

The Use of Enzyme-linked immunosorbant assay in the Detection of *G.Lamblia* Antigens in Children with Persistent Diarrhea

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Abstract

Background: Persistent diarrhea is diarrhea with or without blood, which begins acutely but has long duration (at least 14 days). It may begin as watery diarrhea or as dysentery. It may lead to weight loss and if the volume of stool is great, there is risk of dehydration. Giardial infection is an important cause of persistent diarrhea. Antigen detection is a rapid and specific method for diagnosis of giardiasis.

Aim: Is to evaluate the use of Enzyme- linked immunosorbant assay (ELISA) test (compared to the direct stool examination) as a diagnostic tool for *G.Lamblia* infection among patients with persistent diarrhea.

Patients and methods: A cross sectional study done at Salahaldeen General Hospital among children aged between 6 months and 5 years of age during the period from the 1st of July 2018 to the last of February 2019.

A total of 100 cases were eligible for inclusion in the study sample. Each patient was assessed by a prepared questionnaire including age, sex, residence, type of feeding and duration of diarrhea. Each patient was examined for weight and weight for height. Each patient was sent for general stool examination for 3 consecutive days. All the three stool samples of each patient was examined for the presence of giardia antigen by the use of ELISA test.

Results: Only 23 cases (23%) have *G.Lamblia* infection. Male cases were 13 (56.5%) and Female were 10(43.5%). Most of the infected cases were from rural areas 17 cases (73.9%). The majority of cases were prevalent at the age group between 13-24 months 18 cases (78.3%). Most of the infected cases were on bottle feeding with added solid diet, 13 cases (56.5%). Most of the mothers of the giardia who infected children cases were illiterates, 20 cases (87%). There is no significant difference between the infected and non-infected cases regarding the nutritional state as most of the cases have moderate type of malnutrition for infected 18 (78.3%) and non-infected 55 (71.4%) cases. Most of the giardia cases have only cyst form of *G.Lamblia* 21 cases (91.3%). Regarding the use of ELISA test, only 19 cases (19%) show positive results. According to the results of first stool samples, only 8 cases (8 %) were positive for *G.Lamblia* infection by direct stool examination compared to 19 cases (19%) detected by stool antigens detection (ELISA test) which indicates significant highly sensitive test (82.6%) as compared to the direct stool exam(34.8%). All the cases which were positive for *G.Lamblia* infection by ELISA test were confirmed to be positive by direct stool examination (100%) which indicates very high specificity (100%).

Conclusions: It is concluded that antigen detection is more sensitive and specific than the direct wet stool sample in diagnosis of *G.Lamblia* infection and it is recommended for rapid and easy way for diagnosis of giardiasis.

Keywords: Enzyme-linked immunosorbant, Detection of *G.Lamblia* Antigens, Children with Persistent Diarrhea

INTRODUCTION

Diarrhea has many definitions, but in epidemiological study it is defined as passage of three or more loose (take the shape of the container) or watery stools in 24 hours period.

(1)

Persistent diarrhea is diarrhea with or without blood, which begins acutely but has long duration (at least 14 days) it may begin as watery diarrhea or as dysentery. ⁽²⁾*Giardia lamblia*,

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often referred to simply as “Giardia”, the causative agent of giardiasis, is a flagellated protozoan that was originally observed by Van Leeuwenhoek in 1681. The genus name of this parasite was named after French biologist Alfred Giard.⁽³⁾



Giardia lamblia trophozoite (3)

Regarding transmission of infection, in addition waterborne sources, fecal-oral transmission can occur, for example in day care centers, where children may have poor hygiene practices.⁽⁴⁾

The life cycle begins with a non-infective cyst being excreted with the feces of an infected individual. The cyst is hard, providing protection from various degrees of heat and cold, desiccation, and infection from other organisms. A distinguishing characteristic of the cyst is four nuclei and a retracted cytoplasm. Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes a sexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the feces. While the trophozoites may be found in the feces, only the cysts are capable of surviving outside the host.⁽³⁾

Stool examination for trophozoites or cysts is the traditional method for diagnosing giardiasis. Trophozoites may also be identified in the small intestine. Cysts are oval, measure 8-12 X 7-10 μm , and characteristically contain 4 nuclei. Trophozoites are pear-shaped, dorsally convex, flattened parasites with 2 nuclei and 4 pairs of flagella. The stool sample should be freshly examined (within 6 hours from receiving the sample) for accurate diagnosis as *Giardia lamblia* trophozoite will lose its motility in stored sample by formalin although the shape of the trophozoites and cysts were preserved.⁽⁴⁾ The traditional basis of diagnosis is identification of *Giardia lamblia* trophozoites or cysts in the stool of infected patients via a stool ova and parasite (O&P) examination. Stool examination may be performed on fresh specimens or after preservation with polyvinyl alcohol or 10% formalin (with appropriate staining).⁽⁵⁾

Antigen detection assays are available and often the tests of choice. Various immunologic techniques have been used to detect *Giardia*-specific antigens in fecal specimens. The most common technique is monoclonal antibody-based capture enzyme-linked immunosorbent assay (ELISA). The antigen detection via ELISA is at least as sensitive as microscopic examination. An immunofluorescence assay is available for detecting *Giardia* and *Cryptosporidium* species.

DNA probes for *Giardia* species are available. DNA-based fecal detection assays are being developed.⁽⁵⁾



The *giardia* test is a monoclonal antibody-based ELISA for the rapid detection of *Giardia lamblia* cysts antigen in stool specimens and serves as an in vitro aid in the diagnosis of giardiasis.⁽⁵⁾

Features of The *Giardia* Test

- Rapid (results in < 1 hour)
- Specific for *giardia*
- Easy to perform
- High standardized
- Micro well format.⁽⁵⁾

AIM OF THE STUDY

To evaluate the use of ELISA test compared to the direct stool examination as a diagnostic tool for *Giardia lamblia* infection among patients with persistent diarrhea.

PATIENTS AND METHOD

A cross sectional study was carried out in Salahaldeen General Hospital, from the 1st of July 2018 to the last of February 2019 involved children aged 6 months to 5 years who present with history of persistent diarrhea. For the purpose of selecting patients with persistent diarrhea, the condition defined as diarrhea (loose or watery stool) lasting more than 14 days with no more than 48 hours normal bowel motion. A patient with normal bowel motions during this period is considered a new episode and considered as (recurrent diarrhea) and excluded from the study.

A special designed questionnaire was used through which information taken which include: name, age, sex and area of residence (rural or urban) and feeding pattern (breast, bottle, both breast and bottle, breast and solid, bottle and solid, and solid only) and mothers education level (Illiterate, primary school, secondary school, college and higher education).

Physical examination was done to all patients which includes clinical examination for weight and height measurement and assessment of nutritional status according to weight/age and weight/height by the growth chart, using the Tanner growth chart and the nutritional state which were classified into mild, moderate and severe malnutrition according to the Waterlaw classification for malnutrition.⁽³⁾

Each patient was sent for general stool examination for 3 consecutive days to prove or exclude *Giardia lamblia* infection. The freshly passed stool samples (within 6 hours) were examined by experienced microbiologist. The

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presence of *Giardia lamblia* cyst or trophozoites is considered as symptomatic infection (index case). If the parasite is not identified after 3 consecutive stools, it is considered non infected (control cases). Ideally, 3 specimens from different days should be examined because of potential variations in fecal excretion of cysts. Substances that may interfere with test results include antacids and anti-diarrheal antibiotics., anti-parasite drugs., enemas or laxatives., barium (a contrast material used for X-rays) were stopped 2 weeks before the stool examination.

Giardia lamblia detection using the antigen detection method was done by using commercial ELISA-copro-antigen test (ProSpect *Giardia*-ELISA-Micro plate assay; Remel, Lenexa, KS, USA) kits. Results obtained within 1 hour. The true positive case was that with both positive direct stool examination and ELISA test. The false positive case was that with positive ELISA test and negative stool sample. The true negative cases was that case with both negative direct stool sample and ELISA test. The false negative cases were that cases with positive direct stool examination and negative ELISA test.

Sensitivity was calculated according to the following equation: sensitivity % = true positive / true positive + false negative x 100. Specificity was calculated as the followings: specificity % = true negative / false positive + true negative x 100. ⁽⁵⁾

Statistical analysis was done by using student's chi-square, the P-value less than 0.05 were considered significant.

Results

A total of 100 cases was included in the study. Males were 52 (52%) and females 48(48%).

Figure (1) shows the distribution of cases according to the giardial infection. It is clearly shown that only 23 cases (33%) have giardia infestation.

Figure (2) shows the distribution of infected cases according to sex. male cases were 13 (56.5%) and female were 10(43.5%).

Figure (3) shows the distribution of infected cases according to residence . most of the cases were from rural areas 17 cases (73.9%) and from urban areas 6(26.1%).

Table (1) shows the distribution of giardia infected cases according to the age groups. Most of the cases were prevalent at the age group between 13-24 months 18 cases (78.3%).

Table (2) shows the distribution of infected cases according to the type of feeding. Most of the cases were on bottle feeding with added solid diet, 13 case (56.5%).

Table (3): shows the distribution of cases according to the educational level of the mothers. Most of the giardia infected cases were illetrate , 20 cases (87%) while most of the rest cases have completed only the primary school 63 caees (81.8%).

Table (4) shows the relation of nutritional status to the infection state among the cases. There is no significant difference between the infected and non-infected cases as most of the cases have moderate type of malnutrition for infected 18 (78.3%) and non-infected 55 (71.4%) cases.

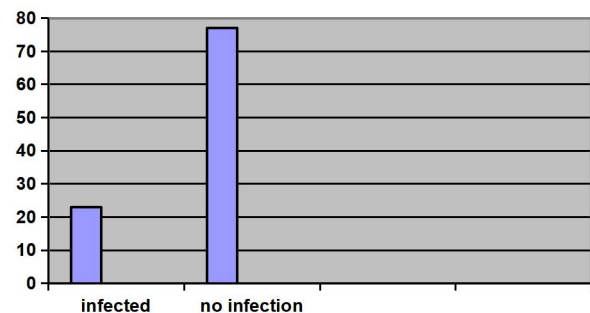
Table (5) shows the distribution of cases according to the presence of *Giardia lamblia* cysts or trophozoits on general stool examination. Most of the study cases have only cyst form of giardia 21 cases (91.3%).

Figure (4) shows the distribution of cases according to the ELISA test. Only 19 cases (19%) shows positive results.

Table (6): shows the distribution of cases according to the

results of first stool samples. There are significant results regarding that the use of ELISA test was more sensitive in diagnosis of *Giardia lamblia* infection 19 cases (19%) than the direct examination of fresh stool samples 8 cases (8%). Sensitivity test was 82.6% and 34.8% for ELISA and direct stool examination respectively.

Table (7): shows the distribution of cases according to the results of three consecutive stool samples. There is nonsignificant results regarding that the use of ELISA test in diagnosis of *Giardia lamblia* infestation 19 cases (19%) than the direct examination of fresh stool samples 23 cases (23%). Sensitivity test was 82.6% and All the cases which were positive for *giardia lamblia* infestation by ELISA test were confirmed to be positive by direct stool examination (100%)



which indicates very high specificity (100%).

Figure 1. shows the distribution of cases according to the *Giardia lamblia* infection

Figure 2. shows the distribution of infected cases according to sex.

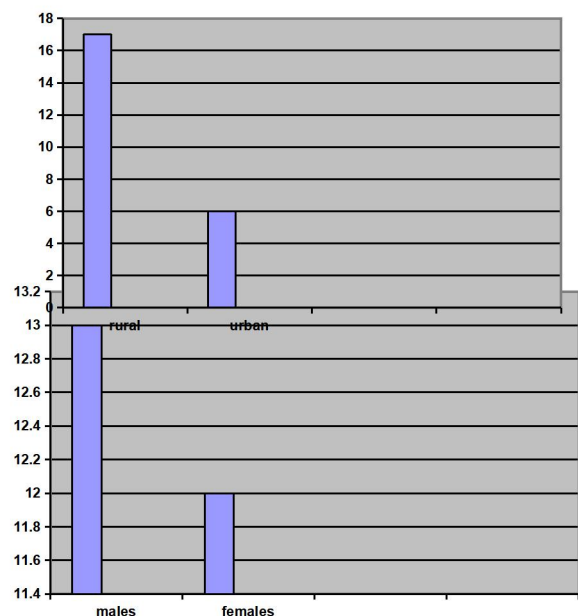


Figure 3. shows the distribution of infected cases according to residence

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Table 1. Distribution of cases in relation to age groups.

Age groups	<i>Giardia lamblia</i> infection	
	No.	%
6-12	0	0
13-24	18	78.3
25-36	1	4.3
37-48	2	8.7
49-60	2	8.7
Total	23	100

Table 2. shows the distribution of infected cases according to the type of feeding.

Type of feeding	<i>Giardia lamblia</i> infection	
	No.	%
Breast only	0	0
Bottle only	0	0
Breast +bottle	0	0
Breast +solid	4	17.4
Bottle +solid	13	56.5
Solid only	6	26.1
Total	23	100

Table 3. The relation of maternal educational level to giardial infection among the study cases.

Educational level	Infected		Not infected	
	No.	%	No.	%
Illiterate	20	87	11	14.3
Primary school	3	13	63	81.8
Secondary school	0	0	3	3.9
University	0	0	0	0
Total	23		77	

Table 4. The nutritional state among the study cases.

	Infected		Not infected	
	No.	%	No.	%
Normal	0	0	0	0
Mild malnutrition	0	0	5	6.5
Moderate malnutrition	18	78.3	55	71.4
Sever malnutrition	5	21.8	17	22.1
Total	23	100	77	100

P-value >0.05 (not significant)

Table 5. shows the distribution of cases according to the presence of giardia cyst or trophozoite on general stool examination.

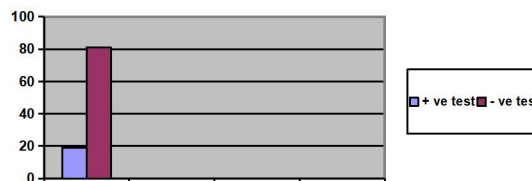
Giardia type	No.	%
Cyst only	21	91.3
Trophozoite. Only	0	0
Cyst and Trophozoite.	2	8.7
Total	23	100

Table 6. shows the distribution of cases according to the results of first stool samples.

Test type	Positive	Negative	Total
Direct stool examination	8 (8%)	92(92%)	100(100%)
ELISA	19(19%)	81(81%)	100(100%)

P- value <0.05 (significant)

Figure 4. Distribution of cases according to the ELISA test.



Test type	Positive	Negative	Total
Direct stool examination	23 (23%)	77(77%)	100(100%)
ELISA	19(19%)	81(81%)	100(100%)

P- value > 0.05 (non-significant)

DISCUSSION

Persistent diarrhea is one of the major causes of malnutrition because food passes too quickly through the gut and therefore cannot be absorbed properly. ⁽⁶⁾

Giardiasis is the most prevalent protozoal infection of the human intestine. *Giardia lamblia* is one of the most common causative agents of epidemic and endemic diarrheal illness throughout the world. ⁽⁷⁾

It is clearly shown that only 23 cases have giardia infestation. This is more than what was reported by other authors ⁽⁷⁾ who reported a lower incidence (5.4%) of giardia infection among patients with persistent diarrhea. This may be due to the fact that giardia infestation is a waterborne disease and is transported by contaminated food and water and as the water supply to the major part of Tikrit city was poorly sterilized and this explains the higher incidence of giardia infestation among the study cases.

Most of the study cases were from rural areas. This is similar to that found by another study ⁽⁸⁾ who give nearly similar results (65 %). This result agrees also with the other study ⁽⁶⁾ that which reported a high percentage of patients from rural areas (67%). This may be due to neglecting and inappropriate feeding habits in addition to the frequent use of non-boiled water which is the most common risk factor for repeated gastroenteritis. *Giardia lamblia* infestation is a waterborne disease that transmitted by contaminated food and water and as the water supply among the study cases from the rural area was from tankers that bring water directly from the river without sterilization explain the high results of giardia infestation among the rural cases.

Most of the cases were prevalent at the age group between 13-24 months. This finding is in agreement with that of the WHO studies ⁽⁷⁾. This can be explained by the fact that immunity derived from the mother decrease in 2nd half of first year of life together with the introduction of contaminated food or water. ⁽⁸⁾ It is also proved that giardiasis occurs mainly in children is particularly at risk for infection through exposure at day-care centers. Many of the epidemics documented over the last 2 decades have originated in day-care centers. Another study by Al-Bederi RR estimates the prevalence of infection, defined by the presence of cyst passage, has been as high as 20-25% in children younger than 3 years. ⁽⁹⁾

Most of the cases were on bottle feeding with added solid diet while no case was on breast feeding only. This explains that the infection of the study cases by *Giardia lamblia* due to the loss of the protective role of breast feeding and the bottles is an important source of infection as it is easily contaminated. Previous studies have demonstrated that continuing breast feeding for more than 6 months markedly decrease the incidence of persistent diarrhea ^(10,11), this is

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because that the breast milk is ready, sterilized all the time, fresh, free of bacteria and also reduce the chance of gastroenteritis, breast feeding also transfer macrophage and immunoglobulin especially in the colostrum, while bottle feeders are more liable for intestinal infections because of more chance of contamination. The protective role of breastfeeding against *Giardia lamblia*, a protozoan parasite endemic in Central Africa, was assessed through an analysis of stool samples from 400 infants aged 1 week-24 months in Libreville. The percentage of infants infected with cysts or trophozoites of *Giardia lamblia* was 1.2% in the exclusively breastfed group, 1.32% among infants who were partially breastfed (at least 2 feedings/day) and 14.7% in the bottle-fed group. In the 0-5-month age group, *Giardia* infection was found in 2.2% of the breastfed infants compared with 9.1% of the bottle-fed infants. In the 6-11-month age group, these percentages were 0 and 11.4%, respectively, while they were 2.2% and 17.6%, respectively, in the 12-23-month age group. These findings confirm an in vivo protective effect of human milk in *Giardia* infection. ⁽¹²⁾

Most of mothers of the *giardia* infected cases were illiterate while most of the rest cases completed only the primary school. A study was done in Spain showed that low level of mother's education associated with increase in percentage of anemia and diarrhea in the children. ⁽¹⁴⁾ This is in agreement with presented study which showed that (49%) of mother's had low education. This goes with other study done by Alammary ⁽⁶⁾ and Al-Bederi. ⁽⁹⁾

There is no significant difference between the infected and non-infected cases in regard to the nutritional state as most of the cases have moderate type of malnutrition for infected and non-infected cases. *Giardia lamblia* has been implicated as the chief cause of growth retardation in infected children, even after control of other agents that cause diarrhea. Approximately 50% of patients infected with *Giardia lamblia* may present with a variety of symptoms, including acute watery diarrhea, chronic diarrhea with malabsorption and weight loss, and abdominal cramping. The pathogenesis of diarrhea in giardiasis is unknown. Proposed mechanisms include occlusion of the mucosa by large numbers of the organisms, competition with the host for nutrients, epithelial damage, immune-mediated absorptive changes such as transient lactase deficiency, altered mucus secretion, and alterations in motility. ⁽¹³⁾

Most of the study cases have only cyst form of *giardia* 21 cases (91.3%). These findings were in agreement with other study ⁽¹⁴⁾ which shows nearly similar results. This is due to the fact that trophozoite presents in the small intestine and with the continuation of the life cycle. They reach the colon in the form of cysts. If the diarrhea is so severe with increase in the intestinal motility, trophozoite may be seen on wet fresh stool sample.

Only 8 cases or proved *Giardia lamblia* infection are detected on first stool sample and in the rest of cases. It is confirmed by the second or third sample. This is similar to the results seen by other study ⁽¹⁵⁾ who they confirm that several samples of stool may be needed to confirm or exclude the presence of *Giardia lamblia* infection. This is due to the fact that *giardia* cyst is not passed in each stool (in fact it is sporadic), and so it needs at least 3 consecutive stool sample on 3 consecutive days to prove or exclude infection. ⁽¹⁵⁾

All the cases that were positive by ELISA test were confirmed (by direct stool examination) for the presence of *giardia* cysts and or trophozoites (100%), this indicates the

very high specificity of this test for *Giardia lamblia* antigens (specificity was 100%). This is nearly similar to the results founded by other study ⁽¹⁶⁾ which confirms the high specificity of ELISA test in diagnosis of giardiasis.

Regarding the first stool sample of examination, and from 100 cases examined for the presence of *Giardia lamblia* infections, less number was positive for *giardia lamblia* cysts and / or trophozoite on direct examination of wet fresh stool sample as compared to that detected by ELISA test. This indicates the high sensitivity of this test as compared to the direct examination test. This is similar to another study. ⁽¹⁷⁾ which shows that antigen testing was of higher sensitivity to that of the direct stool examination test. In this study, a captive ELISA using affinity purified antisera to *Giardia lamblia* antigen was assessed in the early detection of giardiasis in 68 children <6 years old in the village of Wadihaloo in the United Arab Emirates. The proportion of fecal samples positive for *Giardia*, by routine microscopically examination, formol-ether concentration, and fecal antigen capture ELISA were 8.84%, 14.72% and 19.26% respectively. ⁽¹⁷⁾⁽¹⁸⁾

On the other hand, the above results were dissimilar to that of other study ⁽⁵⁾ which show a less sensitivity of antigen detection test as compared to the direct stool sample examination. In this study, the commercial strip test had a sensitivity of 58%, a specificity of 99. These results are comparable to those obtained using microscopy of direct wet-mounted stool. Since the CORIS *Giardia*-Strip test is simpler to perform, it can replace direct wet-mounted stool microscopy for the rapid diagnosis of giardiasis; however, its sensitivity is inferior to that of other immunochromatographic antigen detection tests and fresh stool samples are required for its use. Nevertheless, the results suggest that a positive CORIS *Giardia*-Strip test outcome does not need confirmation, while samples with negative results should be re-examined using another, more sensitive, test. ⁽⁵⁾⁽¹⁹⁾

After 3 consecutive samples, there was no change in the results of ELISA test while direct wet stool sample detect more cases of *Giardia lamblia*. this may be due that several stool samples with repeated concentrated examination by the microbiologist will detect more cases of *Giardia lamblia* cases due to the fact that *giardia* cyst is not passed in each stool (in fact it is sporadic) so several direct stool exam will detect more cases of *giardia* infestation.

CONCLUSION AND RECOMMENDATIONS

It is concluded that antigen detection is a specific and more sensitive than the direct wet single stool sample in diagnosis of *Giardia lamblia*. infection and it is recommended for rapid and easy way for diagnosis of giardiasis.

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