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PREVALENCE AND ASSOCIATED RISK FACTORS FOR *GIARDIA LAMBLIA* AMONG CHILDREN IN ASWAN UNIVERSITY HOSPITALS

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Abstract

Giardiasis is one of commonest intestinal protozoa causing diarrheal worldwide especially in day-care centers. The present study aimed to detect prevalence of *G. lamblia* and its risk factors among children by using stained microscopic smears and immunochromatographic test (ICT). A cross-sectional study was carried out among 100 children (4 to 15 years) suffered from diarrhea at Pediatric Outpatient Clinics, Aswan University Hospitals. Sheet was filled out one each child or his parents as to name, sex, age, and previous history of giardiasis. All samples were examined by ICT, and microscopy

The results showed that the prevalence of giardiasis among children was 49%, & 41% by using stained microscopic examination and rapid ICT (Epituub USA) respectively. Sensitivity and specificity of rapid test were (77.6% & 94.1%) respectively without cross-reactivity with other parasites, with potentially useful rapid test in absence of unqualified lab technician or in epidemiologic surveys. Giardiasis is more prevalent in rural areas' children than in urban ones.

Key words: Children**,** Giardiasis**,** Diagnosis **M**icroscopy, Immunochromatographic test.

Introduction

Giardia lamblia is a protozoan parasite inhabits upper small intestine of man and other vertebrates as one of the causes of intestinal disease worldwide (Fahmy *et al,* 2015). Some giardiasis infected people without symptoms, but spread it to others by stool. For those who do get sick, signs and symptoms appear one to three weeks after exposure include watery, sometimes foul smelling diarrhea alternated with soft, greasy stools, fatigue, stomach cramps, bloating, gas, nausea, and weight loss (Mayo Clinic, 2021). Leung *et al.* (2019) in Canada reported that giardiasis is transmitted by fecal-oral route, via ingestion of contaminated water, or food or pers on-to person, with risk factors to children in day-care centers, and their workers, and institutionalized individuals in endemic areas. Mohamed *et al*. (2020) reported that difference in enteric parasites, as *Giardia*, may due to many factors, as geographic, epidemiologic, socioeconomic status, and environmental sanitation measures. In Egypt giardiasis was reported by many authors (El-Shazly *et al,* 2004; El-Beshbishi *et al,* 2005; Mohammad *et al,* 2012; Sadek *et al,* 2013; Dyab *et al,* 2016; Taha *et al,* 2018), and many others.

Giardiasis infection is diagnosed by micrroscopic stool analysis (Garcia *et al,* 2017) or by stool antigen assays (Ellam *et al,* 2008). Epidemiological authors suggested that microscopy was time-consuming and depended on an expert technician (Alharbi *et al,* 2020). But, Kosack *et al.* (2017) mentioned that one must selected the serodiagnostic test due to differences in sensitivity, specificity and diagnostic value, costs and availability of equipped laboratory and expert man power.

This study aimed to detect the prevalence and risk factors for *Giardia lamblia* infection among children in Aswan Governorate by using microscopy versus immunochrommatographic tests (ICT).

Materials and Methods

Samples collection: One hundred stool samples were collected from children (4 to 15 years) suffered from diarrhea attended Pediatric Outpatient Clinics, Aswan University Hospitals in period from February 2020 to January 2021. Medical sheets were filled out on each child or his/her parents including name, sex, age, residence, and type of water supply, family history with giardiasis or oth-

er gastrointestinal parasites and treatment.

Ethical approval: It was obtained from the Ethics Committee Board of Faculty of Medicine, Aswan University that agreed with the guidelines of Helsinki (2000). Informed consent was obtained from children's parents after explaining the study aim and nature.

Morning stool samples were collected in clean labeled plastic disposable cups, and sample was divided into 2 parts, one was processed for microscopic examination, and second for immunochromatographic test (ICT).

Microscopy stool examination was done as direct wet smear, formol ether sedimentation method stained with modified Ziehl-Neelsen, Gomori's Trichrome, and modified Kenyon's acid-fast stain (El Shazly *et al,* 2006).

The second part was examined by antigen rapid solid phase qualitative immunochromatography kit by monoclonal antibodies *Giardia lamblia* antigen, and solidphase/membrane coated specific anti-*G. lamblia* monoclonal antibody, following manufacturer's instructions (Epi-Tuub® Fecal *Gardia* Antigen test, Epitope Diagnostic, Inc. CA, USA).

Statistical analysis: Data were computerized and analyzed by using the Statistical Package SPSS version 17 (Armitage, 1983).

Results

Giardiasis rapid test were positive in 41% and negative in 59% cases. Microscopy was positive in 49% cases & negative in 51%, males were 51%, and females 49%. Positive cases to negative ones ages were 9.93±2.59 to 10.18±2.35. Patients were 13 from urban areas and 28 from rural ones. Some gave history of giardiasis. All suffered from acute or chronic diarrhea, abdominal pain 66. Stool consistency was watery in 45 and loose in 5t. Cases were more in summer 61%, followed by spring 17%, winter 16%, and least 6% in autumn.

Ages of ICT positive and negatives were not significant (P=.373). Positive cases were 13/27 among urban patients and in 28/45 of rural ones without significant difference.

Risk factors increased to 30/31 among patients' with family giardiasis compared to 11/28 patients without family history. Infection was 18 (43.9%) among children used safe water compared to 23 (56.1%) who consumed unsafe water. Hand-washing positive giardiasis children were 11(26.8%) compared 30 (73.2%) who didn't wash hands. Positive ICT had watery stool in 21 (51.22%), semisolid in 20 (48.78%) and negative ones in 24(40.68%), semisolid in 35 (59.32%) without significant difference. Abdominal pain was in 27 (65.85%) cases compared to negative ICT in 39 (66.1%) without significance. All suffered from diarrhea. Seasonal variation was not significant between ICT cases, 25 & 36 in summer, in winter 6 & 10, in spring 8 & 9 and in autumn just 2 cases and 4 cases (6.78%) for negative ones.

Microscopy and ICT showed 38 positive, 11 by microscopy but negative by ICT (false -ve) and three positive by ICT only (false +ve), but true negatives were 48 cases, with high significant (P <0.001). ICT gave sensitivity (77.6%), specificity (94.1%), PPV (92.7%), NPV (81.4%) and accuracy (86%).

Details were given in tables (1, 2, 3, 4, 5, & 6).

Table 1: Diagnosis of giardiasis in patients.

Diagnostic test	Infection	No.	Percentage
Rapid test	Positive	41	41.0%
	Negative	59	59.0%
Stool examination	Positive	49	49.0%
	Negative	51	51.0%

Table 2: Interpretation between rapid test and demographic data.

Variations		Total	Cases positive	Cases negative	P-value	Significant
Age		100	9.93±2.59	10.36±2.18	0.373[T]	NS
Sex	Male	51	20 (48.78%)	31 (52.54%)	0.711[C]	NS
	Female	49	21 (51.22%)	28 (47.46%)		
Residence	Urban	27	13 (31.71%)	14 (23.73%)	0.377[C]	NS
	Rural	73	28 (68.29%)	45 (76.27%)		

Table 3: Patients clinical presentations.

Variations		No.	Percentage
Stool consistency	Loose	55	55.0%
	Watery	45	45.0%
Abdominal pain	No	34	34.0%
	Yes	66	66.0%
Diarrhea	No	0	0.0%
	Yes	100	100.0%
Previous giardiasis treatment	No	64	64.0%
	Yes	36	36.0%
Seasonal variations	Summer	61	61.0%
	Winter	16	16.0%
	Spring	17	17.0%
	Autumn	6	6.0%

$^{T=}$Student t-test of significance, $^{C=}$Chi-square test of significance.

Table 4: Relation between interpretation of rapid test and associated risk factors

Variations		Positive No. (%)	Negative No. (%)	P-value	Significant
Other family giardiasis	Yes	30 (73.2%)	31 (52.5%)	0.038	Sig.
	No	11 (26.8%)	28 (47.5%)		
Water supply	Safe	18 (43.9%)	40 (67.8%)	0.017	Sig.
	Not safe	23 (56.1%)	19 (32.2%)		
Hand washing	Yes	11 (26.8%)	28 (47.5%)	0.038	Sig.
	No	30 (73.2%)	31 (52.5%)		

Table 5: Interpretation of rapid test and clinical presentation.

Variations		Positive No. (%)	Negative No. (%)	P-value	Significant
Consistency	Loose	20 (48.78%)	35 (59.32%)	0.297$^{(C)}$	NS
	Watery	21 (51.22%)	24 (40.68%)		
Abdominal pain		27 (65.85%)	39 (66.1%)	0.979$^{(C)}$	NS
Diarrhea		41 (100%)	59 (100%)		
Previous giardiasis		15 (36.59%)	21 (35.59%)	0.919$^{(C)}$	NS
Seasonal variation	Summer	25 (60.98%)	36 (61.02%)		
	Winter	6 (14.63%)	10 (16.95%)	0.932$^{(F)}$	NS
	Spring	8 (19.51%)	9 (15.25%)		
	Autumn	2 (4.88%)	4 (6.78%)		

Table 6: Test agreement between stool examination as a gold standard method and rapid test.

(N= 100)		Stool positive	Stool negative	Total	Percent	Kappa	P value	Significant
Rapid test	Positive	38 (92.68%)	3 (7.32%)	41 (41%)	86%	0.719	<0.001	S
	Negative	11 (18.64%)	48 (81.36%)	59 (59%)				
Total		49 (49%)	51 (51%)	100 (100%)				

Discussion

In the present study, among children suffered from diarrhea (aged 4 to 15years, with mean 10.18±2.35), by ICT 41/100 were positive, and stained microscopic examination 49/100 were positive, and thus ICT missed eight positive cases. This agreed with McHardy *et al.* (2014) in USA reported that despite recent advances in diagnostic technology, microscopic examination of stool specimens to diagnosis of most pathogenic intestinal protozoa, and is labor-intensive and requires a skilled technologist. Guswanto *et al.* (2017) in Indonesia reported that ICT usage was preferable in the field situations for babesiosis rapid diagnosis. This disagreed with Ghieth *et al.* (2016), reported that ICT gave high sensitivity (78.6%) than microscopy (76.2 %), but couldn't be used as as a consistent single detection method due to their lowered sensitivities. They added that the nPCR targeting TPI proved a reliable diagnostic test for true *Girdia* prevalence. Also, Atia *et al.* (2016) reported Immunochromatography/copro-antigen test gave sensitivity (100%) and specificity (96.6%) in *G. lamblia* detection. But, El Shazly *et al.* (2004) in Dakhalia found an amplification of 73.3% with fresh feces and concluded that PCR as a routine work for giardiasis diagnosis was not accepted at least in developing and under-developing countries due to its high cost,

high quality of technical staff and advanced laboratory equipment required. Wassef and Abdel-Malek (2019) in Menoufia found that ICT was more sensitive in detecting lower toxoplasmosis antibodies level than ELISA, mainly in immunocompromised patients.

In the present study, giardiasis prevalence in infected children was 49%, &41% by micoscopy and ICT respectively. This agreed with Helmy *et al.* (2009) in Alexandria reported that a rate of 42% in some areas. Sadek *et al.* (2013) in Menoufia & Sharkia Governorates reported rates of 30% & 28.4% respectively. Asher *et al.* (2014) reported that variation in giardiasis prevalence could be due to distribution different *Giardia* assemblages and other risk factors that varied from 1% to 36%, or even up to 72% a country to another. Egyptian giardiasis rates varied between 7.9% (El-Beshbishi *et al,* 2005) up to 35% (Fahmy *et al,* 2015). Also, El Shazly *et al.* (2007) in Dakahlia Governorate examined a total of 840 potable water samples from seven districts from River Nile Demiatta branch, and sub-branch Bahr-El-Saghear the commonest protozoa were *Cryptosporidium parvum* (3.1%) and *G. intestinalis* (2.1%).

In the present study, increased of infection risk in sexes was without significance. This agreed with Mohamed *et al.* (2020) who didn't find giardiasis had age, sex and clinical symptom distributions significant. Al-Mekhlafi *et al.* (2010) in Malaysia reported that *Giardia* was higher in females than males. Zylberberg *et al.* (2017) in USA reported that high prevalence in males as they were more often engaged in predisposing activities such as playing in streams or ponds. Samie *et al.* (2020) in South Africa found that males' children were nearly affected than females due to nutritional deficiencies.

In the present study, giardiasis was higher in children who lived in rural areas than in urban ones. This agreed with Mohammad *et al.* (2012) in Egypt who reported that children in rural were more liable to soil transmitted parasitic infections due to high risk of exposure to contaminated soil in rural settings, bad hygiene, and hand-to-mouth behavior character of early childhood. Al-Mekhlafi *et al.* (2017) attributed this fact due to poverty, poor living and hygienic conditions, drinking sewage contaminated underground water. Bryan *et al.* (2020) in Columbia added that use of human and animal excreta in agriculture fertilization and household wastewater being dropped in irrigation channels, and farm animals contact.

In the present study, giardiasis children suffered from acute and/or chronic diarrhea with malabsorption. This agreed with Atia *et al.* (2016) who reported that diarrhea was the commonest symptom (50%) in giardiasis patients, and Hawash *et al.* (1915) in Saudi Arabia reported acute and transient diarrhea in 71% of intestinal protozoa infection. Nabarro *et al.* (2015) in London reported that the commonest giardiasis clinical signs were diarrhea, with or without mal-absorption syndrome, as infection caused pathophysiological effects on patient's intestine by cellular attachment and excretion of parasite that damages the integrity of intestinal epithelia, function of brush border enzymes, and induced apoptosis in cells. Einarsson *et al.* (2016) in Sweden reported that *G. intestinalis* caused acute, watery diarrhea affected the electrolyte balance causing increased intestinal permeability, and diarrhea.

In the present study, 51.22% of patients had watery stool consistency & 48.78% had loose stool, but without significant difference. Nkrumah and Nguah (2011) in Ghana reported giardiasis was exclusively in loose or semi-formed stools. Helmy *et al.* (2014) reported that stool consistency was not giardiasis indicator. The present children (65.85%) also suffered from abdominal pain. This agreed with Younas *et al.* (2008) in Pakistan reported that abdominal cramps, nausea, vomiting, distension, flatulence/bloating, anorexia & weight loss were the clinical symptom.

In the present study, giardiasis was more prevalent on summer (60.98%), followed by spring (19.51%), winter (14.63%) and autumn (4.88%). This more or less agreed with

Ismail *et al.* (2016) who reported that giardiasis was detected all year around with peaks in mid-summer and late winter. González-Moreno *et al.* (2011) in Spain didn't find seasonality and giardiasis prevalence.

In the present study, positive patients with giardiasis family members (73.2%) were higher than those who didn't have any family infected member (26.8%). This indicated a high level of transmission of infection occurring horizontally within the household and infected family members serving as a source of infection. In this situation, *Giardia* parasites were probably transmitted directly from person-to-person contact or food or water contaminated with infected patient. This was agreed with Delaimy *et al.* (2014) in Malaysia who reported that the population with other family members infected with *Giardia* was a significant infection risk factor. Also, in the present study, children who consumed unsafe water supply were higher than those used piped or boiled water supplies 56.1% & 43.9% respectively. CDC (2022) mentioned that *Giardia* spreads easily from person to person or via contaminated water, food, surfaces, or objects. The most common way people get sick is by swallowing contaminated drinking water or recreational water (for example, lakes, rivers, or pools).

In the present study, little infection was in those who practice hand-washing. Undoubtedly, hand-washing is the most effective hygienic practice for controlling pathogen transmission including bacterial, viral, fungal, & parasitic agents. Talaat *et al.* (2011) in Egypt reported that an intensive hand hygiene campaign was effective in reducing absenteeism caused by influenza-like illness (ILI), diarrhea, conjunctivitis, and laboratory-confirmed influenza. Mahmud *et al.* (2015) in Ethopia concluded that hand-washing with soap at key times and weekly nail clipping significantly decreased intestinal parasite reinfection rates and hand-washing intervention significantly reduced anemia in children.

In the present study, a total of 49 giardiasis cases were proved by wet smear and concentration methods, only 38 were positive by rapid giardia dipsticks (ICT), and 11 were false-negative cases based on true-positive microscopy ones. This was agreed with Weitzel *et al*. (2006) reported 80% sensitivity, Goñi *et al*. (2012) reported 93-97% sensitivity, Regnath *et al.* (2006) and Atia *et al.* (2016) reported 100% sensitivity, but, Bouyou-Akotet *et al.* (2016) reported low sensitivity 63% for ICTs. The variability in the sensitivity and specificity of rapid dipstick test from one study to another may be attributed to the type of the "gold standard" test to which the results compared rapid dipstick test didn't reveal positivity for any stool samples containing intestinal parasites other than *Giardia*.

Generally speaking, the giardiasis itself is one of the gastrointestinal risk disorders especially among children in spite of its different genotypes (Abdel-Moneim and Sultan, 2008). However, Ahmad *et al.* (2020) in Egypt reported more genetic studies were needed to clarify the association between parasite genetic diversity and patient symptomatology. Moreover, Hussein *et al.* (2017) in Egypt found a significant relation between assemblage distributions and heavy infection intensity. They concluded that the higher incidence of assemblage B among symptomatic children compared to asymptomatic could denote its possible pathogenic potentiality. El-Badry *et al.* (2017) in Egypt reported that *Giardia intestinalis* and *Helicobacter pylori* are two intestinal pathogens sharing the same mode of infection, and co-infection was more frequent with assemblage B (50.9%) than A (40%). They added that intestinal parasitism and *H. pylori* association was complex problem necessitated further genomic studies for a better understanding of the epidemiological, clinical impact, and possible strategies for their treatment and control. Elhadad *et al.* (2021) in Alexandria reported that the prevalence of *Giardia* was 18.1% in the examined children. Mixed assemblages A & B was more common (47.4%) than single assemblage B (36.8%) or assemblage A

(15.8%). They added that distribution of different genotypes was significantly associated with the residence area, animal contact, and hand-washing habits. A non-significant association was between the giardiasis assemblages and clinical manifestations, with B genotype predominant among children. El Askary *et al.* (2021) in Benu-Suif University Hospitals reported that among chronic kidney diseases (CKD) patients with giardiasis and *H. pylori* males showed prevalent of (66%), who was from rural areas (66.5%) and using tap water (83.5%). *H. pylori* infection was in 22 CKD patients and in 27 control patients, and the co-infection was found in 10 CKD patients and 19 controls.

Abroad, Ankarklev *et al.* (2012) in Uganda reported that *G. intestinalis* and *H. pylori* infected childrens gastrointestinal tracts early life in endemic locations throughout their lives. Giardia causes diarrhea, bloating, flatulence, and malnutrition, and *H. pylori* colonized in the gastric mucosa causes peptic ulcers, chronic gastritis, and also gastric cancer. Krzyżek and Gościniak (2017) in Poland reported that predisposing factors coincided in *G. intestinalis* and *H. pylori*, as low age, immune suppression status, low socioeconomic and educational status, and consumption of contaminated water sources, and upper gastrointestinal complaints such as upper abdominal pain, abdominal bloating, nausea, vomiting, and epigastric bleeding.

Conclusion

It must be in mind that giardiasis co-infected with *Helicobacter pylori* is a public health risky especially among children. In spite of the controversies about giardiasis and its assemblages, microscopy is still the golden giardiasis diagnosis test. The immunochromatographic test (ICT) is useful in epidemiological surveys in absence of well-equipped laboratory and good technician though with lower sensitivity. Giardiasis was high in rural patients.

There must be sources of clean water, public hygienic education and effective practice for controlling water pathogens in general.

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