Screening of Amebic dysentery in Al-Noor Pediatric Hospital by a Contemporary gene

Saba Fadhil Ali Malaa1, Dhamiaa Maki Hamza*2, Khalid Khalil Alaaraji3
1Al-Furat AL–Awsat Technical University, Iraq
Sabafam6@atu.edu.iq
2, 3University of Kerbala, Collage of Medicine, Iraq
dhamiaa.maki@uokerbala.edu.iq
khaled.khalil@uokerbala.edu.iq

*Corresponding Author: dhamiaa.maki@uokerbala.edu.iq

ABSTRACT

Abstract: Amebic dysentery caused by parasitic protozoan Entamoebahistolytica. This disease transmitted in areas that allow poor sanitation and food with fecal contamination. The absence of delicate techniques is a significant issue connected with identification for this parasite. Polymerase chain reaction (PCR) is efficient in addressing many of the constraints Because PCR's sensitivity depends on the validation of parasitic DNA extractions. The perfect diagnosis system based on morphologic characteristics of the cysts and trophozoites is not efficient molecular methods can deliver genotypic characterization complete. The current study aimed to investigate the E.histolytica in infant's diarrhea depending on unusual gene (CL6EHI-188180). The stool samples were collected from infected children and first diagnosed microscopically and then diagnosed by PCR techniques, DNA was extracted, the CL6EHI_188180 gene used as the target for PCR amplification which rarely used. The samples collected from a total of 170-child aged from one month to two years old attending the hospital and the private laboratories in Al Hila/ Iraq during the period from October 2018 to June 2019, the samples examined by direct microscope and used of Iodine staining to make the internal structures more clearly. The outcomes of the study showed that the rate of infection overall was 35 (20.58%), the highest prevalence in the age group from six months to one-year-old was (11.17 %). The study indicated also that the ratio of infected among males (11.17%) are higher than females (9.41%). In addition, the percentage of infection among children living in the rural population was 12.35%. Also the percentage of infection among children feeding on bottle-feeding was 36.08%. The proportion of infection with the amoeba parasite was 9.41% from 16 positive samples when using the PCR technique by using a new gene as a marker.

Key word: E. histolytica, PCR techniques, CL6EHI_188180 gene

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INTRODUCTION

The parasitic infection is one of the most prevalent parasites which causing childhood diarrhea and it is almost universal with elevated incidence levels, particularly in emerging nations [1]. The diarrhea usually leading to childhood malnutrition, morbidity, and mortality, they are several caused of diarrhea, which induced by eating or may be associated with certain medicines, or may be associated with general diseases such as Tonsillitis, Otitis media, and UTI, as well as to the infection by pathogens parasites amoeba, cryptosporidium, worms in addition to that infection by fungi [2],poor sanitation and contaminated food and water. Despite the significant development in the quality of medical services in terms of diagnosis, treatment, and control of parasitic diseases, which led to a clear reduction in the spread of these diseases in many countries, various parasitic diseases continue to pose a major challenge for Health staff in many developing countries. Amebic dysentery caused by parasitic protozoan E.histolytica. This disease transmitted in areas that allow poor sanitation and contaminated food and water. Despite the significant development in the quality of medical services in terms of diagnosis, treatment, and control of parasitic diseases, which led to a clear reduction in the spread of these diseases in many countries, various parasitic diseases...
continue to pose a major challenge for Health staff in many developing countries [3]. Some studies have revealed that infection by intestinal parasites is one a reason to hormonal and immune impairment of humans [4]. The pathological effects of intestinal parasites, including diarrhea, loss of appetite, flatulence and cause atrophy of intestinal villi, also inhibit the intestinal absorption of proteins, carbohydrates, and vitamins such as vitamin B, and fats [5]. The E. histolytica creates destruction of the tissue that contributes to clinical illness and cause tissue damage by three cases: immediate destruction of the cell in the host, inflammation causes dysentery and maybe invasion by the blood to the liver, lungs, and brain [6]. In general, an outbreak of parasitic diseases depends on classic epidemiologic is the Host, factor, and Environment [7]. All manifestation of diarrhea among infants is a health problem. This paper to detected this parasite infection between the infants (month - 2 years) who are attending the Al- Noor pediatric hospital in Al- Hila city depending on gender, age, type of feeding, Residential district. The absence of delicate and secure techniques is a significant issue connected with identification for this parasite. PCR test is efficient in addressing many of the constraints Because PCR’s sensitivity depends on the validation of parasitic DNA extraction. The current study is the first polymerase chain reaction (PCR) technique in Babylon to detect this parasite (E. histolytica) among the infants by using unusual CL6EHI_188180 genes.

METHODOLOGY

One hundred and seventy samples of stool collected from infants (suffering from diarrhea) attending to Al Noor Pediatric Hospital in Babylon province from during the period from October 2018 to June 2019, the infants diagnosed microscopically infected with amoebiasis.

Ethical Approval

The full clarification about the purpose of the study is given to the parents and assurance about the confidentiality of the information and that the participation is optional.

Samples Collection

The sample is isolated from the stool of infected infants and collected in Eppendorf tubes and stored in freezing (-20 c) until used in DNA extraction. The stools sample examined microscopically to determine the parasite in the cases of acute diarrhea; the samples examined either freshly or by staining with Iodine stain to make the internal ingredient clearer.

DNA Extraction

Genomic DNA extracted from positive stools by using AccuPrep® Stool Genomic DNA Extraction Kit (Bioneer, Korea). A Nano drop spectrophotometer (THERMO. USA) for detecting the DNA by reading the absorbance at a wavelength of between (280-260 nm). This DNA used in thermal cycler machine to amplify the DNA of CL6EHI_188180 gene by using the sense primer 5'- TGGAGGAGCTAAGGTTGCTG -3' and antisense primer 5' - GTAGCAGCAGCAACAGCTTC -3' with 453bp are designed by NCBI site (Primer 3 plus program), according to the PCR program which is shown in the table (1)

<table>
<thead>
<tr>
<th>PCR master mix</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template</td>
<td>5</td>
</tr>
<tr>
<td>Primer F 10 pmol.</td>
<td>1.5</td>
</tr>
<tr>
<td>Primer R,10 pmol</td>
<td>1.5</td>
</tr>
<tr>
<td>PCR Nuclease free water</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>20µl</td>
</tr>
</tbody>
</table>

PCR reaction is performed in 20µl volume containing and 2µM primer at 94 °C for 5 min for 1 cycle, 94 °C for 1min, 55 °C for 30 secs and 72 °C for 2 min for 35 cycles, and a final elongation step of 5min at 72 °C. DNA is Amplified by electrophoresed on 1% agarose gel, stained with Red stain and visualized under UV trans illuminator.
RESULT AND DISCUSSION

Most studies have been based on the microscopic investigation of fecal samples depending on a diagnosis principle of amoebic dysentery by erythrophagocytic trophozoites (pathogenic type) which is rarely seen by the examiner. However, the investigator's experience is finding the cysts only (lack of RBC or non-pathogenic) because of most studies concern carriers of cysts. After examining 170 fecal samples taken from the infants (one month - two years), the incidence of amoeba parasite is 35(20.58%). Many studies about the amebiasis are done in Iraqi provinces like the study in Baghdad which is showed infection percentage 31.8% [8], while the infection with amebiasis in Tikrit is 18.72% [9]. Also, there are many studies done in neighboring countries like the study in Jordan, which showed the percentage of infection with amoebiasis is 80.7 %[10]. While a study is done in Iranian health centers about the prevalence of some intestinal parasites and in which the results are shown the infection percentage is 19.3% [11].

The higher infection rate of amoeba parasites may be due to its direct life cycle, also its transition by contaminated food and water with infective stage of parasites (mature cysts). as well as, the wide spread of the mechanical carrier of the parasite which is represented by insects especially the domestic flies [12] The current study revealed that the highest prevalence of parasite infection occurring in the age group (6 months - 1 year reaching) its percentage (11.17 %) and the lowest in the age group (1 month - 6 month) (3.52%).

The higher incidence of infection in infants may be due to several reasons, including that children in this age are very active and can pick up a lot of objects and put them in the mouth, which may be contaminated, as well as some bad habits in children such as putting a finger in the mouth and these agents make them weaker to get infection. while the source for the low infection rate in the age group one to six months may be due to the immunity of infants are a good level of secretion of immunoglobulin (IgA), which prevents the parasite adhesion to the lining of the gut and thus prevent the parasite from feeding and death [13,14]. The current study showed that infection rate is 11.17% in the males and 9.41 % in the females Table. 2, because of the resemblance of the situations, which exposed to children of both sexes during this age.
The high incidence of infection in the Rif and districts may be due to bad health conditions, poor sterilization, lack of clean drinking water or not available and low educational levels of most mothers, all of which raise the spread of diarrhea [14]. The Fig. 2 appeared that highest rate of parasite infection (13.52%) among infant bottle-feeding; this may be due to the contamination of milk bottles or in cases of continuous neglect of bottle cleaning from mother.

**FIGURE 2.** The distribution of the cases regarding the type of feeding

The most significant methods that confirm the results of phenotypic diagnosis and detection of some feature by molecular methods (because of it the speed and accuracy in the results) that are usually reliable, it has become the best and the most manner with the field of scientific research. The routine techniques have become inaccurate, inconclusive and have more mistakes and they rely on the judgment of researchers and their bias. From sixteen positive samples by PCR detection form the CL6EHI_188180 gene of *E. histolytica* as it is shown in the picture.1 the infection percentage was 9.41%. The grouping between microscopy and molecular technique has enabled specialists to differentiate between nonpathogenic and pathogenic amoeba. Thereby reducing the number of patients who may be unnecessarily treated. In addition, researchers give a broader image of the study of parasite genotypes in humans and useful information to understand the pathogenesis [14].

### TABLE 2: Distribution of cases regarding gender and residence area

<table>
<thead>
<tr>
<th>variable</th>
<th>No. patient</th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>79</td>
<td>19</td>
<td>11.17</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>91</td>
<td>16</td>
<td>9.41</td>
<td>75</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>88</td>
<td>14</td>
<td>8.23</td>
<td>74</td>
</tr>
<tr>
<td>Rural</td>
<td>82</td>
<td>21</td>
<td>12.35</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>35</td>
<td>20.58</td>
<td>135</td>
</tr>
</tbody>
</table>
Cryptic genetic variation is an important characteristic of the parasite *E. histolytica*. Where it is not possible to distinguish between species or genotypes in accordance with the morphological criteria [15]. This criterion is based on the phenotype and does not reflect the true image of genetic variation and susceptibility to change depending on environmental conditions. Here come molecular techniques to resolve this difference. And determine the level of similarity and genetic variation between species belonging to living organisms, such as parasites for understanding pathogenesis and distribution in the hosts [14,16]. The morphologically identical was classified in amoebae depending on size, three genera of amoeba were inhabiting the intestinal tract of humans: *Entamoeba*, Iodamoeba and Endolimax. Members of these genera considered non-pathogenic include *E. hartmani*, *E. gingivalis*, *E. coli* nana, and Iodamoebabutschlii [17].

The disadvantage of the traditional diagnostic in many laboratories has resulted in the misdiagnosis of the intestinal protozoa. The consequence is that there is mistreatment. Furthermore, the prevalence, as well as the exact morbidity and mortality rates caused by these pathogens, are not appropriately ascertained. Thus, why we need new methods that will circumvent the problems associated with the traditional methods is therefore apparent. In the current study, the rate of infection is 20.58% in microscopic diagnosis while the percentage is 9.41% in PCR detection which characterized by high accuracy in detecting low numbers of parasites in fecal samples in comparison to conventional methods such as microscopy [18]. The perfect diagnosis system based on morphologic characteristics of the cysts and trophozoites is not efficient molecular methods can deliver genotypic characterization complete. The pathogenic *E. histolytica* parasite is unable to synthesize fatty acids but possesses its ability to assemblage fatty acids, so 58% of genes in amoeba refer to a variety of metabolic enzymes as phosphoglycerate (CL6EHI_188180) gene.

About 9938 were detected revealed in *E. histolytica* of these, 1439 genes were discovered, nearly 15% of the genes detected in the amoeba which changes through their evolution (Developmental pathway) in the stages of the parasite in both trophozoite or cyst.

Here, we used the unusual target for PCR detection which is known as CL6EHI_188180 as a mark of PCR method to identify the *E. histolytica* in the infants who are infected with amebiasis and the PCR product recognized at 453bp, parasite is observed low rates about 9.41% because gene expression differs or the diversity from one stage to another during the life cycle of the parasite.
CONCLUSION

Any gene was chosen as a marker must be a housekeeping gene and found in all stages and whole individuals of the specie.

RECOMMENDATION

More surveys to detect other aspects of the Entamoebahistolytica like Cryptic Genetic Variation.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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