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Original Article

Genetic Diversity of Human *Blastocystis* Isolates in Khorramabad, Central Iran

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Abstract

Background: There are some genetic differences in *Blastocystis* that show the existence of species or genotypes. One of these genes that help in identifying *Blastocystis* is SSUrRNA. The aim of this study was assessment of genetic diversity of *Blastocystis* by PCR with seven pairs of STS primers.

Methods: This study was done on 511 stool samples collected from patients referred to the health care centers of Khorramabad, Central Iran, in 2012. Genomic DNA was extracted and in order to determine the *Blastocystis* subtype in contaminated samples, seven pairs of primers STS (subtype specific sequence-tagged site) were used.

Results: Out of 511 samples, 33 (6.5%) samples were infected with *Blastocystis*. Subtype (ST) of 30 samples was identified and three subtypes 2, 3 and 4 were determined. Mix infection was reported 10% which 3.33% of the infection was for the mixture of ST 3 and ST5 and 6.67% was for the mixture of ST 2 and ST 3.

Conclusion: The predominant subtype was ST3 that is the main human subtype. The dominance of ST2 and 5 are important in this study. This superiority has been reported in some of the studies in ST 2 which is different from the studies in other countries, because they have announced priorities of the ST1 and ST6 after ST3.

Introduction

Blastocystis is the most common protozoan, anaerobic and zoonosis parasite that live in the gastrointestinal tract of humans and animals (1, 2). *Blastocystis* has a

worldwide distribution and the cyst through contaminated food and water is transferred between hosts. Its Prevalence in the world and even in various communities in each country

is different. Generally, based on many studies, countries in the world have been divided into two groups. A: developed countries With prevalence up to 10-20%; B: developing countries with prevalence up to 50 - 60% that this high prevalence is due to lack of health, contact with animals and contaminated water and food (1, 3).

Blastocystis spp is recognized as a complex of subtypes that have not been characterized as independent species but has genetic differences (4, 5). A large number of studies were done and a variety of data obtained, but still much unknown about the mysterious remain (3). Although many studies argue that parasite is pathogen but since it was seen in people without any symptoms, its pathogenic role is still controversial (6). There are some genetic differences in *Blastocystis* that show the existence of species or genotypes (7). One of these genes that help in *Blastocystis* identification is SSUrRNA. The STS (subtype specific sequence-tagged site) primers can identify seven major and standard genotypes of *Blastocystis* (8, 9). In some of studies on parasite indicators, the virulent indicator concerns parasite subtype (10).

The aim of this study was assessment of genetic diversity of *Blastocystis* by PCR with seven pairs of STS primers in Khorramabad City, Lorestan Province, Iran.

Materials and Methods

This descriptive study was done on 511 stool samples were collected from patients who referred to the health care centers of Khorramabad, Iran, in 2012. Samples were collected in a plastic container without the fixator and sent to the parasitology laboratory.

Genomic DNA was extracted directly from stool specimens using a stool DNA extraction kit (Bioneer Co., Korea) according to the manufacturer's instructions. The concentration of each DNA was measured using spectrophotometer (Biochrom WPA Lightwave II UV/Visible). The extracted DNA was stored at -20 °C until PCR amplification.

Primers were used according to a previous study (11): b11400 FORC (5' – GGA ATC CTC TTA GAG GGA CAC TAT ACA T-3')/ b11710 REVC (5' – TTA CTA AAA TCC AAA GTG TTC ATC GGA C-3').

Table 1: Seven pairs of STS (subtype specific sequence-tagged site) primers and expected PCR product

Subtype	Primer	PCR Product size (bp)	Accession Number in GenBank	Sequences
ST 1	SB ₈₃	351	AF ₁₆₆₀₈₆	F: GAAGGACTCTCTGACGATGA R:GTCCAAATGAAAGGCAGC
ST 2	SB ₃₄₀	704	AY ₀₄₈₇₅₂	F: TGTTCITTGTGTCTTCTCAGCTC R:TTCTTTTCACACTCCCGTCAT
ST 3	SB ₂₂₇	526	AF ₁₆₆₀₈₈	F:TAGGATTTGGTGTTTGGAGA R:TTAGAAAGTGAAGGAGATGGAAG
ST4	SB ₃₃₇	487	AY ₀₄₈₇₅₀	F: GTCTTTCCTGTCTATTCTTTGCA R:AATTCGGTCTGCTTCTTCTG
ST5	SB ₃₃₆	317	AY ₀₄₈₇₅₁	F:GTGGGTAGAGGAAGGAAAACA R:AGAACAAGTCGATGAAGTGAGAT
ST6	SB ₃₃₂	338	AF ₁₆₆₀₉₁	F: GCATCCAGACTACTATCAACATT R:CCATTTTCAGACAACCACTTA
ST7	SB ₁₅₅	650	AF ₁₆₆₀₈₇	F:ATCAGCCTACAATCTCTC R: ATCGCCACTTCTCCAAT

PCR program was performed with an initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 5 min (11). The expected PCR product was 310 bp. PCR products were visualized with ethidium bromide staining after electrophoresis on 1.5% agarose gel.

In order to determine the *Blastocystis* subtype in contaminated samples, seven pairs of STS (subtype specific sequence-tagged site) primers were used (Table 1) (12).

PCR program was performed with an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 40 second, 58 °C for 40 second, and 72 °C for 40 second, with a final extension at 72 °C for 5 min (12). PCR products were visualized with ethidium bromide staining after electrophoresis on 1.5% agarose gel. DNA for sequencing was prepared by PCR, using seven pairs of STS primers.

Results

Out of 511 samples studied, *Blastocystis* sp. was detected in 33 (6.5%) specimens by PCR (Fig. 1). Subtype of 30 samples was identified and three did not respond with any of STS primers (Table 2). Three subtypes including

ST2, ST3, and ST5 were determined by seven pairs of STS primers based on previous study (13). The expected PCR products for ST2, ST3 and ST5 were 704 bp, 526 bp and 317 bp, respectively (Fig. 2). Four subtypes 1, 4, 6 and 7 were not detected. Dominant subtype was ST3 (56.7%), followed by ST5 (20%) and ST2 (13.3%). Ten percent of positive samples had mixed infections and two subtypes were observed simultaneously (Table 2).

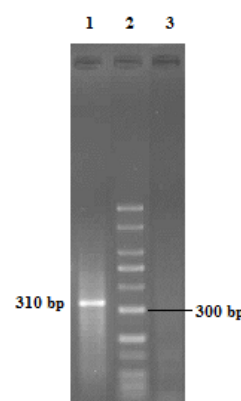


Fig. 1: 1.5% agarose gel electrophoresis of PCR production/ Lane 1: *Blastocystis* isolate/Lane 2: 50 bp DNA ladder marker/Lane 3: Negative control

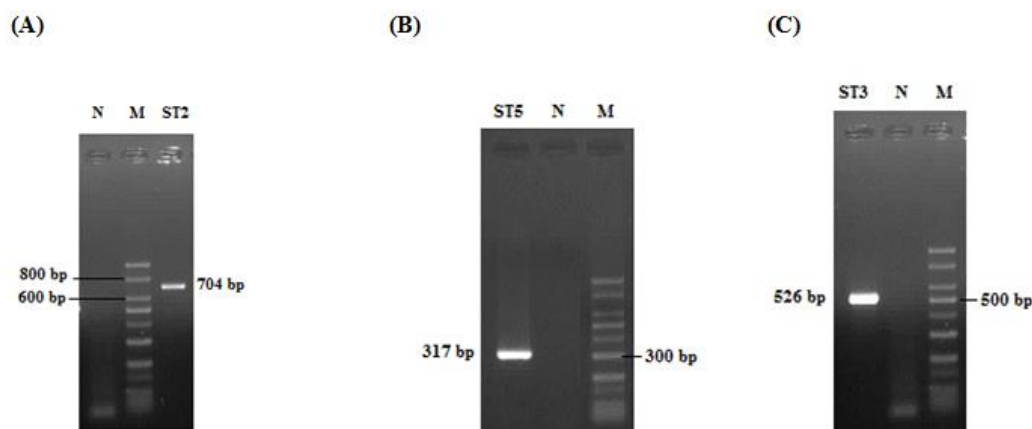


Fig. 2: Identification of *Blastocystis* subtypes. (A) Lane ST2: 704 bp. (B) Lane ST5: 317 bp. (C) Lane ST3: 526 bp. M: 50 bp DNA ladder marker; N: Negative control

Nucleotide sequence data reported in this article have been submitted to the GenBank database with accession numbers, subtype 3 JX483861 and JX483862, subtype 5 JX524458 and subtype 2 JQ976893.

Table 2: Identified subtypes of *Blastocystis* in human stool samples

Subtype	No. (%)
ST 3	17 (56.7)
ST 2	4 (13.3)
ST 5	6 (20)
ST 3 + ST 5	1 (3.33)
ST 2 + ST 3	2 (6.67)
Total	30 (100)

Discussion

PCR was used to determine the subtype of *Blastocystis* in the collected positive samples. PCR compared with other diagnostic methods such as formalin ether concentration technique (FECT) and culture, has priorities for instance higher accuracy, and not necessary for parasite viability. Using this technique in epidemiological studies, more realistic estimates of parasite prevalence are achieved. This method is as fast as FECT but faster than culture (8, 11, 14).

In the present study, for identification of seven subtypes of *Blastocystis*, seven pairs of STS primers were used. Researchers have used these primers in several studies such as the relation of the virulent and subtype (8, 15, 16).

Meanwhile, there are the genotypes that are not determined by seven pairs of STS primers indicating of the polymorphism of parasite, as shown in our study (13). Apparently the number of these subtypes is 13 which have not been applicable since now (17).

Since the studies about *Blastocystis* were based on subtypes, their stability is necessary as a standard but some studies are confusing (18, 19).

In this study ST3 was the most prevalent (56.7%) among the 33 positive. As the predominant subtype in most parts of the world such as Japan, Pakistan, Bangladesh, Germany, Singapore, Greece and Turkey was ST3, believed that this subtype is the main human and has no relation to the geographic area (4, 17).

In the present study, prevalence report of ST2 and ST5 after ST3 were important. This superiority has been reported in some of the studies in ST2 which is different from the studies in other countries (17). In some studies; priorities of the ST1 (7.7-25%) and ST6 (10-22.9%) after ST3 were announced (3).

ST5 is called subtyping of cattle and pig and less human infection with this subtype has been reported (3). The pig is not used in Iran because of doctrinal issues but cow is a major food source for human and Iranian use its meat a lot especially in Lorestan Province, leading to more breeding of this animal and human contact with it. The prevalence of this parasite in cattle was detected 9.6% with the dominant ST5 (Not published). Since *Blastocystis* is zoonosis parasite, so the impact of geographical terms on infection should be considered.

In this study, mix infection that is one of the epidemiological indicators of this parasite, was reported 10% which 3.33% of them was for the mixture of ST3 and ST5 and 6.67% was for the mixture of ST2 and ST3. Mix infection for other studies was between 1.1-14.3% and the most infection was for the mixture of ST3 and ST1 (20).

In a previous molecular study in Shiraz, Iran, classification was done based on seven different ribodeme (I, II, VI, VII, VIII, IX, and X) using RFLP-PCR. Only ribodeme 1 is similar to ST1 called pathogen subtype. Since the samples used in the study were for symptomatic persons thus the result is not unexpected (21). In Hamedan, Iran, 41 human fecal samples infected with *Blastocystis* isolates, were identified by PCR, using seven pairs of STS primers. Three subtypes, including ST1

(56.1%), ST3 (22%) and ST2 (7.3%) were characterized. Coexistence of ST1 and ST3 was detected in 14.6% of cases (22). In another study in Iran, 100 *Blastocystis* isolates were characterized using seven pairs of STS primers. Four subtypes, including ST3 (53%), ST1 (48%), ST5 (33%), and ST2 (7%) were identified. Also ST1 in gastrointestinal patients was significantly more than asymptomatic individuals (23). Thirty two *Blastocystis* isolates obtained from 12 asymptomatic healthy individuals and 20 symptomatic patients were characterized by PCR using known seven kinds of sequence tagged site primers. ST3 was the most dominant genotype in asymptomatic individual (9/12) and ST1 determined all of symptomatic patients (20/20) (24).

In our study genetic diversity and subtype prevalence are approximately similar to other studies except ST1. Based on some studies (23, 24), ST1 is more common in gastrointestinal patients but in present study the samples were collected from patients who referred to the health care centers regardless of gastrointestinal symptoms. So no ST1 report may relate to method of sample collection.

Conclusion

It is suggested that the studies be conducted on individuals infected with gastrointestinal symptoms to characterize *Blastocystis* subtypes. Also the collection of epidemiological data is necessary for a better understanding of *Blastocystis* transmission and the pathogenic subtypes.

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