

SUMMARY

G.lamblia is a widely distributed flagellated protozoan parasite that inhabits the small intestine. It is the most prominent human protozoal enteropathogen with a prevalence rate that varies from 2% to 5% in the industrialized world and 20% to 30% in the developing world. Giardiasis is more prevalent between infant and children.

Giardiasis is recognized as a disease of travelers worldwide, mainly in the developing world, persons at risk include close contacts of infected persons or those who have contact with infected animals.

The parasite exists in two morphological forms, as a trophozoite which is responsible for the production of diarrhea and malabsorption, and as a cyst which is able to exist outside the host in a suitable environment and is the form of the parasite by which giardiasis is transmitted.

The clinical effects of *G.lamblia* infection range from an asymptomatic carrier state to severe prolonged diarrhea more than 10 days, loss of appetite and weight, vomiting and flatulence. The symptoms differ from patient to another according to duration of infection and host and parasite factors.

Diagnosis of *G.lamblia* infection is done classically by microscopic examination of stool samples but the sensitivity of microscopic examination depends highly on the number of the examined samples, the use of concentration techniques and finally on the skills and experience of the technician as a result laboratory diagnosis of *G.lamblia* infections is very time –consuming .

Molecular detection methods based on PCR have been developed to detect *G.lamblia* DNA in faeces these techniques have numerous advantages in terms of sensitivity, speed and specificity in comparison to conventional methodologies. Moreover, they allow the genotyping of the parasites.

The aim of the present work was the evaluation of parasitological and molecular methods in diagnosis of human infection with *G.lamblia*.

In our study diarrheic stool samples of 100 children were examined microscopically and these children were selected from inpatient and outpatient clinics of Benha University Hospital, Benha Educational Hospital and from Children Specialized Hospital, to include those with clinical manifestations that might be suggestive of giardiasis as diarrhea, abdominal pain and abdominal distension then the study was conducted on a total of 40 case (aged from one year up to 15 years) in addition to 10 cases of other parasites.

All cases included in the study were subjected to the following :

- 1- Microscopic examination by direct smear, iodine stained smear and formol ether concentration technique for detection of *G. lamblia* then these cases were divided into three groups according to the results of parasitological examination of stool samples, of these Group I comprised 20 case (*G. lamblia* +ve group), Group II comprised 20 case (*G. lamblia* -ve group) while Group III comprised 10 cases (five cases were positive for *E.histolyica* and five cases were positive for *C. parvum*) .
- 2- All the above groups (50 case) were subjected molecular diagnosis by real time PCR to detect *G. lamblia* DNA and this technique includes three steps which are:
 - Protein extraction and precipitation of DNA.

- Amplification of *G. lamblia* DNA by real time PCR specific primers complementary to the target DNA sequence.
- Detection of the amplified DNA using SYBR Green I Dye.

Based on the results of the present study, it can be summarized into:

- 1- Giardiasis was an important cause of diarrhea in children less than six years of age, as in Group I there were 11 case (55%) out of 20 examined case with age group between 1-5 years, seven cases (35%) out of 20 examined case with age group between 6-10 years, while there were two cases (10%) out of 20 examined case with age group between 11-15 years, while in Group II, there were 10 cases (50 %) out of 20 examined case with age group between 1-5 years, four cases (20%) out of 20 examined case with age group between 6-10 years while there were six cases (30 %) out of 20 examined case with age group between 11-15 years.
- 2- The number of males was higher than females as in Group I the number of male was 12 (60%) out of 20 examined case, while the number of female was eight (40%) out of 20 examined case. While in Group II the number of male was 13 (65%) out of 20 examined case, while the number of female was seven (35%) out of 20 examined case.
- 3- The number of children from rural areas was higher than those from urban areas as in Group I there were 13 case (65%) out of 20 examined case from rural areas, while there were seven cases (35%) out of 20 examined case from urban areas. While in Group II there were 14 case (70%) out of 20 examined case from rural areas while there were six cases (30%) out of 20 examined case from urban areas.

- 4- Regarding the clinical data of cases in the present study abdominal pain was the most prominent symptom as in Group I there were 11 case (55%) out of 20 examined case presented with abdominal pain, 10 cases (50%) out of 20 examined case with abdominal distention, 9 cases (45%) out of 20 examined case with loss of body weight and failure to thrive, 5 cases (25%) out of 20 examined case with loss of appetite and three cases (15%) out of 20 examined case presented with vomiting, while in Group II there were 10 cases (50%) out of 20 examined case presented with abdominal pain, nine cases (45%) out of 20 examined case with abdominal distention, six cases (30%) out of 20 examined case with loss of body weight and failure to thrive, three cases (15%) out of 20 examined case with loss of appetite and two cases (10%) out of 20 examined case presented with vomiting.
- 5- Severe watery diarrhea was more common than mild and moderate diarrhea among cases included in our study possibly as a consequence of malabsorption as in Group I there were 12 case (60%) out of 20 examined case while in Group II there were 10 cases (50%) out of 20 examined case .
- 6- Regarding the diagnostic methods, direct smear detected five cases (10%) out of 50 examined case.
- 7- Iodine stained smear detected eight cases (16%) out of 50 examined case.
- 8- Formol ether concentration technique detected 20 case (40%) out of 50 examined case.
- 9- PCR detected 26 case (52%) out of 50 examined case [20 case related to Group I while six cases related to Group II but there was any case related to Group III] and proved to be more sensitive and accurate than direct methods with a sensitivity 100%.