



Molecular Epidemiology of Human Intestinal Amoebas in Iran

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Abstract

Many microscopic-based epidemiological surveys on the prevalence of human intestinal pathogenic and non-pathogenic protozoa including intestinal amoeba performed in Iran show a high prevalence of human intestinal amoeba in different parts of Iran. Such epidemiological studies on amoebiasis are confusing, mainly due to recently appreciated distinction between the *Entamoeba histolytica*, *E. dispar* and *E. moshkovskii*. Differential diagnosis can be done by some methods such as PCR-based methods, monoclonal antibodies and the analysis of isoenzyme typing, however the molecular study of these protozoa in Iran is low. Based on molecular studies, it seems that *E. dispar* is predominant species especially in the central and northern areas of Iran and amoebiasis due to *E. histolytica* is a rare infection in the country. It is suggested that infection with *E. moshkovskii* may be common among Iranians. Considering the importance of molecular epidemiology of amoeba in Iran and also the current data, the present study reviews the data currently available on the molecular distribution of intestinal human amoeba in Iran.

Key words: Amoeba, *Entamoeba*, Molecular epidemiology, Iran

Introduction

Cases of transmissions for over 18 different human intestinal protozoa have been reported in Iran. Reports on some of these protozoa such as *Microspora* spp, *Cyclospora cayetanensis*, *Sarcocystis hominis*, *Balantidium coli* and *Entamoeba moshkovskii* are as case reports but others including pathogen and non-pathogenic species are more common and prevalent. Human intestinal amoeba contain many species, eight of which (*Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *E. polecki*, *E. coli*, *E. hartmanni*, *Iodamoeba butschlii* and *Endolimax nana*) reside in the human intestinal lumen (1). It is well known that *E. histolytica* is the only pathological species in humans, that causes about 50 million cases of infections with an annual death rate of over 100,000 worldwide (2). The other human intes-

tinal amoeba are considered non-pathogenic and rarely cause intestinal disease in humans (3,4).

Progress in the molecular epidemiology of protozoa infection has been driven by developments in the laboratory techniques, such as PCR and other molecular biological tools. Application of this method to the epidemiology of human intestinal protozoa (e.g. amoeba) revolutionised the knowledge and concept of epidemiology of parasitic infection. Molecular data on the epidemiology of intestinal protozoa, especially pathogenic amoeba, are important for planning the prevention and control programs.

Up to present, many microscopic-based epidemiological studies on the prevalence of human intestinal pathogenic and non-pathogenic protozoa, including intestinal amoeba, have been performed

in different parts of the Iran (5-7). Although several studies (5,7-9) showed the high prevalence of human intestinal amoeba in different parts of Iran, however only a few molecular studies on these protozoa were performed in Iran. This is important due to the recent appreciation on the distinction between *E. histolytica*, *E. dispar* and *E. moshkovskii* and signifying that making differential diagnosis cannot be done by microscopy-based methods.

This review performed to determine and provide an overview of the present information on the molecular epidemiology of human intestinal amoeba in Iran.

Material and Methods

Manual and electronic searches in international and national databases and journals were conducted to find the relevant data reporting molecular studies on human intestinal amoeba in Iran. Searching were performed through in the international database such as PubMed (www.ncbi.nlm.nih.gov), Scirus (www.scirus.com), ISI Web of Science (www.isiknowledge.com), Scopus (www.scopus.com), EMBASE (www.embase.com), Science Direct (www.sciencedirect.com), and Google Scholar (scholar.google.com). National database searched were including: Iranmedex (www.iranmedex.com), IranDoc (www.irandoc.ac.ir), Magiran (www.magiran.com) and Scientific Information Database (www.sid.ir). The search covered the articles published up to the 2011.

In an attempt to identify all related studies, a combination of relevant keywords and MeSH terms including the names of each human intestinal amoeba e.g. "*Entamoeba*," "Intestinal Amoeba" and the following keywords: "Iran", "Molecular" or "genum" were used in search strategy. The searching strategy in Iranian databases was the English and Persian transcription with key words related to the study.

To maximize the sensitivity of search, references of selected articles were checked. Moreover the manual search was carried out in the soft and/or hard copies of articles published in scientific jour-

nals, reports of research projects and post-graduate theses, as well as the abstracts of scientific articles related to this topic presented at scientific congresses by Iranian researchers.

Results

Entamoeba histolytica and *Entamoeba dispar*

Entamoeba histolytica and *E. dispar* are two genetically distinct but morphologically identical species. These two species could be differentiated by some methods such as specific DNA probe, PCR-based methods, monoclonal antibodies and analysis of the isoenzyme typing (10). On the basis of biochemical, immunological and genetic data, a formal re-description of *E. histolytica* was published in 1993 separated this species from the harmless commensal *E. dispar* (11). *E. histolytica* is a potentially pathogenic species, while the *E. dispar* is non-pathogenic one. Hence the differentiation of the two species is important in clinical diagnosis and treatment and also from an epidemiological point of view.

Given the criteria mentioned, the epidemiology of amoebiasis has changed since the separation of *E. histolytica* and *E. dispar* species. Nowadays it is accepted that *E. dispar* is the predominant species and much more common than the *E. histolytica* worldwide (12). However in some regions (e.g. Japan, Mexico, India, South Africa, some Central and South American, and Asian Pacific countries) local prevalence of *E. histolytica* is especially more common among the male homosexuals (13).

Identification and use of molecular tools for accurate differential diagnosis of *Entamoeba* spp. such as *E. histolytica*, *E. dispar* and *E. moshkovskii* displays a satisfactory change in the epidemiology of amoebiasis.

In Iran a number of epidemiological studies have been carried out on *E. histolytica/E. dispar* complex using the routine microscopy stool examination with no separation between them. A prevalence rate of >1% to 30% for *E. histolytica/E. dispar* complex was reported in different parts of Iran (5). A preliminary comparative study of 15 isolates of *E. histolytica/E. dispar* isolated from Hamadan, by

PCR technique showed that all the isolates were *E. dispar* (14). A field study on the distribution of *E. histolytica/E. dispar* cyst passers in northern, central, and southern Iran showed the prevalence of infection with *E. histolytica/E. dispar* was 0.78%, 3.9% and 4.6% for central, northern and southern part of Iran, respectively. The minimum prevalence rate was 0.6% in Tehran, Yazd and Ardekan (central Iran), while the highest rate (8.3%) was seen in rural areas of Ahwaz (southern Iran). This study showed that ratio of *E. histolytica/E. dispar* was higher in southern (tropical and subtropical regions) than the other regions (15).

Data on differential diagnosis of *E. histolytica* and *E. dispar* by molecular methods in different regions of Iran showed that a total number of 92.1% isolates were *E. dispar* and the remaining 7.9% were *E. histolytica* or mixed infection (16). This study demonstrated that *E. dispar* is the predominant species found among the "cyst passers" in Iran (16). The ratio of infection rate with two species in cyst passer in different areas were as follow: In central area (semi temperate zone) 3.5% of isolate were identified as *E. histolytica*, 93% of isolate were *E. histolytica* and 3.5% of individuals were infected by both species.

In northern (temperate region) 5.9% of isolates were *E. histolytica* and 94.1% of isolates *E. dispar*. In southern (tropical and subtropical region) 7.4% of isolates were *E. histolytica* and 88.9% *E. dispar*. 3.7% of person in this area had a mixed infection with two species (16). In Tehran and Karaj, the two major metropolitan areas of central Iran differential diagnosis of 49 isolates of *E. histolytica/E. dispar* using PCR-RFLP revealed that 46 (93.9%) of isolates were *E. dispar*, while only 2 (4.1%) *E. histolytica*. One person (2%) had a mixed infection (10). Another study on 21 isolates of *E. histolytica/E. dispar* complex in fresh stool by PCR in Tehran showed that 95.45% of isolates were *E. dispar* and only one isolate (4.55%) was found to be *E. histolytica* (17). This finding is similar with previous study in this area.

Similar observation that made by Rezaian and Hooshyar(2006), using PCR-RFLP on 21 isolates of *E. histolytica/E. dispar* in rural areas of Ahwaz and Hamidieh (south of Iran) showed that 19

samples (90.48%) were positive for *E. dispar*, one (4.76%) positive for *E. histolytica* and another sample (4.76%) showed mixed infection(5). A local prevalence study in urban and rural areas of Gonbad City (north of Iran) using PCR method showed that 16 samples isolated were *E. dispar* and none of them showed the *E. histolytica* pattern (18). A study by PCR/gel electrophoresis, by Haghighi et al. (2009) on eight microscopical-positive *E. histolytica/E. dispar* samples in Zahedan identified, six of them as *E. dispar* whereas *E. histolytica* was not detected there at all (19). A comparative study of stool antigen detection kit and PCR for the diagnosis of *Entamoeba* sp. infection in asymptomatic cyst passers from western (Luristan), northwestern (West Azerbaijan), and northeastern (Golestan) part of Iran, showed that all of the 88 samples containing *E. histolytica/E. dispar* cysts were negative for *E. histolytica* (20).

All of these studies showed that *E. dispar* is the predominant species in Iran and amoebiasis due to *E. histolytica* is a rare infection in Iran (Table1). Hooshyar et al. (2004) showed that *E. dispar* is much more frequent than *E. histolytica*, and also that the *E. histolytica/E. dispar* ratio is 1:12. Field-based studies in other countries have found the similar ratios: 1:8.5 (Philippines), 1:2.7 and 1:8 (Bangladesh) and 1:46 (Ivory Coast) (16).

The only molecular study on amoeba in Iran revealed that *E. histolytica* was more prevalent than *E. dispar* is a new study that published by Pestechian et al. (2011). In this study From 655 stool samples in Chelgard City (Chaharmahal and Bakhtisry), 11 *E. histolytica/E. dispar* observed. By PCR 10 of them were *E. histolytica* and 1 *E. dispar* (21). This finding is unlike the previous reported data in other parts of Iran and need to more study in this region.

Isoenzyme electrophoresis in starch gel used to distinguish between the *E. histolytica* and *E. dispar*. The chosen isoenzymes were malic enzyme (ME), phosphoglucosmutase (PGM), glucose phosphate isomerase (GPI) and hexokinase (HK). A zymodeme is defined as a group of amoeba strains that share the same electrophoretic patterns and mobility for these enzymes (22).

Table 1: Prevalence of *E. histolytica* and *E. dispar* In Iran

Method of Diagnosis	Year	Region of study (Iran)	No of cases	Prevalence rate (%)			Reference Arthurs (No.)
				<i>E. his</i>	<i>E. dis</i>	Mixed	
PCR based Methods	2001	Hamadan	15	-	100	-	Fallah M et al. (14)
	2001	Tehran	8	-	100	-	Hooshyar et al. (22)
	1999-2001	Different regions of Iran	101	4/9	92/1	3	Hooshyar et al. (16)
		Central region		3/5	97	3/5	Hooshyar et al. (16)
		Northern region		5/9	94/1	-	Hooshyar et al. (16)
		Southern region		7/4	88/9	3/7	Hooshyar et al. (16)
	1999-2001	Tehran& Karaj	49	4/1	93/9	2	Hooshyar et al. (10)
	2003	Ahwaz &Hamidieh	21	4/8	90/5	4/7	Rezaian and Hooshyar (5)
	2006	Gonbad	16	-	100	-	Nazemalhosseini et al. (18)
	2006-2007	Tehran	21	4/55	95/45	-	Nazemalhosseini et al. (17)
Stool antigen detection & PCR Zymodem analysis	2008	Zahedan	8	-	100	-	Haghighi et al. (19)
	2004-2008	Tehran,Zahedan,Gonbad	53	3.54	91/37	-	Nazemalhosseini et al. (27)
	2010	Khorramabad	16	-	93/75	-	Kheirandish et al.(28)
	2011	Chelgerd	10	90	10	-	Pesthechian et al.(21)
	2006	Lurestan,West Azarbayijan and Golestan	88	-	100	-	Solaymani-Mohammadi et al.(20)
	2001	Tehran	8	-	100	-	Hooshyar et al. (23,24)
	2002-2005	South Iran	23	26	74	-	Sahebani et al. (25)
	2006	Tehran and Kazerun	2	-	100	-	Haghighi A (29,24)

By simultaneous the examination of the characteristic banding pattern of PGM and GPI, it was the possible to classify the amoeba in to one of more than 20 zymodemes (12). Twenty-one out of totally of 23 different zymodemes described from human isolates. Eight zymodemes pattern proved to be those of the *E. histolytica* (zymodemes:II,VI,VII,X,XI,XII,XIV and XX), while the rest were belong to *E. dispar*. Prior to the development of DNA-based techniques, isoenzyme

(zymodeme) analysis of cultured amoebae was considered the gold standard for the diagnosis of amoebic infection and differential diagnosis of amoeba species.

Study on zymodemes pattern of 8 isolates of *E. dispar* from Hooshyar et al. (2001) study and two *E. dispar* isolates from Tehran and Kazerun (AS2IR and AS16IR) by Haghighi (2006) in Japan showed that all of them were zymodem I (23, 24).

Isoenzyme analysis of 23 isolate of *Entamoeba* in south of Iran, cultured in Robinson's medium by Sahebani et al. (2002-2005) showed that six samples (26%) were diagnosed as *Entamoeba histolytica* and 17 samples (74%) as *E. dispar*. Zymodemes of *E. histolytica* were II and XIV and, zymodemes of *E. dispar* were I, XVI, XVII and XVIII (25). Based on these results we suggested that zymodeme I of *E. dispar* similar to other subtropical countries is the predominant zymodeme of *E. dispar* in Iran.

Diagnosis of the extra intestinal amoebiasis is important and is most often made by serological methods or detection of the organism at the time of biopsy or autopsy (4). Molecular methods such as PCR are increasingly useful tools for the diagnosis of extra intestinal amoebiasis and can provide rapid diagnosis. The first case of *Entamoeba histolytica* encephalitis diagnosed by PCR of the cerebrospinal fluid of an Iranian patient was done by Solaymani-mohammadi et al. (20). The patient who was born in the Sistan-Baluchestan province of the southeastern Iran, immigrated to the United States, and 4 months prior to the presentation of disease had visited Iran for 3 months. Finally the patient was treated successfully with metronidazole(26).

Entamoeba moshkovskii

Entamoeba moshkovskii also is not distinguishable from *E. dispar* and *E. histolytica* in its cyst or trophozoite forms. For making differential diagnosis nowadays some PCR based methods are available. This organism is considered primarily a free-living amoeba and has been found to be common in sewage, clean and waste-contaminated water worldwide (12). *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to some antiamebic drugs (e.g. emetine), all characteristics that distinguish it from *E. histolytica* and *E. dispar*(30,31,32). Up to present *E. moshkovskii* has rarely been shown to infect humans (33), however a recent study reported a high prevalence (21.1%) of *E. moshkovskii* has been reported in young children from Bangladesh (34). Also *E. moshkovskii* was recently detected in patients between 31 and 50 years of age presented with gastrointestinal symptoms in Australia (35).

To date, human isolates of this species has been reported from many studies all over the world such as South Africa 13% (36), Australia 24.7% (35), India 1.9%(37). These and other studied showed that, at least in some parts of the world, *E. moshkovskii* may be a true human parasite. In Iran, a low prevalence of infection with *E. moshkovskii* of 1.1% was recorded in a healthy person (20). A recent study in Iran reported that out of 3,825 stool samples examined using single-round PCR assay 2(3.5%), 53(91.4%) and 2(3.5%) were reported positive for *E. histolytica*, *E. dispar* and *E. moshkovskii*, respectively. One person (1.73%) had mixed infection of *E. dispar* and *E. moshkovskii*(27). In this study presence of *E. moshkovskii* was confirmed by sequencing. A latest one study in Iran showed that Out of 16 person infected with *E. histolytica*/*E. dispar*/*E. moshkovskii* cysts by microscopic examination in Khorramabad, single-round PCR showed from sixteen samples that were microscopically positive, 1 (6.25%) was *E. moshkovskii*, and 15 (93.75%) were *E. dispar*(28). Considering of this study we suggest that infection with *E. moshkovskii* may be common among the Iranians. This is an important point in molecular epidemiology of the amoeba in Iran.

Despite using PCR for the detection of *E. moshkovskii* in Iran, no attempt was done for its detection by culture methods.

Other intestinal amoeba

Various intestinal amoeba species (e.g. *Entamoeba coli*, *Entamoeba bartmani*, *Iodamoeba butchlii* and *Endolimax nana*) are frequently found in the stool of human in Iran. A prevalence rate between 1% to >70% was reported in different population around Iran (5, 1, 9). Although these amoebas spp. are considered to be harmless, care should be taken to avoid mistaken diagnosis with *E. histolytica*, the causative agents of amoebiasis. There is no reliable data on the molecular epidemiology of the nonpathogenic intestinal amoeba in Iran and all surveys previously done were based on the detection of cysts or trophozoites in stools by using light microscopy.

Discussion

Intestinal protozoan infections (e.g. *Entamoeba* species) are still a public health concern in Iran. There are no comprehensive data on the epidemiology of *E. histolytica* in Iran especially since the separation of *E. histolytica* and *E. dispar* species. The most probable explanation of this condition is due almost to the reliance of all surveys previously done on stool examination by microscopy, not only in Iran, but also in the other parts of the world, because the current epidemiology of amoebiasis is confusing, mainly due to the recently appreciated distinction between the *E. histolytica*, *E. dispar* and *E. moshkovskii*.

A majority of asymptomatic individuals with cysts detected in their stool were actually infected with non-pathogenic *E. dispar*. Based on molecular study, It seems that *E. dispar* is the predominant species in Iran and amoebiasis due to *E. histolytica* is a rare infection in Iran (16, 18, 20), but the definite and true prevalence rate of *E. histolytica* still has become a matter of speculation. Identification of the true prevalence of *E. histolytica* infection in the community is very important to predict the clinical burden of amoebiasis. Thus, the future molecular epidemiological study should be done to determine the true prevalence of *E. histolytica* and *E. dispar*, especially in the tropical area of Iran. Although previous studies revealed that Zymodemes of *E. histolytica* were II and XIV and, zymodemes of *E. dispar* were I, XVI, XVII and XVIII in Iran, subsequent studies suggested that only four zymodemes are valuable for isolate typing (Zymodem II, XIV and XIX for *E. histolytica* and only zymodem I in *E. dispar*) (12,38,39). The others zymodemes appear to be of bacterial rather than amoebal origin. The remaining zymodemes are due to interaction with bacterial enzymes that present in xenic cultures (40). However zymodemes typing have a very important method in recognition of *E. dispar* as a separate species.

According to the results of the study on *E. moshkovskii* in Iran we suggest that human infection with this species may be common among Iranians. This may be considered as an important

point in the molecular epidemiology of amoeba in Iran.

It has been well established that differential diagnosis of some harmless human amoeba such as *E. hartmanni* and other intestinal amoeba may be difficult and controversial. So, molecular diagnostic tools can be useful for the accurate diagnosis of infection with these protozoa.

Based on our knowledge of human intestinal protozoa epidemiology, water and waste water has an important role in transmission of amoeba to human, so these amoebas are considered as the waterborne parasites. Investigating the occurrence of *Entamoeba* species in surface and waste water and also the molecular identification of them in Iran is necessary.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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