Detection ssurRNA gene in children infected by giardiasis

NihadKhalawe Tektook¹, EptissamYounan Pirko² and Tamara Amer Taha³

¹ Middle Technical University- Collage of Medical & Health Technology-Baghdad- iraq.
² Medical College- DiyalaUniversity.Diyala
³ Basic education College- DiyalaUniversity. Iraq.

e-mail: drnihadkhalawe@gmail.com

Abstract. Intestinal parasites that infect humans are widespread health problems, especially in tropical and semitropical zone, is no less important than other microscopic pathogens because it has the ability to infect most of the body's organs and lead to sometimes fatal complications that end in death, in addition to its ability to reproduce and in great numbers, which led to the failure to eliminate it completely, parasitic protozoa and helminthes intestinal are a large variety of parasites that live in the intestine. This study aimed at investigating the infection of Giardia lambila in babies with diarrhea who have been referred to (the women's hospital and children in Babylon, 200 samples of feces were collected form infants patients during the period from January 2018-July 2018, alaboratory microscopictestshowed topositive infection rate50/200 (25%), thise positive samples were examined secondly by Real-Time PCR as a more accurate detection and showed 41/50 (92.41%).

Keyword: Real time PCR, Giardiasis, intestinal diseases.

Introduction

There are many intestinal parasites that cause severe damage when infected with humans and one of the most prominent intestinal parasites primary is Ameba case of the tissue and Giardia, which cause acute diarrhea (1, 2), Giardia lambila is a parasitic whale that is rapidly spread due to the simplicity of its life cycle, its rapid reproduction and its arrival in the host during contaminated foods, its resistance to environmental conditions and chlorine, and its ability to survive for months in moist environments (3,4). Its life cycle is very simple. This parasite causes Giardia, The disease is due to the host eating mature cysts with food and drink and in addition to the method of oral fecal contact (5,6,7).

Giardia contributes to the deterioration of the nutritional status of primary school students, and this deterioration due to malnutrition is negatively reflected on body growth and other vital activities(8,9). The disease is characterized by a number of symptoms including nausea, abdominal pain with bulging, loss of appetite, weakness and weight loss (10). Thus, the current study suggests a molecular diagnosis to investigate the prevalence of G. lamila in babies.
Material and Method

A- Samples collection:

(200) feces sample of infants with diarrhea were collected from 200 infants surveyed to the women's hospital and children in Babylon for the period from January 2018 to July 2018, their ages ranged from (0-2) years.

B- Examination stool samples:

The samples were subjected to microscopic examination by using the direct smear with normal saline solution (0.9%), the Lugol’s Iodine preparation (11), the positive samples are frozen in -20°C until they were examined again by molecular examination, Real-Time PCR test was conducted to investigate G. lamblia by using gene ssuRNA primers, the DNA was then extracted from it by (Stool Genomic DNA extraction) kit supplied by the Korean company Bioneer, and the primers were prepared by the Korean company Bioneer and the method used by (12), table (1):

Table (1): The primers of R.T PCR technique with their nucleotide sequence

| Primer | Sequence                  | PCR product size |
|--------|---------------------------|------------------|
| ssuRNA F | ACG GGT GAA ACA GGA TGA TCC | 73bp             |
| ssuRNA R | TGA TTG ACA GAG GCG GTC TTG |                 |

A Real-Time PCR reaction mix was prepared by the AccuPower® 2X GreenStarqPCR Master Mix, it’s manufactured by the Korean Bioneer Company with all company's instruction implemented.

C- Statistical analysis results

The Data were analyzed by using the statistical program(13), by T-test to determine source of variances between blood and biochemical parameters of patients with control groups (P 0.05).

Results and Discussion

The current study showed that 70/220 were positive for G. lamblia parasite (31.81%), it was higher than (14) in Al-Qadisiyah Governorate when they recorded (22.85%) in infants, and in excess of (15) when he examined the samples of feces for different ages in humans and showed the percentage of infection in the age group (less than two years) was (22.2%), also (16) of the rate of (1.77%) in the Kadhimiya hospital in Baghdad during the examination of 1520 sample feces for children ranging in age from one month to 12 years, we can explain the high incidence of parasitic infection in the current study may be due to ways of transmission to humans are more effective in high degrees and the wet climate condition (17), it is worth mentioning that Al-Basrah governorate under study is located in the far south of Iraq and is known for high temperatures and wet weather most days of the year (18), but this percentage was lower than (19) in Egypt by registering (38.57%) during their examination 70 samples of feces for children with diarrhea, the difference in rates of parasitic infection in the above studies may be due to the difference in the time covered by the study and the difference the size of the sample and the variance in the ages of each study, it may also be attributed to differences in personal hygiene, total number of specimens tested, population density, climatic conditions, examination methods, skill of the examiner and screening techniques (3,11).

we use R-TPCR technique as an ascertained detection of the ssuRNA gene in G. lamblia gene and the results showed positive infection 64/70 (91.42%), as in figure (1):
Figure (1): illustrates the amplification plot of the real-time PCR of the positive results of the *G. lambila* by the ssuRNA gene.

This percentage is higher than in the study of (14) which reached to (87.5%) when they examined 40 samples positive microscopic examination for infants by using the Real-Rime PCR assay, and higher than (12) which recorded (73.07%) *giardia intestinalis* infection in children under 12 years in Al-Qasisiyah governorate, and also what came in the study they showed Of 65/ 41 (63.1%) successfully amplified by using nested-PCR 90f( bg and gdh) genes, also(20) in Germany recorded (60.1%) when examining stool samples of 583 children with diarrhea, as well as (21)recorded41/97 (42.3%) depending on gene (tp1) using Real-Time PCR/RFLP assay, this difference in PCR results may be due to differences in DNA extraction methods from stool samples and PCR methods, the presence of negative samples is due to several causes, foremost among them is the false in microscopic diagnosis for some samples or the presence of inhibitory substances in stool samples may be associated with the DNA polymerase enzyme which inhibits its work and prevents the amplification of DNA(22).

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