Molecular Identification of Entamoeba Histolytica and Entamoeba Dispar in Patients with Diarrhea

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Abstract

Entamoeba histolytica and Entamoeba dispar (also known as E. dispar) are responsible for the parasitism of around ten percent of the world's population. However, only ten percent of those infections cause symptoms. Using Real-Time PCR methods, the purpose of the research was to distinguish between the two strains of E. histolytica and E. dispar. The current study was carried out between the 1st of November 2020 and the 20th of March 2021. We collected stool samples from 455 patients of varying ages (1-60 years) who presented themselves at pediatric clinics in Kirkuk city complaining of diarrhea and/or stomach discomfort. These patients sought treatment at one of the clinics. RT-PCR analysis was then performed on the samples that were found to be positive by microscopy. Stool samples were collected using sterile wide mouth screw cap containers. Fresh samples were analyzed using light microscopy, and after that, samples were stored in the refrigerator until they were analyzed using ELISA and PCR. The assay for the detection of the Entamoeba histolytica genome was carried out with the assistance of a specific Real-Time PCR, the Kestrel-realtime Entamoeba histolytica qPCR kit, and the FAM channel for the detection of target pathogen amplification. According to the findings of the study, 93 (or 48.69%) of the total wet mount positive patients were infected with trophozoites, and 70 (or 36.65%) were infected with both cysts and trophozoites. 14.89% of patients with dysentery were infected with E. histolytica who were diagnosed by PCR. The high incidence of positive cases was seen in males at a rate of 28.57%, compared with 23.46% of females who were E. histolytica / E. dispar positive. The finding was significant. Only 37.36% of the total positive wet mount patients were suffering from dysentery, whereas all E. histolytica infected patients who were diagnosed by PCR were suffering from dysentery and 95.45% of them were suffering from abdominal pain. PCR was used to diagnose all E. histolytica infected patients.

Keywords: PCR, Stool, Dysentry, E. Histolytica, E. Dispar.

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INTRODUCTION

Around ten percent of the world's population is infected with the parasites Entamoeba histolytica and Entamoeba dispar. These two species are responsible for the disease. However, only ten percent of those infections result in symptoms being present in the patient. The goal of the research was to differentiate between the two strains of E. histolytica and E. dispar by utilizing Real-Time PCR technologies. The period of time spanning the 1st of November 2020 to the 20th of March 2021 was utilized for the current investigation. We collected stool samples from 455 patients of varied ages (1-60 years), all of whom presented themselves at pediatric clinics in Kirkuk city, complaining of diarrhea and/or stomach ache. The patients ranged in age from 1 to 60. These patients went to one of the clinics in order to receive treatment. After this, real-time PCR testing was carried out on the samples that had been determined to be positive by microscopy. The collection of stool samples was done with sterile containers that had large mouths and screw caps. Light microscopy was used to examine fresh samples, and after that, samples were refrigerated so that they could be examined using ELISA and PCR later on. With the assistance of a specific Real-Time PCR, the Kestrel-realtime Entamoeba histolytica qPCR kit, and the FAM channel for the detection of target pathogen amplification, the assay for the detection of the Entamoeba histolytica genome was successfully carried out. According to the findings of the study, 70 patients (or 36.65%) were infected with both cysts and trophozoites, while 93 (or 48.69%) of the total wet mount positive patients were infected with trophozoites. 14.89% of patients diagnosed with dysentery were found to be infected with E. histolytica using PCR. E. histolytica and E. dispar positivity was found in males at a rate of 28.57%, while it was found in females at a rate of 23.46%. The high incidence of positive cases was seen in males. The finding had important implications. Only 37.36% of the total positive wet mount patients were suffering from dysentery, whereas all E. histolytica infected patients who were diagnosed by PCR were suffering from dysentery, and 95.45% of them were suffering from abdominal pain. Wet mounts are a more
sensitive method of diagnosing infections than PCR. PCR was used to diagnose all of the patients who were infected with E. histolytica.

**MATERIALS AND METHODS**

This study was carried out between the first of November 2020 and the twenty-first of March 2021. We collected stool samples from 455 patients of varying ages (1-60 years) who presented themselves at pediatric clinics in Kirkuk city complaining of diarrhea and/or stomach discomfort. These patients sought treatment at one of the clinics. RT-PCR was then used to confirm the findings of the positive microscopy tests.

Stool samples were collected using sterile wide mouth screw cap containers, and fresh samples were examined under light microscopy (Olympus CX31RBSPH-Philippines) with the high power magnification 40X and 100X oil immersion lens. Stool specimens ranging from 0.5 ml to 3 ml in volume were collected in two sterile screw cap containers for each sample (one for ELISA and the other for PCR). These containers were then placed in a refrigerator manufactured by VestFrost-Denmark and chilled to -20 degrees Celsius until the ELISA and PCR tests could be performed on them.

There is no requirement for any changes to be made to the conventional collection methods utilized for microscopic exams. Using unpreserved or frozen stool samples are both viable options. For the purposes of this test, preserved stools are not acceptable. After being collected, samples must be examined within twenty-four hours and stored at a temperature between 2 and 8 degrees Celsius. The samples that cannot be analyzed within this time frame should be frozen at a temperature of -20 degrees Celsius or lower until they are needed. The test is not negatively impacted in any way by freezing. Every single one of the dilutions of the stools needs to be done with diluted wash buffer. Getting Ready to Wash the Buffer – Remove the cap and pour the contents of one bottle of Wash Concentrate into a squeeze bottle that already contains 475 ml of distilled water. Mix it up by giving it a good stir. To maximize the amount of washings that can be extracted from a squeeze bottle, the tip should be somewhat thin.

**DNA Extraction and Cleaning**

The NanoDrop ND-1000 spectrophotometer is a full-spectrum instrument that analyzes sample concentrations from 220 to 750 nm and has a high level of accuracy and reproducibility. It does this by utilizing an unique sample retention method that relies solely on surface tension to keep the sample in place. This makes the use of cumbersome cuvettes and other sample containment devices unnecessary and enables the cleanup process to be completed in a matter of seconds. In addition to this, the ND-1000 is capable of determining the concentration of extremely concentrated materials without the need for dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

**Operation**

On the very tip of a fiber optic cable, a 1 l sample is pipetted using a micropipette (the receiving fiber). After that, a second fiber optic cable, known as the source fiber, is brought into contact with the liquid sample, which causes the liquid to cross the gap that exists between the two ends of the fiber optic cables. Both 1 millimeter and 0.2 millimeter pathways are controlled for the gap. The light comes from a pulsed xenon flash lamp, which is also utilized as the light source, and a spectrometer with a linear CCD array is what is used to examine the light after it has passed through the sample. The instrument is controlled by specialized software that is executed on a personal computer (PC), and the data is logged in an archive file on the PC (116). (the appendix titled "Appendix II" has additional information regarding the technique)

Amplification of the pathogen Entamoeba histolytica using RT-PCR

(qPCR Taqman. Kestrel BioSciences, USA).

Kestrel-realtime Entamoeba histolytica qPCR kit was utilized in an assay for the detection of the Entamoeba histolytica genome. This assay was carried out with the assistance of a particular Real-Time PCR (Kestrel Bioscience LLC, USA). The kit includes an Entamoeba spp. specific primer as well as a Taqman probe that targets the 18S ribosomal RNA(18S) gene (Eh 1 5'- AGAGAAGCATTTTCTAGATCTG-3'). The 5'-reporter dye and 3'-quencher labeled oligonucleotide (Taqman probe) hybridizes on a specific area inside the amplified fragment. This is based on the hydrolysis probe detection technology. The reporter dye, also known as fluorophore, is released when the probe is cleaved during the amplification process. The amount of fluorescent signal that is picked up is in direct proportion to the amplicons that are present. Quantification is accomplished with the help of the Ct value, which refers to the cycle at which the rise of fluorescent signal from the baseline becomes first significantly apparent. The FAM channel is used to detect the amplified presence of the target pathogen.

**RESULTS**

Concerning the stages of the E. histolytica / E. dispar infection, the research revealed that 93 (48.69%) of total wet mount positive patients were infected with trophozoites, 70 (36.65%) were infected with cysts and trophozoites, and only 28 (14.66%) were infected with the cystic stage alone, as shown in Figure 1. This information was gleaned from the results of the study.

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**Footer**

Journal of Pharmaceutical Negative Results | Volume 13 | Issue 4 | 2022 | 277
Sanarya Kamal Tawfiq, et al.: Molecular Identification of Entameba Histolytica and Entameba Dispar in Patients with Diarrhea

**Table 1: Relation of E. histolytica excluded by PCR and Microscopy wet mount positive with clinical features of patients**

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>No. of patients from Microscopy wet mount positive (No.=455)</th>
<th>No. of patients from positive PCR (No= 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>No. 312 % 68.57</td>
<td>No. 20 % 90.90</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>No. 411 % 90.32</td>
<td>No. 19 % 86.36</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>No. 366 % 80.43</td>
<td>No. 21 % 95.45</td>
</tr>
<tr>
<td>Weight loss</td>
<td>No. 118 % 25.93</td>
<td>No. 15 % 68.18</td>
</tr>
<tr>
<td>Dysentery while defecates</td>
<td>No. 170 % 37.36</td>
<td>No. 22 % 100</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It is extremely important for accurate diagnosis of these parasites that cause amebiasis for knowing the true prevalence of pathogenic E. histolytica in the community for detecting and differentiating between E. histolytica and E. dispar in stool samples. This can be done in order to prevent the spread of amebiasis. For the purpose of accurate identification of either Escherichia histolytica or Escherichia dispar, a number of different serological and DNA-based molecular approaches have been enrolled (7-9). This result correlated with the study conducted in Kirkuk city by Ismail (10) who discovered that the infection rate of E. histolytica / E. dispar was 47.6% by examining 800 stool samples from children, and with Ibrahim (11) also in Kirkuk who recorded infection rate of 41.12% in children by examining 1250 stool samples. In the current study, the rate of infection with E. histolytica / E. dispar was 41.98% from total of 455 stool samples examined by wet mount. It’s possible that inadequate sanitation and hygiene practices in general are to blame for the high infection rate that was seen in this particular study. Some infection rates records are close to the current study, as is evident from the various studies that were discussed above; this may be due to the similarities of environmental, social, and cultural properties in those areas. On the other hand, diversity in infection rates may be due to population concern, geographical location, difference in total number of specimens examined, methods of examination, techniques of examination, duration of the study, and the age groups of study population (12,13).

This result was consistent with the findings of AL-
Harthi, et al (14) in Saudi Arabia, who found a rate of infection of 59.6% for E. histolytica and E. dispar. It was inconsistent with the findings of Deliali Oglu et al. (15) in Turkey, who recorded a 29.5% infection rate for E. histolytica / E. dispar using Entamoeba ELISA, and with the findings of Braga et al. (16) in Northeastern Brazil, who reported a 25.4% infection rate using Entamoeba TechLab ELISA in 187 of 735 patients. Regarding the distribution of participants by gender, the current study included a total of 237 females and 218 males. This represents a general distribution. This was practically identical to the findings of AL-Harthi, et al (14) who reported an infection rate of 33.3% and 31.4% for males and females respectively. The greater rate of infection was detected in males at 28.57%, compared to females at 23.46%. The result contradicted the findings of AL-Samarray (12), who reported an infection rate of 19.84% in males and 21.85% in females. It also contradicted the findings of Mohammadi, et al. (17), who carried out research in Iran and reported an equal infection rate in males and females. It also stands in contrast to the findings of Samie et al. (18) in South Africa, who reported an infection rate of 44.67% in males and 55.33% in females, as well as the findings of Braga et al. (16) in Northeastern Brazil, who reported an infection rate of 48.1% in males and 51.9% in females. It is possible that the difference in infection rates between males and females can be attributed to the fact that males make up the working population and therefore have more contact with the environment. Additionally, the increased likelihood of being exposed to infectious materials when eating and drinking in public restaurants or from street vendors is due to these factors. In addition, the increased occurrence of E. histolytica in males might be explained as follows: Because males generally demonstrate weaker immune responses and increased intensity of infection compared to females, males are more susceptible to infections caused by parasites than females are. This is because males are more likely to get infected (151). According to the findings of the current study, all of the patients who had been infected with E. histolytica had suffered from dysentery, and 95.45% of them had experienced abdominal pain. In comparison, only 37.36% of the total positive wet mount patients had experienced dysentery, as shown in Table 18. This finding is considered to be highly significant. In contrast to the findings of Ayeh-Kumi et al (170), who calculated that 90 percent of all individuals colonized with E. histolytica never display clinical symptoms, this finding differed significantly. The high rates of the current study may be the result of a true infection of E. histolytica only, which is most commonly represented by dysentery and other associated features, such as fever and abdominal pain. The study didn't contradict the global knowledge, but it may be because of the low age range of the study, low socioeconomic and hygienic conditions of the patients, which may have led to a strong clinical features associated with amebic dysentery. [Citation needed].

CONCLUSIONS

The current study used microscopy to record the infection rate, which came out to be 41.98% for E. histolytica and E. dispar. Regardless of the expense, real-time polymerase chain reaction (RT-PCR) is the most reliable approach for detecting and estimating the true prevalence of E. histolytica in the general population. It is also the ideal technology for use in epidemiological investigations.

RECOMMENDATIONS

Due to the fact that E. histolytica and E. dispar are morphologically similar amoebae that cannot be separated from one another using light microscopy, they should be reported as E. histolytica / E. dispar.

REFERENCES

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