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Full Length Research Paper

Differential diagnosis of *Entamoeba* spp. in gastrointestinal disorder patients in Khorramabad, Iran

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Differential diagnosis of *Entamoeba* spp. has great clinical and epidemiological importance. *Entamoeba moshkovskii* cysts and trophozoites are morphologically indistinguishable from *Entamoeba dispar* and *Entamoeba histolytica*. This study was carried out for the first time to detect *Entamoeba* spp. in stool samples by using molecular method from April 2010 to December 2010 in Khorramabad, Iran. A total of 862 fecal specimens were collected from patients having abnormal gastrointestinal symptoms and who were referred to the health care centers of Khorramabad. Out of 862 stool samples, 16 (1.86 %) showed the presence of *E. histolytica/E. dispar/E. moshkovskii* cysts by microscopic examination. Consequently, single-round PCR was carried out to differentiate the *Entamoeba* spp. OF the sixteen samples that were microscopically positive, 1 (6.25%) was *E. moshkovskii*, and 15 (93.75%) were *E. dispar*. Our results, along with those of other similar study conducted in different parts of Iran, reveal that *E. dispar* is more prevalent.

Key words: *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii,* single-round PCR, Iran.

INTRODUCTIONS

Amoebiasis is still one of the main health problems in tropical and sub tropical regions with low health and economic level (Simonetta et al., 2003). Since micro-scopic diagnosis in differentiation of these species has a low sensitivity and accuracy, so we need much more accurate methods for differentiation of these species (Nazemalhosseini-Mojarad et al., 2010a).

For a tenth of people, *E. histolytica* become an invasive disease and one hundred thousand people die because of amoebiasis disease which makes it the second fatal protozoan disease in the world after malaria (WHO, 1997; Diamond and Clark, 1993).

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Studies have shown that much infection in the world is for *E. dispar* (Heckendorn et al., 2002; Clark, 1998). Also studies in Iran confirmed that prevalence of *E. dispar* is much more than *E. histolyica* (Hooshyar et al., 2004; Nazemalhoseini-Mojarrad et al., 2007; 2010a).

Entamoeba moshkovskii cysts and trophozoites are morphologically indistinguishable from those of the non-pathogenic *Entamoeba dispar* and *Entamoeba histolytica* that is causative agent of amoebiasis and can therefore confound annotation of stool microscopy (Ali et al., 2003). The organism is mainly free-living amoeba that rarely infects humans (Clark and Diamond, 1991; Clark and Diamond, 1997). A high incidence of *E. moshkovskii* infections was reported in stool specimens from Bangladesh and Australia and Turkey (Ali et al., 2003; Foteder et al., 2008; Tanyuksel et al., 2007). Three *E. moshkovskii* infections and one simultaneous infection

with *E. moshkovskii* and *E. dispar* have also been reported in Iran (Nazemalhosseini-Mojarad et al., 2010a; Solaymani–Mohammadi et al., 2006). An expert consultation on amoebiasis in Mexico City recommended the collection of accurate new data on the prevalence of *Entamoeba* species for planning rational control strategies (WHO, 1997). Differential diagnosis of *E. histolytica, E. dispar and E. moshkovskii* has great clinical and epidemiological importance (Nazemalhosseini-Mojarad et al., 2010a). One of the most important advantages of this differentiation is avoiding unnecessary treatment with anti-amoebic chemotherapy and decreasing economic cost, side effects and drug resistance (WHO, 1997; Diamond and Clark, 1993). This study was carried out for the first time to detect *E. moshkovskii, E. histolytica* and *E. dispar* in stool samples from patients clinically suspected to have gastrointestinal infections by using of single–round PCR in Khorramabad, Lorestan province, Iran.

MATERIALS AND METHODS

Study area and clinical samples

The descriptive study was conducted from April 2010 to December 2010 in Khorramabad which is located between valleys of Zagros Mountain at the west of Iran. A total of 862 fecal specimens were collected from patients having abnormal gastrointestinal symptoms (such as diarrhea, abdominal pain, nausea and flatulence) who were referred to the health care centers of Khorramabad, Iran. The stool samples were examined microscopically by using direct slide smear, lugol's iodine, formalin–ether concentration, and trichrome staining. The suspected samples were stored at -20°C for later use.

DNA preparation

Genomic DNA was extracted directly from stool specimens were microscopically positive by using a QIAamp® DNA Stool Kit (QIAGEN) according to the manufacturer's instructions. The extracted DNA was stored at -20°C until PCR amplification.

PCR reaction

A single–round PCR reaction and set of primers were used as described previously (Hamzah et al., 2006). The sequence of the forward primer used was conserved in all three *Entamoeba* spp., but the reverse primers were specific for apiece. The expected PCR products from *E. histolytica, E. dispar* and *E. moshkovskii* were 166 bp, 752 bp, and 580 bp, respectively (Hamzah et al., 2006).

Amplification of each species–specific DNA fragment started 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 7 min (Hamzah et al., 2006). PCR products were visualized with ethidium bromide staining after electrophoresis on 1.5% agarose gels. DNA isolated from axenically grown *E. histolytica KU2*, *E. dispar AS 16 IR* and *E. moshkovskii Laredo* (ATCC accession no. 300 42) (Nazemalhosseini-Mojarad et al., 2010a) were used as positive controls. The study was approved by Medical Research Ethics Committee of Lorestan University of Medical Sciences.

RESULTS

Out of 862 stool specimens, 359(41.65%) of whom were female and 503(58.35%) male, 16(1.86%) showed the presence of *E. histolytica/E. dispar/E. moshkovskii* cysts by microscopic examination. After DNA extraction, the single-round PCR was carried out to differentiate the *Entamoeba* spp. of the sixteen samples that were microscopically positive, 1 (6.25%) was *E. moshkovskii* and 15 (93.75%) were *E. dispar*. Infection of *E. histolytica* was not observed in this study. Amplification produced fragments of 752bp and 580bp corresponding to the expected products from *E. dispar* and *E. moshkovskii*, respectively (Figure 1).

DISCUSSION

E. histolytica, E. dispar, and *E. moshkovskii* are morphologically indistinguishable from each other, except that trophozoites containing ingested red blood cells are more likely to be *E. histolytica* (Hamzah et al., 2006; Haque et al., 2008; Parija and Khairnar, 2005). Because previous studies have shown that *E. moshkovskii* could infect humans, identification of this protozoan parasite to the species level has importance (Ali et al., 2003). *E. moshkovskii* from human samples has already been reported in Bangladesh, India, Australia, Turkey, and Iran (Ali et al., 2003; Tanyuksel et al., 2007; Nazemalhosseini-Mojarad et al., 2010a; Solaymani–Mohammadi et al., 2006; Parija and Khairnar, 2005; Fotedar et al., 2007a, b). 109 stool samples from preschool children in Bangladesh were tested by PCR, 17 (15.6%) were positive for *E. histolytica*, 39 (35.8%) positive for *E. dispar*, 23 (21.1%) were positive for *E. moshkovskii* infection (Ali et al., 2003). In another study 746 stool specimens from patients with gastrointestinal disorder in India were screened, prevalence of *E. dispar*, *E. moshkovskii* and *E. histolytica* were 8.8, 2.2 and 1.7%, respectively (Parija and Khairnar, 2005).

This is the first study to detect *Entamoeba* spp. in stool samples from patients clinically suspected to have gastrointestinal infections in Khorramabad, Lorestan province, Iran. Our results, along with those of other similar study conducted in different parts of Iran, reveal that *E. dispar* is much more prevalent than *E. histolytica* (Nazemalhosseini-Mojarad et al., 2010a, b; Hooshyar et al., 2004; Nazemalhoseini-Mojarrad et al., 2007). From central, southern, and northern Iran in both urban and rural areas 16,592 stool samples were collected and results showed that the average prevalence of infection with *E. histolytica/E. dispar* is 0.78, 4.6 and 3.9%, respectively (Hooshyar et al., 2004).

Also PCR-RFLP analysis showed that 92.1% were *E.*

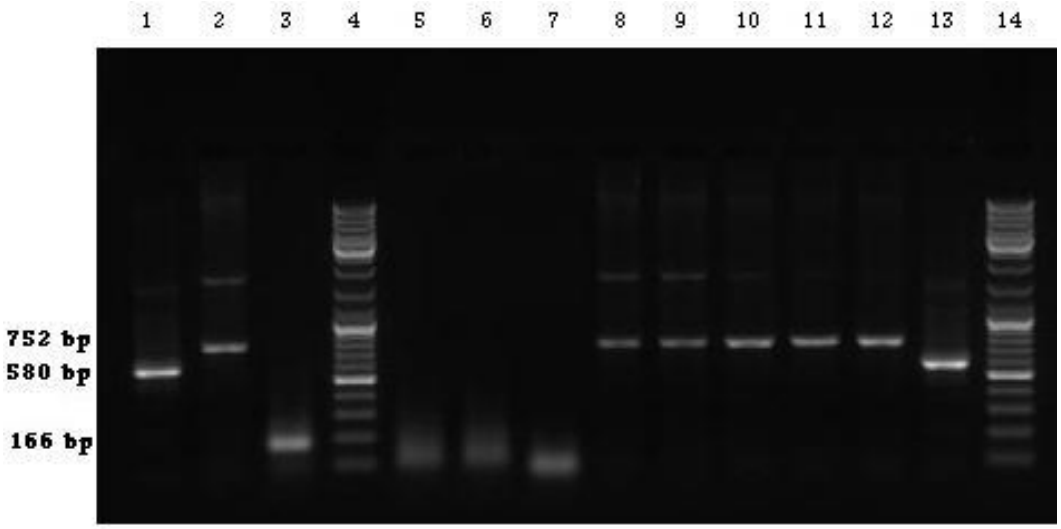

Figure 1. Agarose gel electrophoresis of Entamoeba species using single–round PCR.
Lane 1: *E. moshkovskii* – positive isolate; Lane 2: *E. dispar* – positive isolate; Lane 3: *E. histolytica* – positive isolate; Lane 4, 14: 100bp DNA ladder marker; Lane 5: *E. dispar* negative control; Lane 6: *E. moshkovskii* negative control; Lane 7: *E. histolytica* negative control; Lane 8-12: *E. dispar* isolates ; Lane 13: *E. moshkovskii* isolate.

dispar and only 7.9% were *E. histolytica* and/or mixed infections. The actual prevalence of *E. dispar* and *E. histolytica* in Iran was reported to be 92.7 and 7.3%, respectively (Hooshyar et al., 2004). Similar studies conducted in different countries revealed that the prevalence of *E. dispar* infection is much more than *E. histolytica* (Heckendorn et al., 2002; Clark, 1998). *E. dispar* was estimated that is the cause of 90% of infections in humans with *Entamoeba* spp. (Tanyuksel and Petri, 2003). On the other hand, in some parts of the worlds, such as Mexico and Japan the prevalence of *E. histolytica* infection is high (Petri et al., 2000; Tachibana et al., 2000). Also we found one *E. moshkovskii* isolate in 16 PCR-positive samples that was obtained from a dysenteric stool specimen in which common bacterial agents of dysentery by routine stool culture was negative. Viral dysentery is not common in Iran (Nazemalhosseini-Mojarad et al., 2010a), so this finding supports the opinion that *E. moshkovskii* may has a role in the developing of gastrointestinal symptoms (Tanyuksel et al., 2007; Nazemalhosseini-Mojarad et al., 2010a). But it is not clear *E. moshkovskii* caused the observed symptoms. Some other studies have proposed *E. moshkovskii* to be likely an entropathogen in patients presenting with gastrointestinal symptoms (Foteder et al., 2008; Nazemalhosseini-Mojarad et al., 2010a; Parija and Khairnar, 2005). out of 3,825 stool samples were collected from patients with gastrointestinal disorders in Iran, 2 *E. moshkovskii* (3.45%), and one mixed *E. dispar/E. moshkovskii* infection(1.73%) were detected from dysenteric stool specimens by single–round PCR (Nazemalhosseini-Mojarad et al., 2010a). More reliable data on the prevalence and pathogenesis of *E. moshkovskii* infection are needed to discern the potential role of this amoeba as an entropathogen (Tanyuksel et al., 2007). Conversely, some other reports support the commensal nature (Beck et al., 2008). Solaymani–Mohammadi et al. (2006) found one *E. moshkovskii* from apparently healthy person in Iran (Solaymani–Mohammadi et al., 2006).

Several molecular diagnostic tests have been developed for detection and discrimination of the three morphologically indiscernible *Entamoeba* spp. found in humans, such as conventional and real time PCR, nested multiplex PCR , and single–round PCR assay (Hamzah et al., 2006; Fotedar et al., 2007a, b). In the present study, direct DNA extraction from stool was used, which was recommended by the World Health Organization (WHO/PAHO/UNESCO report, 1997).

By using direct DNA extraction, there is no need to culture; moreover this method is very simple, fast and specific. By using direct DNA extraction from stool sample and with carrying out single-round PCR, the detection of infection is so fast. Because of morphological similarities of *Entamoeba* spp. and lack of differentiation of them in stool samples of macroscopic experiments, using single–round PCR which is a specific method, and also using direct DNA extraction of stool specimen which is an easy, fast and specific method is suggested for routine diagnosis of disease and epidemiological studies (Nazemalhosseini-Mojarad et al., 2010a; Hamzah et al., 2006; Clark, 1998).

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