African Journal of Biotechnology Vol. 11(17), pp. 4079-4082, 28 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3850
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Detection of *Dientamoeba fragilis* in patients referred to Chaloos Medical Care Centers by nested – polymerase chain reaction (PCR) method

Ardavan Ghazanchaei[1], Shohreh Shargh[2], Mohammad Shabani[3], Mohammad Najafi[3] and Seyed Manuchehr Nourazarian[3]*

[1]Department of Parasitology, Tabriz University of medical Sciences, Tabriz, Iran.
[2]Department of Medical Sciences, Health Institute, Chalous Azad University, Chalous, Iran.
[3]Department of Biochemistry, Tehran University of Medical Sciences, Tehran, Iran.

Accepted 20 January, 2012

Dientamoeba fragilis **is a protozoan that inhabits the human colon and is responsible in degrees for clinical symptoms. These symptoms are: Local stomach pains, loss of weight and appetite and vomiting. Treatment with anti-parasite drugs will improve the symptoms. Due to misdiagnosis, prolonged undesired clinical signs can remain in patient. Diagnosis in stool specimens uses standard Iron-Haematoxylin staining and molecular polymerase chain reaction (PCR) and Nested-PCR methods that differ in sensitivity and specifity. The results presented here confirmed the sensitivity and specificity of 85 and 100% respectively. All negative results with staining method were also negative by PCR but six positive reported results were detected by staining and one positive sample was not detected by molecular method. This maybe the result of delay in processing samples for diagnosis which may mean the DNA is destroyed and made undetectable.**

Key words: *Dientamoeba fragilis*, PCR, Chalous region, prevalence.

INTRODUCTION

Dientamoeba fragilis is an amoeboid parasite that inhabits in the large intestine and lives in crypts of large intestine (Levine et al., 1980). This parasite was discovered at 1907 by Wenyon (Schwartz and Nelson, 2003). Their specification was described by Jepps and Dobell (1918). The size of the parasite differs between 4 to 19 µm and trophozoites in colored stool specimens have two nuclei with fragmented chromatin (Johnson et al., 2004). No cyst stage is identified yet. Originally, *D. fragilis* was considered an amoeba, but based on ultra structural characteristics it has been established that it is a trichomonad (Johnson and Clark, 2000; Banik et al., 2011).

The transmssion of *D. fragilis* is unknown until this day. Most intestinal protozoa with a fecal-oral transmission require a cystic phase to be able to live in the outside environment. Some researchers have reported precystic, pseudocystic or cystic phases of *D. fragilis* but generally it is accepted that this parasite does not have a cystic phase (Stark et al., 2008; Barrat et al., 2011). Dobell (what year) believed *D. fragilis* like *Histomonas meleagridis* does not have a cyst stage and its transmission may be through nematode eggs like *Ascaris lumbricoides* or *Trichuris trichiura*. Burrows and Swerdlow (1956) believed that this nematode is *Enterobius vermicularis*. They found that the rate of simultaneous infection with pin worm is 20 times more than the expected rate. In addition amoeboid small cells similar to *D. fragilis* were observed in the eggs of worm confirmed their belief. Uncertainty about the pathogenicity of *D. fragilis* means it is often considered a nonpathogen (Lagace et al., 2006). Studies demonstrated that in patients, *D. fragilis* causes fatigue, poor appetite, nausea, swelling, weight loss and diarrhea which were improved with anti amoebic treatment (Yang and Scholten, 1977; Stark et al., 2010a). Prevalence rate are reported between 0.4 to 91% that show the difficulties of diagnosis

*Corresponding author. E-mail: m-nourazarian@razi.tums.ac.ir. Tel: +98(21) 88622742.

(Peek et al., 2004; Stark et al., 2005; Barratt et al., 2011). This parasite is not detectable by wet mount and since it does not have a cystic form it will be destroyed by concentration methods; so use of preservatives permanent staining are recommended for diagnosis (Johnson et al., 2004; Stark et al., 2005; Chan et al., 1993; Estevez and Levine, 1985). Prolonged staining methods used and accuracy of investigator caused focus on use of molecular methods. In the limited studies, published sensitivity and specificity of PCR was reported respectively 88/9 to 100% and 93% to 100%. In comparison, the staining and microscopic method study showed a sensitivity and specificity of 92/4 and 98/7% (Stark et al., 2005, 2006; Verweij et al., 2007). The aim of this study was the detection of *D. fragilis* in stool samples by PCR and Iron-Haematoxylin staining from patients referred to Chaloos medical centers.

MATERIALS AND METHODS

The present study is a Cross-sectional study. The samples were obtained randomly from referred patients to Imam Reza and Taleghani hospitals of Chaloos.

During June to February 2010, 302 stool samples were collected from referred patients. After sampling and completion of questionaire form, the sample was immediately investigated for trophozoites of *D. fragilis* and other parasites by light microscopy and the results were recorded on the questionaire form. Then a small part of the sample was placed into two microtubes; one of them contained Poly Vinyl Alcohol preservative (for staining) and the second one included 70% ethanol (for molecular study). After fixation of samples on slides, they were stained with Iron-Haematoxylin and then investigated for presence of pathogens.

For DNA extraction, the CTAB, SDS and proteinase k method were used. 300 µl of stool in alcohol was transferred to a microtube and 60 µl 10% SDS, 150 µl TE (10/1 mM) buffer and 5 µl proteinase k (20 mg/ml) were added. After vortexing the microtube, it was incubated at 56°C for one day. Next day using CTAB and NaCl (5 M) followed by chloroform, isoamyl alcohol was added and DNA extracted. After sedimentation in isopropanol, DNA was washed with 70% alcohol. DNA was dissolved in 30 µl TE buffer and stored frozen for PCR. The initial PCR was performed with external primers causing the amplification of one part of the 18s rDNA family of trichomonads. The sequence of external primer was as follows:

Forward: 5´-GATACTTGGTTGATCCTGCCAAG-3´
Reverse: 5´- GATCCAACGGCAGGTTCACCTACC-3´

The program of thermal cycler for initial PCR was as follows: Initial denaturation 60 s at 94°C followed with 30 cycles of denaturation at 60 s at 94°C, annealing 90 s at 55°C extension 120 s at 72°C and finally the final extension 5 min at 72°C. These primers were designed by Silberman et al. (1996). Then 2 µl from initial PCR product was subjected to second PCR with the use of inner primers as following:

Forward: 5´-GGTTGGATACTCCTACTCTCGC-3´
Reverse: 5´-TTGTAACCTAGCAGAGGGCCAG-3´

Program for thermal cycler for second PCR was: Initial denaturation 1 min at 94°C followed by 30 cycles of denaturation 60 s at 94°C, annealing 60 s at 55°C extension 90 s at 72°C, and the final extension 5 min at 72°C. These primers were designed by Menghi et al. (2006). Negative control was used in the first and second PCR. For analysis of second PCR products, electrophoresis was in 2% agarose gel followed by ethidium bromide staining and visualization under UV.

RESULTS

From 302 collected samples, 145 samples were from male and 157 samples were from female. The range of ages for selected individuals was 10 months to 79 years old. In direct examination by wet mount, no positive case was observed. Study of stained samples showed six cases of *D. fragilis* infections. Two cases were from females and four cases from males. Other parasites identified were as follows: *Entamoeba coli* was observed in 12 cases. This was the highest percent of infection and *Taenia saginata* eggs in 1 case was the lowest level of infection. *Entamoeba histolytica* / *dispar* 2 cases (0.66%), *Giardia lamblia* 7 cases (2.3%), *Iodamoeba butschlii* 4 cases (2.9%), *Blastocystis sp.* 5 cases (1.7%) and *E. vermicularis* eggs in 2 cases (0.66%). Simultaneous infection of *D. fragilis* with *E. coli* in 2 cases and with *Blastocystis sp.* in one case was observed. All patients that were infected by *D. fragilis* had diarrhea and stomach pain signs. All microscopic positive cases and 100 negative samples that were selected randomly were subject to PCR and Nested PCR. Study of Nested PCR products showed that only 5 samples gave a 414 bp band that was considered positive samples. PCR was not able to identify one of the positive samples. All negative samples were negative by PCR.

DISCUSSION

D. fragilis inhabits the human large intestine and is closely related to flagellated trichomonads. This organism causes gastroenteritis in humans such as diarrhea, stomach pain, weight loss, poor appetite and etc (Norberg et al., 2003, Yang and Scholten, 1977; Cuffari et al., 1998). This protozoon often is seen with two nuclei and a cystic form is not known.

This study used nested PCR for the identification of *D. fragilis* infection. From 302 samples that were stained with Iron-Haematoxylin, six positive cases (1.4%) of *D.fragilis* were identified. In an investigation from Iran, Rezaian and Hooshyar (2006) showed that the infection rate of *D. fragilis* was 0.5% (Soleymani et al., 2006) and in another study in Northern villages of Iran, the infection rate was reported 1.1% (Kia et al., 2008). In another study in Ahvaz (South of Iran), the infection rate was reported 0.76% (Rezaian and Hooshyar, 2006).

The findings show that diarrhea and stomach pain were common signs in all positive cases identified in this study. This result agrees with other results from other studies that showed that *D. fragilis* is the reason for different

gastroenteritis signs (Norberg et al., 2003; Yang and Scholten, 1977). By nested PCR, five positive cases were identified. In a study of 100 negative cases by PCR and nested PCR, *D. fragilis* was not observed in any case. According to the obtained results in this study if the staining method is accepted as gold standard, the sensitivity and specificity of PCR were determined as 85 and 100% respectively. Comparison of results of our study with other studies noted the agreement of this study with others. The sensitivity and specificity of PCR method were reported as 88.9 to 100% and 93 to 100% respectively in comparison to the staining method and microscopic study that had sensitivity and specificity of 92.4 and 98.7% (Stark et al., 2005a, 2006, 2010b; Verweij et al., 2007).

The reason for the low sensitivity of PCR in comparison with staining method could be due to the delay in examination of samples by these methods. As *D. fragilis* does not have a cystic form and the trophozoite form dies soon after passing from the body; consequently any delay in processing samples for diagnosis may mean the DNA is destroyed and made undetectable.

To get more accurate results, it is better to shorten the time of storage of the specimens, DNA extraction and PCR of fresh samples should be used.

The transmission of *D. fragilis* is unknown. It is possible that it is transmitted in a nematode egg. *D. fragilis* infection is commonly seen with other intestinal protozoa and investigation has shown 5% of infection with *E. vermicularis*, but with *Blastocystis hominis* in 40.3%, *Endolomax nana* in 24%, *E. coli* in 6% and *Giardia lamblia* in 5.7%. Existence of a high rate of infection with other organisms that are transmitted via oral-fecal way shows there is similar transmission for *D. fragilis*. In a study on 6750 patients, 60 cases were positive by staining/microscopy and 54 cases were positive by PCR. The prevalence rate was 0.9% and simultaneous infection of *D. fragilis* with *E. vermicularis* was not observed (Stark et al., 2005b). In a study in Turkey from 2007, 217 infected children with *E. vermicularis*, 99 cases (45.6%) were infected with *D. fragilis* (Girginkardesler et al., 2008). A study of wet mount and stained slides of 2206 patients from Durham city demonstrated that any *D. fragilis* was not seen in wet mount and in stained slides only one case was seen (Estevez and Levine, 1985). The role of Enterobius and other helminthes, if any, in the transmission of *D. fragilis* is still not clear.

AKNOWLEDGEMENT

This work was fully supported by a research grant from the Chalous Azad University which authors are thankful.

REFERENCES

Banik G R, Birch D, Stark D, Ellis J T (2011). A microscopic description and ultrastructural characterization of Dientamoeba fragilis: an emerging cause of human enteric disease. International Journal for Parasitology. (In press). Published online.

Barratt J L N, Harkness J, Marriott D, Ellis J T, Stark D, (2011). A review of Dientamoeba fragilis carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes, 2: 3-12.

Barratt J N, Harkness J, Marriott D, Ellis J, Stark D (2011). The ambiguous life of Dientamoeba fragilis: the need to investigate current hypotheses on transmission. Parasitology, 138: 557-572.

Burrows R B, Swerdlow M A (1956). *Enterobius Vermicularis* as a Probable Vector of *Dientamoeba Fragilis*. Am. J. Trop. Med. Hyg. 5(2):258-265.

Chan FT, Guan MX, Mackenzie A.M (1993). Application of Indirect Immunofluorescence to Detection of *Dientamoeba fragilis* Trophozoites in Fecal Specimens. J. Clin. Microbiol. 31(7): 1710-4.

Cuffari C, Oligny L, Seidman E G (1998). *Dientamoeba fragilis* masquerading as allergic colitis. J Pediatr Gastroenterol Nutri. 26(1): 16-20.

Estevez E G, Levine J A (1985). Examiniationof Preserved Stool Specimens for Parasites: Lack of Value of the direct wet mount. J. Clin. Microbiol. 22(4): 666-7.

Girginkardeşler N, Kurt O, Kilimcioğlu A A, Ok U Z (2008). Transmission of *Dientamoeba fragilis*: evaluation of the role of Enterobius vermicularis. Parasitol. Int. 57(1):72-5.

Johnson E H, Windsor J J, Clark C G (2004). Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. Clin. Microbiol. Rev. 17(3): 553-70.

Johnson J A, Clark C G (2000). Cryptic Genetic Diversity in *Dientamoeba fragilis*. Clin. Microbiol. 38(12): 4653-4.

Kia E B , Hosseini M, Nilforoushan M R, Meamar A R, Rezaeian M (2008). Study of intestinal protozoan parasites in rural inhabitants of mazandaran province, northern iran. Iranian. J. parasitol. 3(1): 21-25.

Lagace-Wiens P R, Van Caeseele P G, Koschik C (2006). *Dientamoeba fragilis*:an emerging role in intestinal disease. C.M.A.J. 175(5): 468-469.

Levine ND, Corliss JO, Cox FE, Deroux G, Grain J, Honigberg BM, Leedale GF, Loeblich AR , Lom J, Lynn D, Merinfeld EG, Page FC, Poljansky G, Sprague V, Vavra J, Wallace FG.. (1980). A newly revised classification of the protozoa. J. Protozool. 27: 37-58.

Menghi C I, Gatta C L, Makiya R, Mendez O C (2006). Molecular Detection of *Dientamoeba fragilis* in Faeces: Removal of DNA-Polymerase Inhibitors. Parasitol. Latinoam, 61: 146-151.

Norberg A, Nord C E, Evengard B (2003). *Dientamoeba fragilis*--a protozoal infection which may cause severe bowel distress. Clin. Microbiol. Infect. 9(1): 65-8.

Peek R, Reedeker F R, Van Gool T (2004). Direct Amplification and Genotyping of *Dientamoeba fragilis* from Human Stool Specimens. J.Clin. Microbiol. 42(2): 631-5.

Rezaian M, Hooshyar H (2006). Differential diagnosis of Entamoeba histolytica from Entamoeba dispar and a study on the intestinal parasites in rural areas of Ahwaz and Hamidieh. J. Sch. Pub. Health Ins.Pub. Health Research, 4(4): 33-38.

Schwartz M D, Nelson M E (2003). *Dientamoeba fragilis* infection presenting to the emergency department as acute appendicitis. J. Emerg. Med. 25(1): 17-21.

Silberman J D, Clark C G, Sogin M L (1996). *Dientamoeba fragilis* shares a recent common evolutionary history with the trichomonads. Mol. Biochem. Parasitol.76:311-314.

Solaymani M S, Rezaian M, Babaei Z, Rajabpour A, Meamar A R, Pourbabai A A, Petri WA Jr. (2006). Comparison of a stool antigen detection kit and pcr for diagnosis of entamoeba histolytica and entamoeba dispar infections in asymptomatic cyst passers in Iran. J. Clin. Microbiol., 44(6): 2258-61.

Stark D, Beebe N, Marriott D, Ellis J, Harkness J (2005a). Detection of *Dientamoeba fragilis* in fresh stool specimens using PCR. Int. J. Parasitol. 35(1): 57-62.

Stark D, Beebe N, Marriott D, Ellis J, Harkness J (2005b). Prospective study of the prevalence, genotyping, and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. J.Clin. Microbiol. 43(6): 2718-23.

Stark D, Beebe N, Marriott D, Ellis J, Harkness J (2006). Evaluation of

Dientamoeba fragilis in Stool Specimens. J.Clin. Microbiol. 44(1): 232-5.

Stark D, Phillips O, Peckett D, Munro U, Marriott D, Harkness J, Elis J. (2008). Gorillas are a host for *Dientamoeba fragilis*: an update on the life cycle and host distribution. Vet. Parasitol., 151(1): 21-26.

Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J(2010a). A review of the clinical presentation of dientamoebiasis. Am. J. tropical med. hygiene. 82: 614-619.

Stark D, Barrat J, Roberts T, Marriot D, Harkness J, Ellis J (2010b). Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of Dientamoeba fragilis in clinical stool samples. Euro. j. clinical microbiol. infect. Dis. 29: 411-416.

Verweij J J, Mulder B, Poell B, Van Middelkoop D, Brienen E A, van Lieshout L (2007). Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. Mol. Cell. Probes. 21(5-6): 400-4.

Yang J, Scholten T (1977). *Dientamoeba fragilis*: a review with notes on its epidemiology, pathogenicity, mode of transmission, and diagnosis. Am. J. Trop. Med. Hyg. 26(1): 16-22.