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Research Article
Identification of Serious Clinical Amebic Dysentery Cases in the Middle Euphrates Region of Iraq

[1]Hadi Fadhil Alyasari, [2]Hayder O. Hashim, [2]Alaa Hamady Obeid Altaei and [3]Mohammed Baqur S. Al-Shuhaib

[1]College of Medicine, Babylon University, Babil 51001, Iraq
[2]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Babil 51001, Iraq
[3]Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil 51001, Iraq

Abstract

Background and Objective: A lot of enteric parasites are responsible for causing morbidity and mortality outcomes in the worldwide individuals, especially in poor hygienic countries. The present study was conducted to detect amebic dysentery-causing *Entamoeba* species in the middle Euphrates regions in Iraq. **Materials and Methods:** A total of 155 diarrhea admitted-females (aged from 10-70 years old) who underwent parasitological examination were included in the study. After its classical confirmation, the presence of amebic dysentery was detected by PCR. **Results:** It was found that the overall prevalence of amoebic species infection in the infected females was 62% (96/155). Comparison of age groups showed that 30-39 aged females had a more susceptibility rate than other age groups since the highest levels of amoebic infection were shown in the 30-39 aged females. Three forms of amoebic infection were observed, including *E. histolytica, E. dispar* and *E. coli*, which, as long as other painful clinical symptoms were concentrated in rural and low educated level of the studied areas. **Conclusion:** This pilot present study discovered a remarkable percentage of amoebic dysentery infection in the females aged 30-39 years old, which may imply serious precautions for this group throughout the developing world.

Key words: Dysentery, PCR, bloody diarrhea, *Entamoeba* species, enteric parasites, amebic dysentery, co-infection

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Corresponding Author: Mohammed Baqur S. Al-Shuhaib, Department of animal production, College of agriculture, Al-Qasim Green University, Al-Qasim, Babil 51001, Iraq Tel: 009647707115693

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Enteric parasites are responsible for causing intestinal disorders, various morbidities and mortalities among a lot number of individuals worldwide, especially in low-income countries and in low hygienic levels and a large number of infectious diseases can be transmitted by many and various contamination routes and eventually reach to human beings and their own animals to cause effective diseases[1]. The protozoan parasite *Entamoeba histolytica* is one of the main alimentary tract protozoa of human beings[2]. There area variety of species pertaining to the genus *Entamoeba* and all these species can infect human's alimentary tract, including *Entamoeba histolytica*, *Entamoeba coli*, *Entamoeba hartmani*, *Entamoeba dispar*, *Entamoeba gingivalis,* and *Entamoeba polecki*[3]. The *Entamoeba histolytica* caused amoebiasis, which is a global health problem as it is responsible for more than 100,000 deaths per year and it is the second leading cause of global death due to protozoa after malaria[4,5]. *Entamoeba histolytica* is an enteric protozoa parasite that lives as a colonizer in the lumen of the human intestinal tract and has the ability to destroy the epithelium tissue. Most frequently observed clinical symptoms of this disease are dysenteric diarrhea, alternating with periods of constipation, flatulent stomach, colitis, fatigue, fever, malaise, abdominal pain and weight loss[6]. Extra-intestinal infection of amoebiasis is taken place by parasite when invading other organs, such as lung, bone and brain to cause abscesses in these invaded organs[7]. Thus, several experimental methods have been used to obtain accurate and reliable diagnostic outcomes for amoebiasis detection[3,7-9]. The diagnosis of enteric amoebiasis is primarily based on the detection of parasites in the smear, but in the case of epidemiological complication by *E. histolytica* that accompanied with other amoebic species or enteric parasite, the claimed infection may be fully realized only if there is an accurate method that can distinguish the targeted species among all other closely relatedorganisms[10]. This notion is particularly applicable when great numbers of related organisms are encountered in the smear[8,11,12]. Though the preliminary diagnosis of this parasite relied on the detection of cysts and trophozoites of *E. histolytica* with a light microscopy-based technique[4], such general identifications are no longer reliable as it's so difficult to distinguish between two identical morphologies of two closely related organisms, as in the case of pathogenic *E. histolytica* and non-pathogenic *E. dispar*. Thus, the genetic option is the best applicable tool to differentiate between the closely related parasites that live in the same intestinal habit[13]. Moreover, the highly specific molecular techniques have been widely available nowadays to differentiate between extremely related micro-organisms, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)[12]. However, several recent diagnostic methods for *E. histolytica* in stool samples are costly because they need a long time and outclass microscope experts for detection[14]. However, PCR specificity is usually greater than that of other techniques, such as ELISA, which command isolation or antigen capture procedures. The PCR tool still the method of choice particularly in the low budget laboratories[13,15]. So that, PCR is a highly discriminating technique between the pathogenic *Entamoeba* species from that of non-pathogenic counterpart sinan accurate diagnosis for the causative agent of dysenteric amoebiasis. Though PCR techniques have been widely been used in the accurate identification of such organisms, it's still limited in detecting the age of the assessed samples. On the other hand, though several reports have measured several pathogenic *Entamoeba* species by implementing several infections-contributing factors, no sufficient data were available, in terms of dividing the accurate grouping of amoebic-infected patients[6-8,16,]. Therefore, the present manuscript aimed to combine the high accuracy of PCR detective power, with further details of the age of patients, to provide an accurate time management of patients ages regarding it as an essential contributing factor to assess the susceptibility of the infected females age to develop such amoebic dysenteries.

MATERIALS AND METHODS

Study population and sampling: A cross-sectional study was conducted from March 2014 to September 2016 and enrolling 155 amoebic dysentery-suspected females, aged from 10 to 70≥years, who attended to the Al-Zahraa Hospital for Maternity and Children in Al-Musaib district/Babel city, Iraq. The number of samples was155 of fresh stool. Out of 155 samples, 95 were confirmed to have amoebic dysentery on the basis of a previous SAF-fixed sample using conventional techniques, including the formalin-ethyl acetate concentration and iron hematoxylin staining[17]. All the procedures used in the present study received prior approval from the Committee on the use of Human Research Subjects at University of Babylon. Oral consent was obtained from all participants of the present study. All admitted infected females were itemized in specialized questionnaire papers. Most clinical symptoms and signs of the study infected females were reconfirmed by the aid of the specialist clinicians in the targeted study hospital and from all admitted hospital patient's records. The samples were preserved in 5%

potassium dichromate to avoid fungal growth and for the preservation of protozoa cysts and oocysts. Subsequently, cysts in stool samples were determined based on the protocols[4].

Extraction of *Entamoeba* genomic DNA: Genomic DNA was extracted from each microscopically positive faecal sample according to the manufacturers' instructions (QIAGEN, Hilden, Germany). The extracted DNA was quantified by a Nano drop (BioDrop-UK), then stored at -20°C until performing PCR amplification[13].

PCR: The PCR amplification was used to genetically characterize *E. histolytica* and *E. dispar* according to Khairnar and Parija[9], with several modifications. Both negative control (DNase-free water, Sigma Cat. No. W4502), and positive control (*Entamoeba* species genomic DNA) were included in each PCR run. The PCR was carried out in a 25 µL volume with the final mix containing 10×PCR buffer, 1.25 mM dNTPs, 25 mM MgCl2, 10 pmole of each primer, 2.5 U of *Taq* polymerase and 2.5 µL of DNA template. To detect *E. histolytica* (439 bp), amplification was carried out using primer sets EH-1 (5'-AAGCATTGTTTCTAGATCTGAG-3') and EH-2 (5'-AAGAGGTCTAACCGAAATTAG-3'). The samples were denatured at 96°C for 2 min, followed by 30 cycles of 92°C for 1 min (denaturing), 56°C for 1 min (annealing), 72°C for 1 min 30 sec (extension) and a final extension at 72°C for 7 min regarding the detection of *E. histolytica* (439 bp), amplification was carried out using the primers set ED-1 (5'-TCT AAT TTC GAT TAG AAC TCT-3') and ED-2 (5'-TCCCTACCTATTAGACATAGC-3'). The PCR conditions were performed with the following conditions; denaturation (95°C for 1 min), annealing (48°C for 1 min) and extension (72°C for 1 min). In both amplifications, samples were incubated in a thermal cycler (MyCycler, Bio-Rad, Hercules, USA). The generated PCR amplicons of *Entamoeba* species were subjected to electrophoresis in 2% agarose gelsand visualized in a UV Transilluminator (ChemiDoc-Bio-Rad, USA).

Statistical analysis: Detection of *Entamoeba* species was determined on the basis of morphological characteristics of the cysts under microscopy. The data entry and analysis was carried out using the SPSS software (Statistical Package for the Social Sciences) program for Windows ver. 17[18]. Qualitative data were estimated and presented as frequencies and percentage. The prevalence and 95% confidence intervals (CIs) were calculated for each parasite according to Ngui *et al.*[19]. The statistical percentages were estimated using Chi-Square and a p-value of 0.05 was regarded as an indicator for a statistical significant differences in the analyzed samples.

RESULTS

The present study had shown a high infection ratio caused by amoeba species to 30-39 years old (28%) and parallel low infection ratio (1%) at 70 years old (Table 1). Similarly, the amoebic infection had got the highest level (28%) in the age group 30-39 years old, while the low amoebic infection was at the age group of ≥70 years old (Table 2).

The current study has provided information about the clinical symptoms and signs of the patients-confirmed dysentery of Table 3. These results indicated a high level of abdominal pain (94.7%) and a parallel low level of clinical symptoms as sporadic constipation (8.3 %). On the other hand, the current study had obviously revealed a prevalence of three types of *Entamoeba* parasite which are *E. histolytica, E. dispar* and *E. coli.* Furthermore, the presently observed amoebic mono-infections had a higher percentage than the amoebic co-infection (Table 4). Moreover, the present study has provided further information regarding the residence of patients that infected with amoebiasis, in which patients in rural areas had shown a high level of parasitic infection

Table 1: Assortment of the overall parasitic infections among study patients according to their age groups

Patients age group	Amoeba group	%	*G. lamblia*	%	*E. coli*	%	*H. nana*	%	*A. lumbricoides*	%	Hook worms	%	Co-infection	%
Overall parasitic infection														
10-≥19	18	19	2	29	2	17	4	40	3	50	2	50	5	25
20-≥29	23	24	3	43	3	25	2	20	2	33.3	1	25	6	30
30-≥39	27	28	1	14	4	33.1	2	20	1	16.7	0	0	4	20
40-≥49	15	16	0	0	1	8.3	1	10	0	0	1	25	4	20
50-≥59	8	8	1	14	1	8.3	0	0	0	0	0	0	1	5
60-≥69	4	4	0	0	1	8.3	1	10	0	0	0	0	0	0
70≥	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Sum	96	100	7	100	12	100	10	100	6	100	4	100	20	100

Co- infection refers to infection caused by amoeba group associated with other of non-amoebic parasite

Table 2: Assortment of the overall parasite infection among study patients according to the type of parasitic infection

	Type of parasitic infection (Among 155 patients)					
Patients-age groups	Amoebic species (infected number)	%	Non-amoebic species (infected number)	%	Amoebic co-infection (infected number)	%
10-≥19	18	19	13	33.3	4	20
20-≥29	23	24	11	28.2	5	25
30-≥39	27	28	8	20.5	5	25
40-≥49	15	16	3	8	4	20
50-≥59	8	8	2	5	2	10
60-≥69	4	4	2	5	0	0
70≥	1	1	0	0	0	0
Sum	96	100	39	100	20	100

Table 3: Presentation of clinical signs and symptoms among all studied patients

	Symptomized number			
Clinical signs and symptoms	Dysentery-suspected patients	%	Dysentery-confirmed patients	%
Bloody diarrhea	120	77.4	86	89.5
Fever	125	80.6	83	86.4
Headache	100	64.5	81	84.3
Vomiting	98	63.2	70	72.9
Malaise	107	69	70	94.7
Abdominal pain	140	90.3	91	94.7
Hepatomegaly	41	26.4	38	39.5
Itching	32	20.6	21	21.8
Weight loss	89	57.4	60	62.5
Anemia	80	51.6	71	73.9
Anorexia	77	49.6	50	52
Flatulence	42	27	22	22.9
Dehydration	113	72.9	89	92.7
Sporadic constipation	19	12.2	8	8.3
Abdominal dilation	16	10.3	9	9.3
Sum	155	100	96	62

Table 4: Assortment the type of amebic complex-co-infection by use of PCR technique and microscopic examination

	Type of amebic complex co-infection			
Amebic species	Case numbers solely amebic infection	%	Case numbers mixed amebic co-infection	%
E. histolytica	60	62.5	20	13
E. dispar	27	28.1		
E. coli	9	9.4		
Sum	96	100		

Table 5: Assortment of amoebiasis according to the patient's residence

	Patients residence			
Amoebiasis	Urban	%	Rural	%
Dysentery-confirmed Infected number (96)	30	31.2	66	68.8
Sum	100			

Table 6: Assortment of amoebiasis according to the origin of patient's residences

Age group of Amoebiasis infected females	Total infected number	Origin of residence			
		Genuine	%	Novel	%
10-≥19	18	8	8.3	10	10.4
20-≥29	23	10	10.4	13	13.5
30-≥39	27	10	10.4	17	17.7
40-≥49	15	8	8.3	7	7.2
50-≥59	8	3	3.1	5	5.2
60-≥69	4	2	2	2	2
70≥	1	0	0	1	1
Sum	96	41	42.7	55	57.3

Table 7: Assortment of amoebiasis according to the patient's educational level

Amoebiasis (Patients age groups)	Total infected number	Patients educational level					
		High	%	Middle	%	Low	%
10-≥19	96	20	20.8	46	47.9	30	31.3
20-≥29							
30-≥39							
40-≥49							
50-≥59							
60-≥69							
70≥							
Sum	100						

Table 8: Assortment of amoebiasis according to patient's social levels

	Patients social level					
Amoebiasis infected number	High	%	Middle	%	Low	%
96	14	14.6	32	33.4	50	52
Sum	100					

(68.8%), while patients in urban areas have shown a low level of infection (31.2%) (Table 5). As well, the obtained results of the current study had revealed that a novel residence of the population had an effect on infection percentage with *E. histolytica* parasite (57.3%), while the genuine residence was only 42.7% (Table 6). The current study had observed a significant effect of the educational level can affect infection levels since it was found that people at a high, intermediate, and low educational level had 20.8, 47.9 and 31.3%, respectively (Table 7). Furthermore, the present study had observed that the social level had affected on the level of infection, as the parasitic infection at a high social level was lower than its level in the middle and low social levels, respectively (Table 8).

DISCUSSION

The present study has identified remarkable levels of amoebic infections in the females aged between 30-39 years, while other aged groups have not attained such serious levels of infections. In agreement with our results, it was found that adult had a significantly higher amoebic infection rate than children[19]. However, several reasons may lie behind such sensitivity, which may be attributed to the fact that this age group is more likely to be engaged in many regular activities that might increase their exposure to amoebic infection through undergoing more interaction with many contaminated subjects, such as soil, water and food[20,21]. In contrast to several related studies that had dealt with age as a suggestive factor with such infections[22,23], the specific age-grouping that conducted by this study has provided more detailed information regarding the accurate effect of age of the patients' susceptibility to amoebic infection. Such details were accurately arranged, in such away all the studied time scales were separated from each other and each decade was dealt with as an individual unit. Such separation had enabled us to provide confirmatory results regarding the particular involvement of the 30-39 aged females in these infections. In addition to the age-related factors, these obtained results may be due to the differences in the living and the geography of the position or to the socio-economic factors of the study populations places and regions[24].

The causes for our obtained results of high abdominal pain and low level of clinical symptoms might due to the level or nature of patient's immunity and infection period at a chronic or acute stage[25]. The currently obtained results of the significant effect of the level of education on the infection ratio were in accordance with the results of Hailegebriel[26], who found a proportional correlation between educational level and infection with intestinal parasites. This result may be related to the association between patients' knowledge and their hygienic awareness, which includes the source of drinking water, the level of environmental sanitation and the absence of cleaning or the cutting of fingernails and the incorrect using of toilets, as the major factors contributing to high infection[27]. Such low educational level may be considered as the best-suited environment to enable the amoebic cyst to survive for a long time[5]. Regarding the social level effect, the reason that leads for our obtained results may be associated with the presence of pit or pore in the walls of muddy rooms that considers as a location contain parasites that pollutes drinking water, persons and contact with flies and items contaminated with flies, and other hygiene issues[28]. Eventually, the present study highlights the importance of regular intestinal parasites screening in diarrhea suspected females with amoebic dysentery. As well, our results of higher levels of amoebic mono-infections were adjacent to the results of Fotedar *et al.*[4], who found that the ratio of mono-infection was 70.8 %, while only 61.8% of the co-infection was observed in the same study. This result may be due to *E. histolytica* when it has been recognized as a nurse at the first, while the ability of the other accompanying species, such as *E. moshkovskii*[29] and *E. dispar*[30], to cause the disease is unclear. On the other hand, our results of higher infection ratios in rural areas may be attributed to the type of drinking water and the use of polluted water too. Virtually, the population/personal low hygienic level, improper use of latrines and contact with contaminated articles in rural areas compared to urban areas can bring about exhibiting such preceded results[26].

Our observation of the presence of higher infection ratios in the novel residents may be referred to the loss of treatment for drinking water, lack of cleaning, poor hygiene and education levels, contaminated animals and neglect of hand hygiene before meals at a new residence[27].

CONCLUSION

Amongst several observations the current study had observed, it was found that the amoebic infection is the most prevalent in females aged between 30-39 years old. Thus, the specific age-grouping that conducted by this study has provided further information regarding the accurate effect of age on the patients' susceptibility to amoebic infection. Accordingly, the current study is highly recommending this aged female group to take a special precaution to prevent such infection as they exhibit an extra-ordinary susceptibility to be affected with several forms of amoebic dysenteries.

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