly reported in various studies (15-19). Even some researchers have considered the role of different subtypes and alleles of *Blastocystis* in the development of chronic urticaria symptoms.

In the study conducted by Aykur et al. (20) in Türkiye, stool samples were collected from patients with chronic spontaneous urticaria and healthy individuals as control group and investigated using different conventional and molecular methods. The results revealed a relationship between chronic spontaneous urticaria and *Blastocystis* subtypes as well as a significant difference in total IgE levels between the *Blastocystis* ST2 and ST3 positive group and the negative group (20).

Based on this, and in terms of the high importance of this disease, as well as insufficient information and many uncertainties about this emerging pathogen, the present study was designed to determine the prevalence of *Blastocystis* infection in people referred to health centers in Bushehr city, Iran, in order to take positive steps towards solving the existing ambiguities about pathogenicity and infection control by investigating the relationship between infection with this protozoan and skin urticaria and various epidemiological factors and clinical symptoms.

## METHODS

### Ethical Approval and Informed Consent

This study was approved in Ethical Committee of Bushehr University of Medical Sciences with ethics code: IR.BPUMS.REC.1400.145. Informed consent was obtained from the patients and in the case of minors, from their parents and confidentiality of the information was guaranteed.

### Study Area

This study was carried out in Bushehr city, located in the northwest margin of the Gulf and southwest of Iran. The population of the



**Figure 1.** Geographical location of Bushehr city on the map of Iran

city is close to three hundred thousand people and this area has hot and humid climate most of the year (Figure 1).

### Sample Collection

In this descriptive-analytical study, samples were taken between December 2021 and June 2022, from 180 patients referred to Bushehr medical centers with various gastrointestinal symptoms as well as those suffering from skin disorders. A questionnaire containing demographic information and variables related to *Blastocystis* infection was completed for each patient.

### Stool Examination

The collected samples were first examined by direct microscopic examination using the wet mount method, and in the next step, in order to increase the sensitivity of the test, were evaluated using the modified formalin-detergent concentration method. The detergent used in this research was a liquid with the formula of dodecyl benzene, coconut diethanolamide, and antibacterial, viscosifying agent, essential oil, urea and dye.

Commercial kits (Parasite Test, KaraTeb Nur, Iran) were used for this purpose. Briefly; first, 3.5 mL of working solution (containing formalin 10% and detergent) was added to the cylindrical part of the kit, and then, with the spoon of the brain part, about 2 grams of stool sample is taken and entered into the cylindrical tube. After completely closing the two parts, the tubes were vortexed for 30 seconds until the samples were completely suspended. The next step was the incubation of the tests for 24 hours at room temperature. Then, the tubes were inverted and centrifuged at 1000 rpm for 1 minute. Finally, the two parts were slowly separated and the sediment remaining in the conical part was transferred to the slide with Lugol and examined under the light microscope.

### Statistical Analysis

The results of the tests along with the questionnaire data were analyzed using the statistical software SPSS (Chicago, IL version 22, SPSS Inc.) and the chi-squared test.

## RESULTS

In this study, out of a total of 180 examined patients, 20 samples (11.1%) had blastocystosis. Table 1 shows the prevalence of *Blastocystis* infection by sex and as it can be seen, there is no significant relationship between *Blastocystis* infection and the gender of the examined patients (Table 1).

The results of the present study showed that the highest rate of infection is in the age group of 21-40 years and the lowest rate is in the group under 20 years, which in fact, considering that the most and least examined patients were in these age groups respectively, this difference was not statistically significant (p-value 0.208) (Table 2).

**Table 1.** Distribution of *Blastocystis* in patients referred to Bushehr medical centers by sex

Sex	Pos		Neg		p-value
	n	%	n	%	
Male	8	4.4	47	26.1	
Female	12	6.7	113	62.8	0.234
<b>Total</b>	<b>20</b>	<b>11.1</b>	<b>160</b>	<b>88.9</b>	

Despite the fact that the majority of cases infected with *Blastocystis* were village residents (10.5%) and only 0.6% of them lived in the city, statistical analysis showed that there is no significant relationship between the place of residence and *Blastocystis* infection (Table 3).

Out of all 20 patients with blastocystosis, 11 patients (55%) had various gastrointestinal symptoms such as anorexia, nausea, vomiting, abdominal pain and diarrhea, which is very significant, but as can be seen from Table 3, in the separate analysis of each of these variables, there was no significant difference between the prevalence rate and clinical symptoms (Table 3).

Other variables analyzed in this study were respiratory symptoms, skin itching and acute or chronic urticaria. Statistical

**Table 2.** Distribution of *Blastocystis* in patients referred to Bushehr medical centers by age

Age group	Pos		Neg		p-value
	n	%	n	%	
<20 y	0	0	11	6.1	
21-40 y	17	9.4	112	62.2	
41-60 y	3	1.7	32	17.8	0.208
>60 y	0	0	5	2.8	
<b>Total</b>	<b>20</b>	<b>11.1</b>	<b>160</b>	<b>88.9</b>	

**Table 3.** Distribution of *Blastocystis* in patients referred to Bushehr medical centers by some effective factors

Variables	Results				p-value
	Pos		Neg		
	%	n	%	n	
<b>Residence</b>					
Rural	10.5	19	77.8	140	0.288
Urban	0.6	1	11.1	20	
<b>Abdominal pain</b>					
Yes	2.8	5	28.3	51	0.365
No	8.3	15	60.6	109	
<b>Anorexia</b>					
Yes	3.3	6	32.8	59	0.367
No	7.8	14	56.1	101	
<b>Diarrhea</b>					
Yes	3.3	6	37.2	67	0.220
No	7.8	14	51.7	93	
<b>Nausea and vomiting</b>					
Yes	3.3	6	19.4	35	0.287
No	7.8	14	69.4	125	
<b>Respiratory symptoms</b>					
Yes	3.9	7	28.9	52	0.502
No	7.2	13	60	108	
<b>Skin itching</b>					
Yes	5.6	10	22.8	41	0.025
No	5.6	10	66.1	119	
<b>Acute urticaria</b>					
Yes	3.9	7	10	18	0.010
No	7.2	13	78.9	142	
<b>Chronic urticaria</b>					
Yes	2.2	4	7.8	14	0.121
No	8.9	16	81.1	146	

analysis showed that there is a significant relationship between *Blastocystis* infection and these variables (Table 3).

## DISCUSSION

The ability of *Blastocystis* to cause various gastrointestinal disorders as well as skin disorders is still being discussed by researchers, although recent clinical and epidemiological studies emphasize the pathogenicity of this parasite (3-5). Some researchers also believe that eradication of *Blastocystis* is not necessary and it is essential just in some cases where it is the only infectious agent and patients show significant symptoms (21).

A wide range of non-specific gastrointestinal disorders, such as diarrhea, abdominal pain, bloating, nausea, vomiting, constipation, and weight loss can appear in patients with varying severity from acute to chronic, are known as blastocystosis. There are even hypotheses about the relationship between this parasite and colorectal cancer (12).

Despite the fact that limited studies have reported the role of *Blastocystis* in the occurrence of acute urticaria, many other studies have mentioned this important role in the development of chronic urticaria symptoms (16). It is assumed that the amoeboid form of ST1, ST2 and ST3 subtypes of the parasite acts as a factor in stimulating the host's immune responses through binding to the intestinal epithelium and affecting its homeostasis, and finally leads to the secretion of mediators such as histamine which can cause allergic reactions and chronic urticaria in sufferers (15,18,22-26).

In the present study conducted in Bushehr clinical centers in order to determine the rate of *Blastocystis* infection and also to investigate the relationship between *Blastocystis* with various epidemiological factors and various clinical symptoms such as urticaria, a prevalence of 11.1% was obtained, which is significant. Other studies conducted in Iran and other parts of the world have announced different results. In a study in Tabriz, Iran, 558 stool samples were collected from those who referred to medical centers and tested by direct microscopic methods, formal ethyl acetate concentration and trichrome staining. The results indicated a 26.17% prevalence of *Blastocystis* and the most common clinical symptoms were reported as abdominal pain, anorexia and nausea. The *Blastocystis* of the mentioned study have shown that *Blastocystis* is one of the most common intestinal parasite, which if neglected, it can cause many problems for patients (27).

During the years 2010 to 2014, Rahimi et al. (28) analyzed the reports of parasitic infections of 70,978 people who referred to the Baqiyatullah Hospital in Tehran and among the detected parasites, *Blastocystis* with a prevalence of 2.49% had the highest rank. In a similar study by Memar et al. (29), which was conducted on healthy people and AIDS patients, they reported a higher prevalence of *Blastocystis* than other intestinal parasites in both investigated groups.

In analyzing the results of the present study and investigating the correlation of some variables involved, there was no significant relationship with the gender, age, place of residence and education level of the patients. These findings are contrary to the reports of some studies conducted in this field; Shaker et al. (11) found a significant relationship in their study between *Blastocystis* and the variables of age, gender, educational level, place of residence and history of contact with animals, but

with some other evaluated factors such as consumable water and symptoms such as diarrhea, bloating and weight loss, this relationship was not significant (11). In the present study, although 55% of patients had various gastrointestinal symptoms such as anorexia, nausea, vomiting, abdominal pain and diarrhea, but in the separate analysis of each of these variables, there was no significant difference between the prevalence rate and clinical symptoms, which is consistent with the results of the study conducted by Shaker et al. (11).

Another valuable finding of this study was the existence of a significant relationship between *Blastocystis* and some allergic symptoms such as skin itching and acute urticaria. Most of the researches conducted in this field have reported the correlation between *Blastocystis* infection and urticaria not completely confirmed, but ambiguously and possibly (18,19,30,31).

In a study that aimed to evaluate the presence of clinical symptoms and skin manifestations in 80 patients with a positive *Blastocystis* test, 11.25% of them showed the relationship between the infection and skin disorders, although these symptoms were mainly reported in women more than men. Also, 73.75% of the examined people had various gastrointestinal disorders. Examination of routine hematological and biochemical tests has shown an increase in C-reactive protein levels in infected patients, but the amount of blood eosinophils was within the normal range. Finally, researchers have recommended that stool exam be done routinely in patients with skin disorders of unknown cause (14).

## CONCLUSION

According to the results obtained and the high importance of this emerging pathogen in terms of causing allergic and gastrointestinal disorders, it is necessary that patients with urticaria be evaluated for blastocystosis along with other diagnostic procedures and physicians should request a test before any medical intervention. Thus, diagnosis and treatment of these people can play an important role in improving the health of society.

Also, considering that the severity and type of pathogenicity of this parasite apart from the factors related to the host as well as the number of parasites, has been attributed to different subtypes of the parasite, therefore, in order to respond to many contradictions in this field, it is recommended to carry out additional studies of molecular epidemiology in the region and the dominant subtypes on human and livestock samples be identified relying on advanced molecular techniques.

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### \* Ethics

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### \* Authorship Contributions

Concept: M.F., M.R., A.B., Design: M.F., M.R., A.B., Data Collection or Processing: M.G., H.M., B.A., Analysis or Interpretation: M.G., H.M., B.A., Writing: M.G., M.F., H.M., M.R., B.A., N.S., A.S., N.K.G., A.B.

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