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**CLINICAL-EPIDEMIOLOGICAL, IMMUNOLOGICAL, MICROBIOLOGICAL ASPECTS
AND PREVALENCE OF BLASTOCYSTOSIS AMONG THE POPULATION
OF THE REPUBLIC OF AZERBAIJAN**

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Blastocystosis is one of the least studied infectious diseases among intestinal protozoans in the Republic of Azerbaijan; only several studies have been conducted in this field, dating back 30–35 years. The attitude towards blastocystosis worldwide, including in the Republic of Azerbaijan, has not been unambiguous. We have discovered epidemiological features and regional characteristics. As a result, three subtypes of *Blastocystis hominis* have been identified – ST3, ST2, and ST4. For example, ST3 was found the most, followed by ST2, and relatively less ST4. Therefore, it is essential to study the prevalence of *B.hominis* among populations in different regions and countries and to study the role in human pathologies, its pathogenesis, and clinical course caused by different species and genotypes, which lead to the effectiveness of treatment.

Key words: blastocystosis, genotypes, morbidity, opportunistic infection, cytokines, intestinal microbiota, conditionally pathogenic microflora, diarrhea

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**КЛІНІКО-ЕПІДЕМІОЛОГІЧНІ, ІМУНОЛОГІЧНІ, МІКРОБІОЛОГІЧНІ АСПЕКТИ
І ПОШИРЕНІСТЬ БЛАСТОЦИСТОЗУ СЕРЕД НАСЕЛЕННЯ РЕСПУБЛІКИ
АЗЕРБАЙДЖАН**

Бластоцистоз є одним з найменш вивчених інфекційних захворювань серед найпростіших кишкових в Азербайджанській Республіці, в цій галузі проведено лише кілька досліджень, датованих 30–35 роками тому. Ставлення до бластоцистозу у світі, зокрема й у Азербайджанській Республіці, було однозначним. Ми знайшли такі епідеміологічні особливості, як регіональні особливості. В результаті було ідентифіковано три підтипи *Blastocystis hominis* – ST3, ST2 та ST4. Наприклад, найбільше було виявлено ST3, далі ST2 і дещо менше ST4. У зв'язку з цим, актуальним є вивчення поширеності *B.hominis* серед популяцій у різних регіонах та країнах, а також вивчення ролі у патології людини, її патогенезу та клінічного перебігу, зумовлених різними видами та генотипами, що зумовлюють ефективність лікування.

Ключові слова: бластоцистоз, генотипи, захворюваність, опортуністична інфекція, цитокіни, мікробіота кишечника, умовно-патогенна мікрофлора, діарея.

Blastocystis is a genus of unicellular protozoan parasite belonging to *Blastocystae* class and order of *Blastocystida* and *Blastocystidae* family. It is estimated that as many as 1 billion people worldwide are infected with *Blastocystis* [11]. The parasite comprises several species, living in the gastrointestinal tracts of humans and animals including farm animals, birds, rodents, reptiles, amphibians, fish and cockroaches [7, 9]. Pathology of the gastrointestinal tract has a great impact on growing body and such infections as *Blastocystis hominis* (*B.hominis*) leads not only disruption of normal intestinal microflora, but also can be a cause of complications in liver and pancreas [6, 8].

Blastocystosis is a relatively underexplored infectious disease among intestinal protozoans in the Republic of Azerbaijan. Only a handful of research studies have been conducted in this area, and they date back to approximately 30–35 years ago. The global and local perspectives on blastocystosis, including within the Republic of Azerbaijan, have been somewhat unclear and inconsistent. Therefore, it is imperative to conduct studies on the prevalence of *B.hominis* among various populations in different regions and countries. Furthermore, it is crucial to investigate its role in human pathologies, understand its pathogenesis, and examine the clinical course associated with different species and genotypes, as this knowledge can significantly impact the efficacy of treatment.

The purpose of the study was to evaluate the epidemiological and clinical features of different species and genotypes of *Blastocystis hominis* in Azerbaijan.

Materials and methods. The study was performed according to scientific plan of Department of Infectious diseases of Azerbaijan Medical University. In total 8002 people were examined randomly to assess the prevalence of blastocystosis among population in the different regions of Azerbaijan. All people included in the study were analyzed according to place of residence (cities or countryside) and age groups: 12–17-years-old, 18–40-years-old, 50–61 years-old and >61 years-old.

The detection of blastocystosis was performed in faeces using microscopic stool analysis, types were identified by genotyping method.

To assess the features of blastocystosis course the clinical symptoms, as well as cytokines (measured in the serum by ELISA method), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the condition of intestinal microflora were also examined.

For evaluating the numeral data descriptive statistical methods (mean, standard deviation, frequency, percentage) were used. A Mann-Whitney U-test was used for comparisons between two groups of non-normally distributed quantitative variables and a Student t-test – for comparisons between two groups of normally distributed data. Statistical significance was accepted as $P < 0.05$.

Results of the study and their discussion. *B. hominis* occurred in 2079 of 8002 people (25.98 ± 0.49 %). According to prevalence among every age group, blastocystosis was the most common in 12–17-year-old children (31.58 ± 1.63 %), 18–40-year-old adults (33.86 ± 1.10 %), and the least common in elderly people, 61 years and older (14.21 ± 2.63 %; $P < 0.001$).

Notably, Blastocystosis as diagnosis was significantly detected more among rural (31.88 ± 1.17 %) than urban residents (24.52 ± 0.54 %; $P < 0.001$). Nevertheless, on average the morbidity of blastocystosis according to the geographic area occurred almost at the same level in the whole country (Kur intermountain region – 24.72 ± 1.44 %, Lankaran region – 27.4 ± 3.69 %), except Araz province (5.41 ± 3.72 %).

In addition, considering the variety of types and genotypes of *B. hominis*, led us to investigate the genotypes characterization of this parasite among residents of Azerbaijan.

As a result, 66 human fecal samples detected with *B. hominis* were examined by PCR to identify genotypes. ST3 subtype was found in 39 cases (59.1 ± 6.1 %), ST2 subtype – 16 (24.2 ± 5.3 %) and ST4 subtype – 11 (16.7 ± 4.6 %).

Markedly, only the ST3 subtype in comparison with others is more common in women than men (62.0 ± 7.7 % and 55.0 ± 8.0 %, respectively).

As a matter of fact, the attitude towards the role of blastocystosis in human pathologies is ambiguous, some researchers have shown that the course of infection mostly passes without any clinical symptoms in humans.

In that case, it makes to be difficult for diagnostics and treatment in time. Therefore, it is vital to clarify specific clinical signs of infection.

For this reason, clinical signs before and after treatment were compared in 198 patients with blastocystosis.

For example, observations have shown that there was abdominal pain before treatment, approximately 40.93 ± 3.54 %, and after treatment – 12.44 ± 2.37 % ($P < 0.001$).

In particular, nausea was detected in 38.34 ± 3.50 % of patients before treatment and 11.40 ± 2.29 % – after treatment ($P < 0.001$). In the same way, diarrhea was observed in 35.23 ± 3.44 % of cases before treatment and it was decreased after treatment – 10.88 ± 3.24 % ($P < 0.001$). Such symptoms as strong fecal odor (47.15 ± 3.59 %), skin rashes (44.04 ± 3.57 %), allergy symptoms (41.45 ± 3.55 %), hypersalivation (35.23 ± 3.44 %), unpleasant breath (31.51 ± 3.35 %) were revealed among patients before treatment and these symptoms were significantly reduced after treatment (21.24 ± 2.94 %, 19.69 ± 2.86 %, 15.54 ± 2.61 %, 8.29 ± 1.98 % and 12.95 ± 2.42 % respectively).

On the other hand, those indicated symptoms are not pathognomic, consequently, blastocystosis should not be overlooked by a doctor while observing at least one of these clinical signs.

Likewise, the level of liver enzymes was examined to detect how blastocystosis affects on liver function. As it was shown that only 12 out of 45 patients with blastocystosis (26.7 ± 1.24 %) have had a higher level of ALT. Although the level of AST was not elevated as much as ALT among examined practically healthy individuals and patients with blastocystosis. In the same manner, the level of amylase was increased in 5 patients (8.9 ± 0.6 %). As can be seen, blastocystosis has a negative effect on the liver and pancreas altogether.

It is known that blastocystosis is related to opportunistic infection hence such factors as the state of the immune system and its components affect on occurrence, clinical course, pathogenesis of disease, and the effectiveness of treatment. It was equally important to examine the amount of cytokines IL4 and IL8 in the blood, which showed that the level of IL4 did not exceed the norm among 83 patients with blastocystosis. On the contrary, it was demonstrated that IL8 was higher than normal in 75.9 % of cases. All things considered, it would be recommended to study other cytokines and indicators of immune system in blastocystosis as well.

Moreover, the course of blastocystosis can lead to changes of intestinal microbiota which is very essential. However, this issue has not been adequately studied before in Azerbaijan. For this reason, we have examined the intestinal microflora of 117 patients with blastocystosis and 46 practically healthy individuals. As a result, this study has demonstrated that the normal microflora of the intestine was reduced

in 35.9±4.44 % of patients with blastocystosis and in 10.9±4.6 % of practically healthy individuals (P<0.001). In comparison with practically healthy individuals pathogenic and conditionally pathogenic microflora were found in patients with blastocystosis much more (8.7±4.16 %, P<0.001 and 47.0±4.61 %, respectively).

In blastocystosis (82.91±3.48 %) compared to practically healthy individuals (19.57±5.85 %, P<0.001), dysbacteriosis is also detected significantly more.

Interestingly, detection of *Candida* fungi was five times more frequent among people with blastocystosis (22.22±3.84 %), *Staphylococcus aureus* – by three times, Hemolytic *E.coli* (23.08±3.9 %), lactose negative *E.coli* (19.66±3.67 %) were four-five times more detectable than among practically healthy individuals.

Whereas *Proteus*, *Klebsiella*, *Ps.Aeruginosa* were not detected among practically healthy individuals, they were only found in patients with blastocystosis (5.98±2.19 %; 3.42±1.68 %; 6.84±2.33 %, respectively).

In order to clarify the impact of blastocystosis on the intestinal microflora, it was also examined after etiological treatment.

Before treatment, reduction of normal microflora was observed in 35.9±4.44 % of patients with blastocystosis and after treatment – 9.40±2.7 % (P<0.001). Pathogenic and conditionally-pathogenic microflora also occurred significantly less (47.0±4.61 % and 10.26±2.81 %, respectively, P<0.001) after treatment.

The conducted study has shown quantitative and qualitative changes in the normal intestinal microflora, which can negatively affect on the clinical course, pathogenesis, and effectiveness of treatment of blastocystosis among infected patients.

In some studies of other researchers the similar results were showed. So, Seyer A, et al (2017) with the purpose to investigate the prevalence of *Blastocystis* and its subtypes (STs) in North Cyprus studied the stool samples from 230 volunteers. They assessed the prevalence according to demographic, socioeconomic, and epidemiological factors, as well as gastrointestinal symptoms. Prevalence of *Blastocystis* infection in North Cyprus was found to be 10.5 % by direct microscopy (25.98±0.49 % in our study). But the authors from Cyprus additionally detected *Blastocystis* by polymerase chain reaction and revealed them as 27.8 %, that is close to our results. The researchers noted that presence of *Blastocystis* was not significantly related to any of the demographic, socioeconomic, and epidemiological factors, as well as gastrointestinal symptoms among symptomatic and asymptomatic individuals [10]. We also found out that clinical symptoms observed in participants were not pathognomic.

In common cases infection in humans is mild and self-limiting. It is considered that up to 50 % of infected people may be asymptomatic carriers for months or years [2, 4]. On the other hand, many people with *Blastocystis*-only invasion report symptoms, such as abdominal pain, lack of appetite, nausea, loose stools, flatulence, constipation, etc.) [1, 3]. In our study the gastrointestinal symptoms were partially associated with blastocystosis and changed with treatment. Thus, blastocystosis should not be ignored by medical professionals if at least one of these clinical signs is observed.

Rudzińska M, et al (2023) studying the prevalence of *Blastocystis* in Poland revealed that in humans, the prevalence ranged between 0.14 and 23.6 %. The noted about seven subtypes which were identified in humans (ST1-ST4, ST6, ST7, and ST9), and as in our work, ST3 was the most common [7]. But in contrast with our study the authors evaluated the prevalence of *Blastocystis* also among animals (wild, livestock, and pet animals), and concluded about the possibility of *Blastocystis* transmission between animals and humans.

It is known that changes in microorganism stains in gastrointestinal tract mucosa can cause opportunistic infections under certain conditions [5]. So, this conception is supported by the quantitative and qualitative changes in the normal intestinal microflora obtained by us, which can negatively affect the course of blastocystosis.

Conclusions

1. Blastocystosis is very widespread among the population in the Republic of Azerbaijan. This infection is mainly registered among young people and people of working age.

2. Three subtypes of *Blastocystis hominis* have been identified – ST3, ST2, and ST4. ST3 was found the most, followed by ST2, and relatively less ST4.

3. The level of IL8 was higher than normal in 75.9 % of cases.

To summarise, to fully assess the role of this disease in human pathologies in Azerbaijan, it is essential to continue studying this field more deeply.

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THE IMPACT OF ABLATIVE LASER THERAPY ON VARIOUS TYPES OF SKIN AGING

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The purpose of the study was to evaluate changes in facial skin during the use of an ablative fractional laser depending on the morphotype of ageing. 72 patients with involutive skin changes were involved. Group I included 29 patients with a deformative morphotype, Group II included 20 patients with a finely wrinkled morphotype, and Group III – 23 patients with a mixed morphotype. The ablative fractional photothermolysis procedure was carried out using a CO₂ laser. To assess the results visual analogue scale and FACE-Q Satisfaction with Skin were used. After therapy, the total score on the FACE-Q Satisfaction With Skin in Group I increased by 42.5 %, in Group II – increased by 40.7 %, in Group III – increased by 41.7 %. After 6 months in the Group I the percentage of patients with preserved effect was 93.1 %, in Group II – 95 %, in Group III – 95.6 %, after 12 months – 86.2 %, 85 % and 87 %, respectively. Thus, fractional laser has high effectiveness in therapy for all types of aging.

Key words: morphotype of aging, fractional photothermolysis, dermatological scale, wrinkles

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ВПЛИВ АБЛЯЦІЙНОЇ ЛАЗЕРНОЇ ТЕРАПІЇ НА РІЗНІ ТИПИ СТАРІННЯ ШКІРИ

Метою дослідження було оцінити зміни шкіри обличчя під час використання абляційного фракційного лазера залежно від морфотипу старіння. У дослідженні взяли участь 72 пацієнти з інволютивними змінами шкіри. До I групи увійшли 29 пацієнтів з деформаційним морфотипом, до II групи – 20 пацієнтів з дрібноморщинистим морфотипом, до III групи – 23 пацієнти зі змішаним морфотипом. Процедура абляційного фракційного фототермолізу проводили з використанням CO₂-лазера. Для оцінки результатів використовували візуальну аналогову шкалу та FACE-Q Satisfaction With Skin. Після терапії загальний бал FACE-Q Satisfaction With Skin у I групі збільшився на 42,5 %, у II групі – на 40,7 %, у III групі – збільшився на 41,7 %. Через 6 міс у I групі відсоток хворих із збереженим ефектом становив 93,1 %, у II групі – 95 %, у III групі – 95,6 %, через 12 міс – 86,2 %, 85 % та 87 % відповідно. Таким чином, фракційний лазер має високу ефективність в терапії всіх типів старіння.

Ключові слова: морфотип старіння, фракційний фототермоліз, дерматологічна шкала, зморшки.

Skin aging is a complex biological process that occurs under external and internal factors. External factors, first of all, mean various factors that affect the skin from the outside, including the harmful effects of sunlight. In addition, factors such as cigarette smoke, alcohol, physical inactivity, poor diet, hormonal dysfunction, etc. play an important role in the occurrence of photoaging Internal factors are associated with