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Frequency of Intestinal Parasites in Patients With Gastrointestinal Disorders, in Different Parts of Iran During 2012-2013

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Background: Intestinal parasites of humans are one of the most important health problems worldwide, especially those located in tropical and subtropical areas.

Objectives: The aim of this study was to determine the frequency of intestinal parasites in patients with gastrointestinal disorders, in different parts of Iran.

Patients and Methods: A total of 1520 stool samples were collected from patients with gastrointestinal disorders. The stool specimens were examined by direct wet mount, formalin-ether concentration and a modified version of the Ziehl-Neelsen staining technique. Amoeba-positive samples were cultured for further differentiation of *Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*. DNA-based methods were used to differentiate these amoebas and to detect Cryptosporidium- positive samples. Statistical analysis was carried out by SPSS ver. 16.

Results: Out of the 1520 individuals studied, 153 (10.06%) were infected at least with one intestinal parasite. 781 (51.4%) of patients were male and 738 (48.6%) were female. The prevalence of protozoan parasites 148 (9.7%) was significantly higher than helminth parasites 5 (0.3%) ($P < 0.001$). The frequency of intestinal parasites was as follows: *Blastocystis sp*., 72 (4.73%); *Giardia intestinalis*, 35 (2.30%); *Entamoeba coli* 21 (1.38%); *Endolimax nana* 10 (0.92%); *Cryptosporidium spp*., 1 (0.06%); *Entamoeba dispar*, 1 (0.06%); *Dientamoeba fragilis*, 1 (0.06%); *Hymenolepis nana*, 3 (0.19%); *Dicrocoelium dendriticum*, 2 (0.13%). In five (0.32%) of the positive samples, co-infections with two parasites were found. *G. intestinalis* was more prevalent in male 22/35 (62.86%) than female 13/35 (37.14%) as well as in 0-9 years old group. In one sample *Heterodera ova* contained larva were seen.

Conclusions: *Blastocystis and G. intestinalis* were the predominant intestinal parasites detected in patient with gastrointestinal disorders. The results indicated that the intestinal parasites, particularly helminth infections have been significantly declined in recent years.

Keywords: Parasitic Intestinal Disease; Gastrointestinal Diseases; Frequency; Iran

1. Background

Intestinal parasitic infections are being considered among the most common infections in the world. It was estimated that 60% of people worldwide were infected with intestinal parasites (1, 2). Recent estimates indicate that at least more than one-quarter of the world's population are infected with intestinal parasites and most of them are people who live in developing countries (2, 3). These parasites can cause severe illness and the mortality in endemic areas (4). Parasitic diseases are important hygiene problems and the impediment to economic and social development in many countries, particularly in developing world. Poverty, illiteracy, low levels of hygiene, lack of approach to safe drinking water and humid tropical climate are the most common factors of intestinal infections. In Turkey, people who had unsanitary disposal of feces the prevalence of pathogenic parasites were high (5).

Parasitic infections of the gastrointestinal tract are a major cause of morbidity in developing countries and are increasingly important in certain populations from the developed countries (6). Approximately 39 disability adjusted life years (DALY) has been ascribed to intestinal parasitic infections (7, 8). Therefore represents a considerable economic burden and death particularly among invasive amoebiasis patients (8). Epidemiology of intestinal parasitic infections suggests that these parasites are found in every age group and both sexes. But in some areas, the incidence is higher in children (9). Intestinal parasitic infections caused by worms and protozoan par-

asites are the most common human infections in developing countries (10).

The pathogenic protozoa *Entamoeba histolytica*, *Giardia intestinalis*, and *ryptosporidium spp.* have a worldwide distribution and are a common cause of diarrhea in human. In Pakdasht, Tehran Province, protozoan parasites infections were reported the highest rate (41.5%) (11). In Ardabil, northwestern Iran, a total of 10 (14%) species were identified with *Giardia intestnalis*, *Blastocystis hominis* (10%) and *Entamoeba coli* (4.1%) being the most common parasites (12). According to the World Health Organization estimates, 50 million of people worldwide suffer from invasive amoebiasis per annum (13). It is prevalent in West and South-east Africa, China, and Mexico (14). *E. histolytica* is more abundant in the tropical and subtropical, however, the range of infection in temperate climates and cooler, with poverty of sanitary is the same as what is found in tropical climate. As in the United States of America, *G. intestinalis* is the most common intestinal parasite of humans; also it is a very common infection in developing countries (10).

In recent years, the isolation of parasitic DNA from fecal samples and PCR techniques, have been improved and simplified. Moreover, the introduction of real-time PCR has made it possible to multiplex different targets into one reaction (14). Recently, in different parts of Iran, several studies have been conducted to reveal the intestinal parasites prevalence. According to literature review, there is a sharp decline in the prevalence of human helminthes infections (15).

2. Objectives

The aim of the study was to determine the frequency of intestinal parasites in patients with gastrointestinal disorders, in different parts of Iran.

3. Patients and Methods

3.1. Population Study

During April 2012 to May 2013, by non-randomize simple sampling selection, 1520 stool samples were collected from patients with gastrointestinal disorders who had been referred to clinical laboratories of: Fardis central laboratory, Fardis, Shahid Taleghani hospital, Tehran, Health care centers of Amol and Varamin and Tamin Ejtemaee hospital, Ahvaz, Iran (Table 1).

Table 1. Clinical Laboratories Which Stool Samples Were Collected From Patients With Gastrointestinal Disorders

Location	No. (%)
Fardis central laboratory, Fardis	1115 (73.4)
Shahid Taleghani hospital, Tehran	53 (3.4)
Health care center, Amol	72 (4.7)
Health care center, Varamin	10 (0.7)
Tamin Ejtemaee hospital, Ahvaz	270 (17.8)
Total	1520 (100)

3.2. Stool Examination

3.2.1. Direct Microscopy (Wet Mount)

The stool samples were macroscopically examined for consistency, color, the presence of blood and mucus, adult intestinal helminthes. All specimens examined by using normal saline (0.85% NaCl) for the presence of trophozoites and lugol's iodine staining was used for detection of cysts of intestinal protozoa under light microscope (× 400).

3.2.2. Formalin-Ether Concentration

About, 5 g of preserved stool sample by using an applicator was put in a clean beaker containing 7 mL formalin. The sample was dissolved and blended thoroughly with applicator stick. The resulting suspension was filtered through cotton gauze into a 15 mL conical centrifuge tube. The debris captured on the sieve was discarded. After adding 3 mL of ether to the mixture and hand shaken, the content was centrifuged at 2000 rpm for 3 minutes. The supernatant was discharged. The pellets put on slide and covered with cover slip. The complete area under the cover slip was examined using 100 objective magnifications. Also the sediments were examined for the ova of helminthes, as well as for the cysts and the trophozoites of protozoa under light microscope (× 400).

3.2.3. Ziehl-Neelsen Staining

Second smear from sedimentation was made and stained using the modified Ziehl-Neelsen staining method for the detection of *Cryptosporidium*. Finally, the stained smears were microscopically examined using 1000x magnification (16).

3.3. Culture Method

Positive stools specimens of amoeba were cultured in Horse Serum ringer- starch medium (HSr-s) (17) to detect the presence of *Entamoeba histolytica|dispar|moshkovskii*. Cultures were incubated at 35-37°C for four days, and a drop of culture medium was examined under a 400 × objective magnification.

3.4. Polymerase Chain Reaction (PCR)

DNA-based method used to detect the three species of amoeba. Genomic DNA was extracted from samples using the QIAamp DNA stool mini kit (QIAgen, Germany). 100 mg of the positive stool samples of infected patient mixed with 1 mL distilled water (DW) in a 1.5 mL micro tube, washed with DW twice by centrifugation at 4000 rpm and 14000 rpm for *Entamoeba* and *Cryptosporidium*, respectively. Then extraction of DNA from pellets was done according to the manufacture's recommendation. The multiplex PCR assay was used to detect and differentiate the *Entamoeba spp.* A conserved forward primer

Table 2. Details of Oligonucleotide Primers Used in This Study

Protozoan Parasites/Primer Name and Sequence	Amplicon Size	Annealing Temperature
Entamoeba histolytica/E. dispar/E. moshkovskii		55°C
EntaF: 5'-ATGCACGAGAGCGAAAGCAT-3'		
E. histolytica		
EhR: 5'-GATCTAGAAACAATGCTTCTCT-3'	166 bp	
E. dispar		
EdR: 5'-CACCACTTACTATCCCTACC-3'	752 bp	
E. moshkovskii		
EmR: 5'-TGACCGGAGCCAGAGACAT-3'	530 bp	
Cryptosporidium	749 bp	55°C
Cry F: 5'-CTGACCTATCAGCTTTAGA-3'		
Cry R: 5'-GCTGAAGGAGTAAGGAACA-3'		

Table 3. Prevalence of Intestinal Parasites in Patients With Gastrointestinal Disorders, in Different Parts of Iran

Parasites	No. (%)
Protozoa	148 (9.7)
Blastocystis spp.	72 (4.73)
Giardia intestinalis	35 (2.30)
Entamoeba coli	21 (1.38)
Endolimax nana	10 (0.92)
Entamoeba dispar	1 (0.06)
Cryptosporidium spp.	1 (0.06)
Dientamoeba fragilis	1 (0.06)
Iodomoeba butschlii	1 (0.06)
Chilomastix mesnili	1 (0.06)
Helminthes	5 (0.3)
Hymenolepis nana	3 (0.19)
Dicrocoelium dendriticum	2 (0.13)
Co-infection	5 (0.3)

was derived from the middle of the small-subunit rRNA gene, and reverse primers were designed from signature sequences specific to each of these three *Entamoeba* species. The sequence of a forward primer used was conserved in all three *Entamoeba spp*., whereas the specific reverse primers were specific for *E. histolytica*, *E. dispar*, *E. moshkovskii*, respectively (Table 2) (18). Also we used the single-plex PCR for detection of *Cryptosporidium*. Forward and reverse primers were derived from the small-subunit rRNA gene (Table 2) (19).

PCR was performed using amplicon (Bie and Berntsen A/s, Denmark) as a ready-made solution. The reaction mixture contained 5 μL distilled water, 7.5 μL amplicon, 20 pmol forward and reverse primers, and about 5-10 ng of extracted DNA template to achieve a final volume of 15 μL. Amplification of each species-specific DNA fragment started with an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. Amplified products were visualized after electrophoresis on 1.5% agarose gels.

3.5. Statistical Analysis

All data from stool study was collected and analyzed with Statistical Package for the Social Sciences (SPSS) version 16.0. Statistical significance was determined with the chi-square (x^2) test. Data analyzed allowed us to compare prevalence of intestinal parasites compared according to age and sex.

4. Results

Out of 1520 studied individuals, 153 stool specimens (10.06%) were infected with intestinal parasites. Of these, 782 (51.4%) patients were male and 738 (48.6%) patients were female in the age range of 1-92 years old. Microscopic examination of stool specimens showed that there was a significantly higher prevalence ($P < 0.001$) of protozoan parasites 148 (9.7%) in compare to the helminthic infection 5 (0.3%). The positive rate of intestinal parasites was: *Blastocystis spp*. 72 (4.73%), *Giardia intestinalis* 35 (2.30%), *Entamoeba coli* 21 (1.38%), *Endolimax nana* 10 (0.92%), *Cryptosporidium spp*. 1 (0.06%), *Entamoeba dispar* 1 (0.06%), *Dientamoeba fragilis* 1 (0.06%), *Iodamoeba butschlii* 1 (0.06%), *Chilomastix mesnili* 1 (0.06%), *Hymenolepis nana* 3 (0.19%), *Dicrocoelium dendriticum* 2 (0.13%) (Table 3). *Heterodera ova* along with its larva were seen in one of the samples. In five (0.32%) of the positive samples mixed infections with 2 or three parasites were found. Prevalence of *G. intestinalis* was highest in the ≤ 1-10 years age group 15/35 (42.86%), and in males and females was 22/35 (62.86%) and 13/35 (37.14%) respectively. From the single-round PCR amplification, we obtained the following products with the predicted sizes: 752 bp and 749 bp for *E. dispar* and *Cryptosporidium*, respectively (Figure 1). The microscopy result of positive *cryptosporidium spp*. was confirmed with the PCR method.

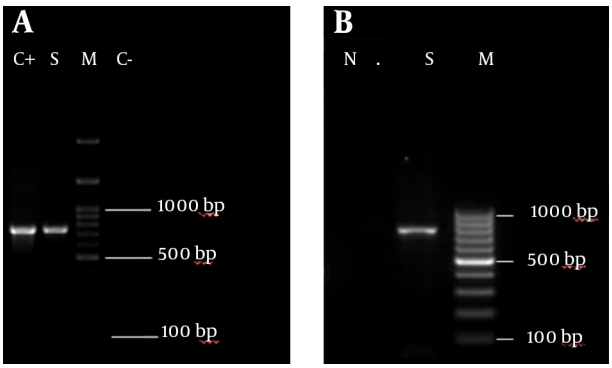

Figure 1. Single-Round PCR Amplification Products With the (A) 752 bp and (B) 749 bp Sizes for *E. dispar* and *Cryptosporidium spp*. Respectively

5. Discussion

Intestinal parasites comprise some of the most common and important parasites of human and they are regarded as an important public health problem of most communities, especially those situated in tropical and subtropical areas. However, patterns of intestinal parasites infections in the population may be altered on account of changes in man behavior and life styles during a time and prevalence of these varies with different geographical regions (7, 20, 21).

Several studies have carried out on prevalence of intestinal parasites in Iran. The current study was performed to determine the frequency of the parasitic infections during 2012-2013 in patients with gastrointestinal disorders. The prevalence of intestinal parasites was 10.06%. Our results showed that the prevalence of protozoan infection (9.7%) was higher than the (0.3%) of helminthes infection. The higher incidence rate of protozoan than helminthes may be attributed to the fact that being infected with the protozoan cyst through contaminated water and food will be readily, thus make easy their acquisition of the infections. Some studies from Iran and some parts of the world found that intestinal protozoan infection had higher prevalence than intestinal helminthes infection (12, 22-25).

The relatively high incidence of intestinal protozoan infections in the study highlights the methods of preventing the transmission and their expansion must be considered. Results of this survey in the population in order of protozoa frequencies indicated that *Blastocystis spp*. (4.73%) and *G. intestinalis* (2.3%) were the most commonly detected organisms. Several studies in other areas of Iran have previously revealed rates of intestinal protozoan parasitic infection of 0.5 to 30% (21, 26-31). In period of 2004-2005, the distributions of intestinal protozoan parasites were characterized, in rural population of Mazandaran, northern Iran and results proved that the *G. intestinalis* (10.2%) and *Blastocystis spp.* (9.8%) were the most detected protozoan (22).

Based on our findings the frequency of *G. intestinalis* was highest in children with peak values among the ≤ 1-10 years age group (42.86%). Also in the sex-related incidence and intensity of the infection, male gender 22/35 (62.86%) recorded a higher incidence and intensity of the infection than female gender 13/35 (37.14%). However age and sex did not show any significant correlation with the prevalence of *G. intestinalis* ($P > 0.05$).

In this study for one microscopy-positive *E. histolytica|E. dispar|moshkovskii* sample, multiplex PCR was performed for the accurate differential diagnosis of *Entamoeba spp*. It was identified as *E. dispar.* Studies show that *E. dispar* is maybe 10 times more prevalent than *E. histolytica* worldwide (32). It seems that *E. dispar* is main species particularly in the central and northern areas of Iran and amoebiasis as a result of *E. histolytica* is a rare infection in the country. Haghighi et al. (27) showed that of the eight microscopy-positive *E. histolytica|E. dispar* samples, six were identified as *E. dispar* by PCR/gel electrophoresis, whereas *E. histolytica* was not detected at all. Nazemalhosseini Mojarad et al. in a study among patient with gastrointestinal complaint indicated that of 22 microscopy-positive samples for *histolytica|E. dispar* complex, 21 (95.45%) samples were diagnosed as *E. dispar* by PCR (33).

In our study, *Cryptosporidium* as a coccidian parasite was detected by microscopy (modified Ziehl Neelsen staining method) in one patient (0.06%). We also used DNA-based methods to detect *Cryptosporidium spp*., when this case was analyzed with single round PCR; the presence of *Cryptosporidium* was confirmed. Ghobadi and et al. from Sanandaj, Iran reported 8% frequency for *Cryptosporidium* in their study (34). Kia et al. and Berenji et al. in their studies did not find any *Cryptosporidium* in patients (21, 24). Currently, there are various methods for the detection of *Cryptosporidium*, including special staining, IFAT, and molecular techniques. In the present study, among helminthic intestinal infection we found *Hymenolepis nana* 3 (0.19%), *Dicrocoelium dendriticum* 2 (0.13%). *Heterodera ova* along with its larva were also seen which is consistent with Ashrafi study (35).

With regard to the egg of these parasites in fecal samples can not necessarily confirm the real infection, therefore, the foregoing report is provided only for laboratory observations. Meanwhile, due to the lack of adequate access to information about their supply of *Dicrocoelium* eggs found in the feces of patients cannot be accurately commented on the true occurrence of the infection caused by this parasite.

Blastocystis and *G. intestinalis* were the predominant intestinal parasites detected in patient with gastrointestinal disorders. The results indicated that the intestinal parasites, particularly helminth infections have been significantly declined in recent years.

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