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Original Article

Molecular phylogenetic identification of *Blastocystis* sp. isolated from humans in the Northwest of Iran

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Abstract

***Background & Aims*:** *Blastocystis* sp. is a common parasite of humans with a vast variety of nonhuman hosts and global distribution. Based on molecular methods, distinct subtypes are isolated from different hosts. In this study, we detected human-isolated *Blastocystis* subtypes in the northwestern of Iran.

***Materials & Methods*:** A total of 600 stool samples referred to health centers in Urmia and Maragheh cities in the northwest of Iran was randomly collected and examined using direct wet mount, formalin-ether concentration, a modified version of the Ziehl–Neelsen staining technique for the detection of parasitological items and using barcoding method (18S rRNA gene) for the molecular survey.

***Results*:** From 600 samples studied, 496 (82.7%) and 104 (17.3%) were male and female, respectively. Three subtypes, including ST_1, ST_2 and ST_3, were identified from collected samples, and the frequent subtypes were ST_3, ST_1, and ST_2, respectively.

***Conclusion*:** While genetic similarity of *Blastocystis* isolated from human and animal did not show in this region, the zoonotic cycle of this parasite, probably by fecal contamination, exists in the study area. By identifying *Blastocystis* subtypes, it is possible to prevent the transmission and related complications.

***Keywords*:** *Blastocystis* sp., 18S rRNA, Northwestern of Iran

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Introduction

Blastocystis, an anaerobic protozoan, lives in the human gastrointestinal tract and many mammals, birds, amphibians, and insects and has a worldwide distribution (1). Epidemiological studies of *Blastocystis* have been carried out to understand its relationship with humans and gastric disorders, with little focus on other hosts of this parasite (2, 3). The prevalence of *Blastocystis* often varies based on geographical region and method of detection (4).

Some studies have suggested that the pathogenicity of *Blastocystis* could lead to traveler's diarrhea, but some other did not report such observation (5, 6). In human disease, *Blastocystis* is found in both symptomatic and asymptomatic individuals, meaning that it may cause clinical manifestations (7). In this regard, many studies have been conducted on the signs and symptoms of this protozoan (8-11).

Molecular surveys have been carried out in several countries to elucidate the genetic diversity of *Blastocystis*. The variety of *Blastocystis* STs depends on different types of hosts, climate, breeding, ecology and mode of transmission (6).

The genetic diversity of the genus *Blastocystis* sp. and its several distinct ribosomal lineages known as subtypes (STs) have already been described. Molecular methods based on variations of small subunit ribosomal RNA (SSU rRNA) gene have been developed, and accordingly, 17 subtypes have been detected in different hosts, including mammals (12). ST_1-ST_9 have been identified in humans, with ST_1-ST_4 being the most common (13).

To our knowledge, to date, few studies have been conducted in Iran to illuminate the distribution of subtypes of this mysterious parasite with various methodologies in humans (6-8, 10, 14-16). Apparently, knowledge about this parasite and its potential diseases in the northwest of Iran seems to be very limited due to the climate and ecological diversity of this region. This study was designed to identify *Blastocystis* subtypes in the northwestern Iran.

Materials & Methods

Parasitological analysis:

The current descriptive cross-sectional study included 600 human stool specimens that were randomly referred to health centers in Urmia and Maragheh cities in the northwest of Iran from February 2016 to September 2018 (Figure 1). These geographical areas are considered as agricultural and livestock poles. The specimens were examined based on direct macroscopic observation (17), formalin-ether concentration (18), and a modified version of the Ziehl–Neelsen staining technique (19) to detect larvae, ova, cysts, trophozoites, and worms. This study has been approved by the Ethics Committee of Maragheh University of Medical Sciences, Maragheh, Iran (ethical code: IR.MARAGHEHPHC.REC.1396ac). Informed consent was obtained from the participants of this study.

DNA extraction and PCR amplification:

DNA of positive samples was extracted using a DNA Extraction Kit (Cinnagen, Iran). Primers used to amplify were RD5 (ATCTGGTTGATCCTGCCAGT) and BhRDr (GAGCTTTTTAACTGCAACG). These primers were designed for different regions of the small ribosomal subunit (18S rRNA) containing ~620 bp and specific for *Blastocystis* and amplified the DNA and subtyped by DNA barcoding approach (20). PCR was performed using the Taq DNA Polymerase Master Mix Red (Amplicon, Denmark). The reaction mixture contained 5.5 µl of distilled water, 7.5 µl of master mix, 2 µl of forward and reverse primers, and about 100-500 ng/µl of extracted DNA in a final volume of 15 µl. PCR products were visualized on 1.5% agarose gels stained with ethidium bromide.

Sequence and statistical analysis:

Sequences of *Blastocystis* subtypes obtained in our study were aligned and compared with those extracted from the GenBank. Sequencher 4.1.1 software (Gene Codes Corporation, USA) and Choromas 2.2 were used to analyze sequences and edit the sequenced fragments, respectively. Alignment gaps were treated as missing data. Bootstrap analyses were conducted using 1000 replicates, and phylogenic analyses were conducted by maximum likelihood using MEGA6 (11, 21). Sequences obtained in this study were submitted to the GenBank under the following accession numbers: MG515328-MG515343.

Results

From 600 samples studied, 496 (82.7%) were male, and 104 (17.3%) were female. The samples were collected randomly from intestinal parasites. Among 600 samples, 40 were diagnosed directly (without using

culture medium) with *Blastocystis*, and 32 were analyzed by molecular method and crop sequence analysis. Three subtypes, including ST_1, ST_2 and ST_3, were sequenced (Table 1). In brief, different varieties of *Blastocystis* subtypes were found, but similarity was observed within subtypes. According to molecular and phylogenetic analysis presented in Figure 2, three subtypes (ST_3, ST_2, and ST_1) of *Blastocystis* were identified in the present study, with ST_3 as the most prevalent subtype. The phylogenetic tree was designed by using neighbor-joining (NJ) using MEGAX software. The differences of nucleotides and sequence alignments of isolated subtypes are depicted in Figure 3.

Table 1. Percentage and frequency of *Blastocystis* subtypes obtained in this study

Subtypes	N (%)	Accession number
ST_1	6 (18.75)	MG515328, MG515336, MG515341
ST_2	6 (18.75)	MG515329, MG515334, MG515340
ST_3	20 (62.5)	MG515330, MG515331, MG515332, MG515333 MG515335, MG515337, MG515338, MG515339 MG515342, MG515343

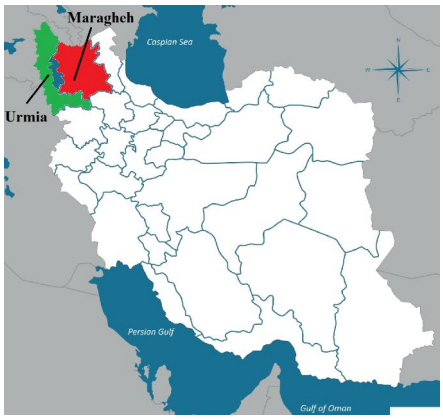

Fig. 1. Map of the study area

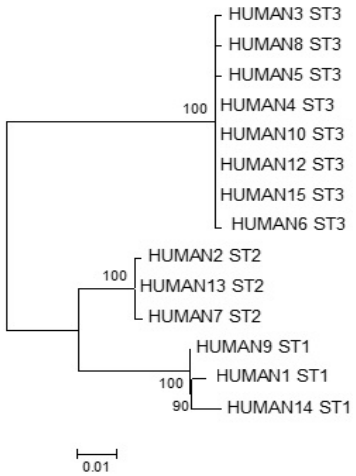

Fig. 2. Phylogenetic tree derived from 18s rRNA genes for *Blastocystis* specimens.

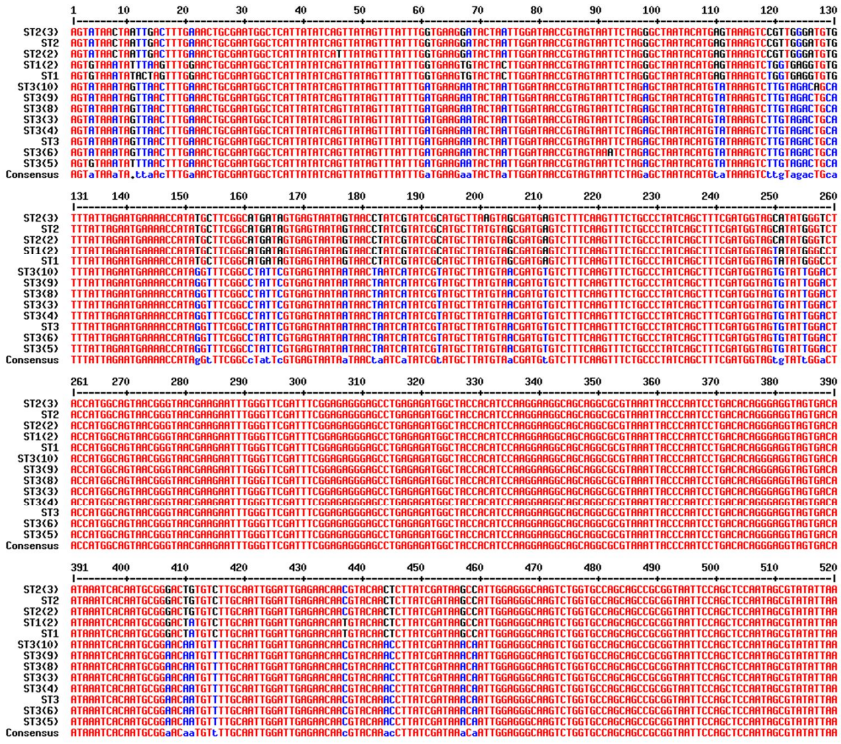

Fig. 3. Sequence alignments of isolated study subtypes of *Blastocystis*

Discussion

In this research, we attempted to study the distribution of *Blastocystis* subtypes in humans in the northwest of Iran. The results of our study may provide a basic information for the genetic study of *Blastocystis* in animal hosts in this part of the country. Prevalence of *Blastocystis* may differ in various regions of the world. Epidemiological study showed that the animal handlers have a higher risk of infection, and there is always the possibility of zoonotic transmission (8).

Sequencing of barcoding region was the last consensus method to find *Blastocystis* subtypes (13). Stensvold *et al.* reported the genetic differences within the same ST (22). Three subtypes, including ST_1, ST_2 and ST_3, were identified in our samples; however, most of the *Blastocystis* subtypes belonged to ST_3, ST_1 and ST_2 subtypes, respectively.

There are numerous reports that have introduced ST_3 as the main subtype isolated from human in different countries in Asia, including Turkey, Pakistan, Bangladesh, Singapore and Japan (23-25). ST_3 and ST_2 are often found in humans, while ST_1 is correlated with pathological alterations in humans (2, 6).

The result of our study showing ST3 as the most abundant subtype is similar to those of Alfellani et al. (7). Similarly, Dogruman *et al.* have reported that ST3 is the dominant subtypes in Turkey and found no association between ST_2 and the presence of clinical symptoms (26). Some other studies in different parts of the world identified ST4 or ST1 as the main subtypes of *Blastocystis* (8, 27). ST4 is a common and important *Blastocystis* subtype in humans in Europe countries. We did not detect ST_4 in northwest of Iran.

There is a hypothesis that animal sources are likely one of the most important sources of human infection (28, 29). ST_5-ST_9 have rarely been isolated from humans. ST_5 is called subtyping of cattle and pig, and ST_{10}-ST_{17} have not been found in humans (7, 30, 31).

These variations of the results arise from ecological diversity, host life, and impacts of human development index, and climate conditions (8).

Numerous studies conducted in Iran have affirmed our results and indicated a natural pattern of transmission for *Blastocystis* (16, 32). It has also exhibited that water resources have a potential role in the prevalence of this parasite (8). Due to the presence of specific human and animal subtypes, the clinical symptoms of this parasite should be investigated in more details. However, it is not easy to relate these symptoms to the presence and genetics of *Blastocystis*.

Conclusion

In this study, we could not depict the precise mode of circulation of Blastocysts subtypes between humans; however, water contaminated with stools may cause infection. While genetic similarity of *Blastocystis* isolates from human and animals did not show in northwest of Iran, but more investigations are suggested to illustrate the role of education in promoting general public health. The zoonotic cycle of this parasite exists in the study area. By identifying of *Blastocystis* subtypes, it is possible to prevent the transmission and its complications.

Acknowledgments

None declared.

Ethical considerations

This study has been approved by the Ethics Committee of Maragheh University of Medical Sciences, Maragheh, Iran (ethical code: IR.MARAGHEHPHC.REC.1396ac). Informed consent was obtained from the participants of this study.

Conflict of interest

The authors have no conflict of interest in this study.

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Data availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

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