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Original Article

Frequency of Entamoeba histolytica and *Entamoeba dispar* prevalence among patients with gastrointestinal complaints in Chelgerd city, southwest of Iran*

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Abstract

BACKGROUND: Differentiation between *Entamoeba histolytica* and *Entamoeba dispar* is very important for both clinical therapy and epidemiological studies. Although these two species are morphologically identical, they have differences in genetic, chemical specifications and pathogenicity. This study was carried out to differentiate *E. histolytica* from *E. dispar* and also to find out frequency of the two species.

METHODS: Fecal samples were collected three times from 655 patients with gastrointestinal complaints (47.3% male and 52.7% female), who were referred to the primary health care centers of Chelgerd, Chaharmahal and Bakhtiary province. Samples were examined microscopically with direct smear, formalin-ethyl-acetate concentration and trichrom staining methods to distinguish *E. histolytica* from *E. dispar* complex and differentiate them from non-pathogenic intestinal amoeba. Genomic DNA was extracted from microscopy positive isolates and polymerase chain reaction (PCR) was carried out to different the two morphologically identical Entamoeba isolates.

RESULTS: Among the 655 recruited patients, eleven subjects with *E. histolytica* / *E. dispar* isolates (1.7%) were identified by microscopy methods. Ten of the positive isolates (90.9%) were identified as *E. histolytica* by PCR and one isolate (9.09 %) was positive for E. dispar.

CONCLUSIONS: This study revealed that E. histolytica was more prevalent than *E. dispar* in the studied area. This result was different from the previously reported data in other parts of Iran.

KEYWORDS: Gastrointestinal Complaints, Entamoeba Histolytica, Entamoeba Dispar, Polymerase Chain Reaction, Iran.

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Amoebiasis is defined as an intestinal or extra intestinal disease due to the protozoan parasite E. histolytica. Patients with amoebiasis may suffer from a wide range of symptoms including diarrhea, fever, and cramps. The disease may also affect liver as well as some other organs of the body. The parasites are found in all parts of the world but most frequently in tropical and subtropical regions where the socio-economic status and environmental sanitation are poor.[1,2] It has been estimated that infection with E. histolytica results in 34 million to 50 million symptomatic cases of amoebiasis worldwide each year, causing 40 to 100 thousands of deaths annually.[3,4]

Since 1925 existence of two species of

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amoeba has been reported, one is pathogen and the other one is a non-pathogen commensal organism.[5,6] However, it was not accepted until 1993 when Clark and Diamond gave the ultimate re-description of these two species. Finally, these cumulative evidence were formally accepted by expert committee of the World Health Organization in 1997.[7,8]

Indeed, the recommendation of the WHO-Pan American Health Organization to develop improved methods for the specific diagnosis of E. histolytica infection is very important for the establishment of accurate prevalence data of E. histolytica and E. dispar infections worldwide.[9]

Differential diagnosis of these two species is of great clinical and epidemiological importance, considering that no treatment is recommended for E. dispar infections.[10] However except for the cases of haematophagous trophozoites in acute dysentery, it is not possible to differentiate E. histolytica from E. dispar using microscopy.[11]

To address the need for a specific diagnostic test for amoebiasis, a substantial amount of work has been carried out over the last decade in the world. Molecular diagnostic tests are increasingly being used for both clinical and research purposes. In order to minimize undue treatment of individuals infected with other species of Entamoeba such as E. dispar, efforts have been made for specific diagnosis of E. histolytica infection and not to treat based on the microscopic examination of Entamoeba species in the stool.[12]

This study was conducted to determine frequency rate of amoebiasis in patients with gastrointestinal disorders in Chelgerd city in southwestern of Iran, by using molecular methods.

Methods

Study area

A cross-sectional study was conducted from April to October 2009. Fecal samples were collected three times from 655 patients with gastrointestinal complaints (47.3% male and 52.7% female) who referred to the primary health care centers in a township of Chaharmahal and Bakhtiary province. Medical School Council of Isfahan University of Medical Sciences confirmed the ethical issues of the study.

Patients who suffered from all or some of gastrointestinal symptoms such as abdominal pain, fever, tenesmus, diarrhea and/or dysentery were entered to this study.

Chelgerd is located in south west of Iran at the vicinity of Zagros Mountain rang. Temperature in spring and summer is temperate and in winter is very cold (0-20◦C). In spring, this area accept the biggest immigrant tribal populations who come from south of Iran, mostly Khozestan province. The people who live in this region have inadequate hygiene facility and they have close contact with domestic animals especially goat, lamb and dog. They generally are employed in animal husbandry and agriculture and have poor socio-economic status.

Microscopic examination of stool samples

Stool samples were microscopically examined by direct smear examination of fresh stool, formalin-ethyl acetate concentration and thrichorom staining. The presence of one to four nuclei cysts and/or trophozoites of amoeba was detected as E. histolytica/E. dispar. Trophozoite containing ingested red blood cell was not seen in fecal samples. Positive E. histolytica/E. dispar isolates were kept frozen into 2 ml tubes at -20◦C until used for DNA extraction.[13]

Molecular characterization of E. histolytica and E. dispar species

DNA was extracted directly from fecal samples using DNA extraction kit (CinnaGen inc, Iran) according to the manufacturer instruction with a small modification for extraction of DNA from cysts. One ml acid pepsine was added to the stool samples, incubated for 1 hour at 37◦C. Samples were washed 3 times with 1% buffered saline phosphate (pH = 7). The tubes were frozen and thawed 5 times.[14]

Polymerase chain reaction (PCR)

Two sets of oligonucleotide primers: HSP1

(GAG TTC TCT TTT TAT ACT TTT ATA TGT T) and HSP2 (ATT AAC AAT AAA GAG GGA GGT) for E. histolytica and DSP1 (TTG AAG AGT TCA CTT TTT ATA CTA TA) and DSP2 (TAA CAA TAA AGG GGA GGG) for E. dispar were used for PCR method.[15] These primers amplify a region of about 340 bp and 430 bp of the locus D-A gene of tRNA (also known as locus 1-2) for E. histolytica and E. dispar respectively. E. histolytica HM1: IMSS strain was used as standard control. PCR was carried out in a 25µl reaction mixture; containing 1µl DNA, 1.5 µM concentration of each primers, 12.5 µl Master mix (CinnaGen, Iran) and 10.5 µl DW in a Corbett Thermocycler.

Descriptive statistics and frequency tables were used to describe the results. Chi-square test was preformed to compare the proportion of binominal variables among groups of patients. A p-value of < 0.05 was accepted as statistically significant.

Results

Among the 655 patients with age range from zero to 69 years old (47.3% male and 52.7% female) who participated in this study, the high incidence of infection with E. histolytica/E. dispar complex was in10-19 years age group. Most of the patients with gastrointestinal disorder were in the groups of 0-9 years. Among them,11(1.7%) isolate were identified as E. histolytica/E. dispar complex by microscopic examination (Table 1). Trophozoite containing ingested red blood cell was not seen in fecal samples. Ten (90.9%) of the positive isolates showed a fragment of about 360 bp for STR D-A locus and were identified with PCR as E. histolytica. One of the samples (9.09%) with a PCR fragment of about 430 bp was distinguished as E. dispar (Figure 1). Therefore E. histolytica was observed in 10 (1.5%) and E. dispar was observes in 1(0.2 %) of the studied samples.

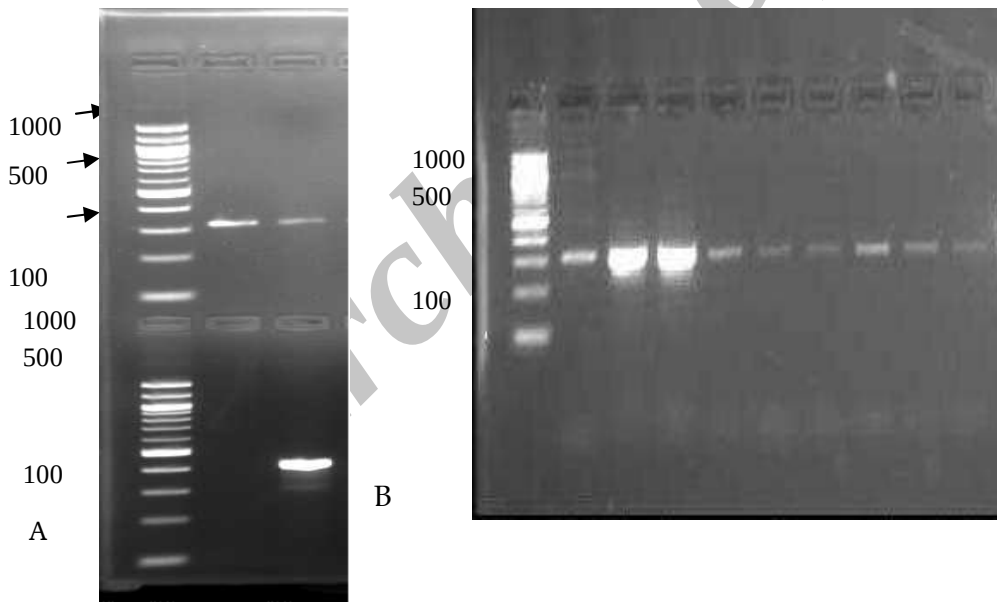

Figure 1. Agarose gel electrophoresis of positive *Entamoeba dispar* and *Entamoeba histolytica* isolates
A:
Lane 1, 4:100 bp marker
Lane 2: *Entamoeba histolytica* positive control (HM-1: IMSS) with HSP1-HSP2 primers
Lane 3: *Entamoeba histolytica* positive isolate with HSP1-HSP2 primers
Lane 5: Negative control
Lane 6: *Entamoeba dispar* positive isolate with DSP1-DSP2 primers
B:
Lane 1:100 bp marker
Lane 2-10: *Entamoeba histolytica* positive isolates with HSP1-HSP2 primers

Table 1. Frequency of positive *E. histolytica/ E. dispar* in 655 patients in Chelgerd city, southwest of Iran

Entamoeba Species	Number of positive patients	Sex		Age Group(years)			
		Female	Male	0-9	10-19	20-29	30-39
E. histoletica	10	6	4	3	3	1	3
E. dispar	1	0	1	0	1	0	0
Total	11	6	5	3	4	1	3

Discussion

Amoebiasis is still a serious health problem in many tropical and sub tropical areas of the world, especially in developing countries such as Iran. Several epidemiological studies have already been conducted to determine the prevalence rate of amoebiasis in Iran.[16,17]

Our results showed that E. histolytica is more prevalent than E. dispar in the Chelgerd city in southwest of Iran. These results were in contrast with the results obtained from other studies in Iran. It is not surprising to find E. histolytica more prevalent in different geographic situation, climate condition and culture. Based on molecular identification, E. histolytica was more prevalent than E. dispar in Thai/Myanmar border region of Thailand.[18] A similar trend of E. histolytica infection was reported in a highly endemic region in Mexico.[19] However, in the most of countries, prevalence of E. dispar is higher than E. histolytica. For example, in Australia 3.4% of the patient's were infected with E. histolytica, while 33.7% of the samples identified as E. dispar, and 24.7% were infected with Entamoeba moshkovskii.[20]

Of the 8 microscopy-positive E. histolytica / E. dispar samples isolated from gastrointestinal disorder patients in Zahedan, six were identified as E. dispar by PCR method, while E. histolytica was not detected.[21] PCR-RFLP analysis of 101 isolates of E. histolytica /E. dispar obtained from asymptomatic people in three regions of Iran conducted by Hooshyar et al., showed 92.1% E. dispar, 7.9% E. histolytica and/or mixed infection.[16] E. dispar was also reported more frequent in patients with gastrointestinal symptoms in Gonbad and Tehran.[17]

In this study, no significant differences were seen between the different age groups and sexes for E. histolytica/E. dispar distribution ($p > 0.05$).

The populations that live in Chelgerd area in southwest of Iran have special socio-demographic conditions. These results are probably due to the low level of individual and public hygienic conditions which might be considered as cause of more prevalent E. histolytica infections in this area. Perhaps the difference between our results and other studies in Iran is due to climatic conditions and height of the region, or because of the tribal's migration from other provinces.

Our results clearly demonstrated that E. histolytica is also present and more prevalent in at least some parts of Iran. However, more molecular studies are recommended to verify the real prevalence of the Entamoebas in different climate regions of Iran.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

NP participated in the design and administration of the study, drafted and edited the manuscript. MN carried out the design and coordinated the study, participated in all of the experiments and prepared the manuscript. AH participated in the design of the study and molecular and parasitological exam and prepared the manuscript. MS participated in the design of the study and molecular exam and prepared the manuscript. HY participated in the design of the study and parasitological tests. All authors read and approved the final manuscript.

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