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Prevalence of Intestinal Parasitic Infections during Upper Gastrointestinal Endoscopy

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A B S T R A C T

Helminthic and protozoal infections of gastrointestinal tract remain a major health problem worldwide especially in tropical and subtropical areas**.** Light microscopy of stool specimens is the most frequently used for diagnosis of parasitic infections. However, it is time-consuming, and requires experienced staff. The aim of this study was to evaluate the frequency of parasitic infections during routine upper gastrointestinal endoscopy. Patients with different age groups admitted for upper endoscopy for any indication were subjected to informed consent, questionnaire including name, age, gender, residence, and indication for endoscopy. Duodenal aspirate samples were examined microscopically by direct smear examination, iodine, modified Ziehl-Neelsen stain, and immunodiagnostic technique for detection of *Cryptosporidium* and *Giardia* antigens. Histopathological examination of duodenal biopsies by hematoxylin and eosin stain for detection of parasites was also performed. This study was conducted on 70 patients. Most of parasitic infections were more prevalent in males than females, especially in age group from (40−60) years. *Giardia duodenalis* and *Cryptosporidium parvum* were the most prevalent parasites. *Cyclospora cayetanensis* and *Entamoeba histolytica* were also detected. *Ascaris lumbricoides* was identified in one case by direct visualization by endoscope. Iodine and modified Ziehl-Neelsen stains for diagnosis of *Giardia* and *Cryptosporidium* respectively were more accurate than direct smear examination and rapid immunoassay test. Histopathological examination of duodenal biopsies had detected parasites and their pathological features of inflammation. Upper gastrointestinal endoscopy proved a useful tool in the diagnosis of parasitic infections.

Introduction

Gastrointestinal parasitosis is a significant cause of morbidity and mortality. Although it is particularly more common in underdeveloped regions with poor sanitary conditions, they are present throughout the whole world. Clinically, it may vary depending on the parasite type and the affected regions of gastrointestinal tract (GIT)

(Ali *et al*., 2008).Usually, the diagnosis of alimentary tract parasites is made by characteristic findings as eosinophilia and egg shape appearance on examination of fecal samples (Mohamed *et al*., 2000). However, misdiagnosis may be due to absence of eggs in stool. Moreover, there are some reports of parasitic diagnosis during routine upper endoscopy (Zaher *et al*., 2012).

Intestinal protozoa are particularly important due to difficulty in their diagnosis. As *Giardia* cysts can be excreted intermittently, many cases (>50%) of giardiasis will be missed. Therefore, when giardiasis is suspected and stool specimens are negative, the string test, duodenal aspiration, or biopsy can be performed.

In a fresh specimen, trophozoites usually can be visualized on direct wet mount. Moreover, in *Cryptosporidium parvum* infection, oocyst shedding in feces can be intermittent, and several fecal specimens (at least three for an immunocompetent host) should be collected for microscopic examination (Washam and Frenck, 2018).

Some cases may require tissue evaluation in order to rule out other GIT pathologies. Although pathologists are usually familiar with common parasites, there is no adequate knowledge about direct microscopic visualization of parasites in biopsy samples (Gupta *et al*., 2009).

In addition, it is not known whether there are specific clues to suspect a parasitic infection or not, especially in cases where the microorganism is either very sparse or not visualized on small biopsy material or when there is no relevant clinical information upon the possibility of a parasitic infection (Pehlivanoğlu *et al*., 2016).

Obtaining of duodenal fluid during the upper gastrointestinal endoscopy and microscopic examination of it can help in the diagnosis of parasites like *Giardia lamblia* especially in patients with persistent diarrhea. Also intestinal aspirates from immuno-compromised patients may show protozoa: *Giardia*, *Cystoisospora*, *Cryptosporidia*, and helminths as *Strongyloides stercoralis* and *Fasciola* (Wahnschaffe *et al*., 2007; Bhaijee *et al*., 2011).

The biopsy sample can be used to make touch preparations for identifying *Giardia* in tissue sections and for histological examination. In addition, *Cryptosporidium parvum* can be found along the microvillus region of the epithelia that line the gastrointestinal tract. Histolopathological examination may reveal villous atrophy and blunting, and inflammation of the lamina propria (Robert *et al*., 2016).

This study aimed to determine the frequency of intestinal parasitic infections among patients undergoing upper gastrointestinal endoscopic procedures in the endoscopy units of Tanta University Hospitals.

Materials and Methods

Subjects

In the period from May 2018 to July 2019, a total of 70 patients underwent upper gastrointestinal (GI) endoscopy for various upper GI symptoms. They were subject of the present study. They were randomly selected from Endoscopy unit, Tanta University.

Inclusion criteria

The study enrolled patients of different age groups with different clinical presentations who were admitted for upper gastrointestinal endoscopy during one year from the start of the study.

For conduction of this study all patients were subjected to the following

Informed consent was obtained from all participants.

Interviewing questionnaire including demographic data such as age, gender, name, level of education, occupation, housing conditions regarding crowding index and source of water; clinical data such as symptoms of abdominal colic, vomiting, diarrhea and also the indication for endoscopy.

Technique of upper gastrointestinal endoscopy (Koch and Zurad, 2020)

Duodenal aspirate samples were collected from the second part of the duodenum through a polythene tube passed via the biopsy channel. The polythene tube was specially prepared with multiple perforations on the sides. A sterile syringe was attached to the polythene tube and duodenal juice collected in a clean tight fitting container. Then every sample was labeled properly with the patient's name.

Biopsies were taken from the duodenal mucosa using biopsy forceps.

Parasitological study

Duodenal aspiration samples (El-Hady *et al*., 2018)

After performing concentration by centrifugation of the fluid at 500 ×g for 5 min and the supernatant was decanted. Microscopic examinationwas done on the sediment as direct wet mount as well as after staining the samples with Lugol's iodine and modified Ziehl- Neelsen stain for detection of oocysts of coccidian.

Direct smear examination (Carleton *et al*., 1980)

Saline wet mount is used for the detection of trophozoites and cysts of protozoa, also it is used for detection of eggs and larvae of helminths.

Modified Ziehl-Neelsen stain (Rosenblatt *et al*., 2009)

The modified Ziehl-Neelsen stain for duodenal aspirate smears was employed for detection of coccidian protozoa oocysts namely *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Cystoisospora belli.*

Immunodiagnostic technique for detection of coproantigen, according to R-Biopharm AG, Darmstadt, Germany, which is certified by the Quality Control.

Fresh or frozen duodenal aspirate samples were subjected to rapid immunochromato-graphic test for detection of *Cryptosporidium* and/or *Giardia* antigens(Rida quick *Cryptosporidium*/*Giardia* Combi Art. No. N1122).

This test is a quick immunochromatographic test for the qualitative determination of *Cryptosporidium parvum* and/or *Giardia lamblia* in samples. Antigens from clinical specimens that are specific for these two parasites are isolated and immobilized on a membrane using specific antibodies. An antibody-enzyme conjugated then binded to specific sites on these antigens.

The antigens were detected after the addition of substrate by the formation of color bars in different areas depending on the parasite present and showed on the test device as red and blue bands.

Results and Discussion

Positive

A) *Cryptosporidium parvum* positive: a blue test band appears along with the green control band.
B) *Giardia lamblia* positive: a red test band appears along with the green control band.
C) *Cryptosporidium* and *Giardia* positive: blue and red bands appear along with the green control band.

Negative

Only the green control band appears.

Not valid

No green control band. In this case, the test must be repeated with a new strip.

Histopathological examination

Collection of biopsy samples (Koch and Zurad, 2020)

Biopsy samples are taken when visible changes are seen in the duodenum during upper endoscopy.

Specimens were then subjected to microscopic examinations of the section stained by hematoxylin and eosin stain to detect inflammation, granuloma, eosinophilia and parasitic infections of the duodenum (Allen, 1992).

Statistical analysis

Statistical presentation and analysis of the present study was conducted, using chi-square test by SPSS V.22. For categorical variables, Chi-square test was used for analysis and when it was found inappropriate, it was replaced by Ficher Exact Test. P value was considered statistically significant when < 0.05

Results and Discussion

Demographic data of cases with parasitic infections

Parasitic infections were detected in only 15 patients (21.4%). Two patients (13.3%) were < 40 years, nine patients (60%) were from (40−60) years and four patients (26.7%) were > 60 years.

Moreover, 11 patients (73.3%) were males and four patients (26.7%) were females. Concerning residence, 14 patients (93.3%) were from rural areas and only one patient (6.7%) was from urban area (Table 1).

Parasites detected via direct observation by endoscopy

Ascaris lumbricoides adult worm was directly observed in one case only (1.4%) by the endoscope in the duodenum. The worm was extracted during endoscopy and was then identified by its gross macroscopic features (Figure 1).

Parasites detected by examination of duodenal aspiration and biopsy samples

By laboratory examination of duodenal aspirate and biopsy samples, the following parasites were detected (Table 2).

G. duodenalis was detected in 8 cases including one case was mixed *G. duodenalis* and *Cryptosporidium* spp. infection.

Cryptosporidium spp. was detected in 6 cases including one case mixed *G. duodenalis* and *Cryptosporidium* and another case mixed *Cryptosporidium* and *Cyclospora cayetanensis* infection.

Performance of different diagnostic techniques

Regarding different methods of duodenal aspiration and biopsy examination used in the diagnosis of parasitic infection, only three cases (4.3%) were positive by direct smear examination of duodenal aspirate and nine cases (12.9%) were positive by using iodine staining. By modified Ziehl-Neelsen stain, six cases (8.6%) were found positive.

As regards the immunoassay technique for antigen detection, seven strips (10%) showed positive results, while by using histo-pathological examination of biopsies, 13 specimens (18.6%) were found positive (Table 3).

Direct smear examination

Regarding direct smear examination, this study had detected three positive cases (4.3%) for *G. duodenalis* with a statistically significant difference ($P< 0.001$). No positive cases were detected for *Cryptosporidium* spp., *C. cayetanensis* and *E. histolytica* infection by direct smear examination (Table 4).

Iodine stain examination

Using iodine stain, *G. duodenalis* cysts were detected in eight cases (11.4%) (Figure2). In addition, *E. histolytica* metacyst was detected in one case (1.4%). These results were statistically significant ($P< 0.001$ in *G. duodenalis* and 0.009 in *E. histolytica*) (Table 5).

Intestinal coccidian infections by modified Ziehl-Neelsen (Z.N.) stain

Regarding modified Z.N. stain, *Cryptosporidium* oocysts were detected in six cases (Figure 3), while *C. cayetanensis* oocyst was detected in one case only (Figure 4) with a rate of 8.6% and 1.4%, respectively. Ziehl-Neelsen stain showed statistically significant results in the diagnosis of both *Cryptosporidium* and *Cyclospora* infection ($P< 0.001$)(Table 6).

Immunoassay technique for antigen detection

By the use of the immunoassay technique, seven strips were found positive. Four strips (5.7%) were positive for *G. duodenalis* only, two strips (2.9%) were positive for *Cryptosporidium* spp. only and one strip was positive for both infections (1.4%). The detection of antigen was found to be statistically significant for the diagnosis of *G. duodenalis* and *Cryptosporidium* spp. ($P< 0.001$ and $P< 0.02$, respectively) (Table 7, figure5).

Histopathological examination of biopsy samples

Among the 70 duodenal mucosal specimens, pear-shaped *G. duodenalis* trophozoites were detected in eight cases (11.4%). These specimens also showed histopathological features of infection such as infiltration by chronic inflammatory cells and eosinophils as well as villous atrophy (Figures 6 and 7). In addition, goblet cell hyperplasia is found (Figure 6). *Cryptosporidium* intracellular stages were also found in six cases (8.6%). These cases showed infiltration by chronic inflammatory cells and eosinophils, flattened fused villi (Figures 8 and 9) as well as goblet cell hyperplasia (Figure 8). *Cyclospora cayetanensis* and *E. histolytica* were both negative on histopathological examination. These results were statistically significant ($P< 0.001$) for the diagnosis of *G. duodenalis* and *Cryptosporidium* spp. infection (Table 8).

Table.1 Demographic data of cases with parasitic infections

Demographic data			Positive cases
Age	**< 40**	**N**	2
		%	13.3%
	40−60	**N**	9
		%	60%
	> 60	**N**	4
		%	26.7%
Gender	**Male**	**N**	11
		%	73.3%
	Female	**N**	4
		%	26.7%
Residence	**Rural**	**N**	14
		%	93.3%
	Urban	**N**	1

Table.2 Parasites detected by examination of duodenal aspiration and biopsy samples

Parasites detected	No. of cases
Giardia duodenalis	8
***Cryptosporidium* spp.**	6
Cyclospora cayetanensis	1
Entamoeba histolytica	1

Table.3 Performance of different diagnostic techniques

Methods		Direct smear	Iodine	Z.N.	Immunoassay	Biopsy
Positive	**N**	3	9	6	7	13
	%	4.3%	12.9%	8.6%	10%	18.6%
Negative	**N**	67	61	64	63	57
	%	95.7%	87.1%	91.4%	90%	81.4%
Total	**N**	70	70	70	70	70
	%	100.0%	100.0%	100.0%	100.0%	100.0%

Table.4 Findings of direct smear examination

			Direct smear		X^2	*P* value
			+ve	-ve		
G. duodenalis	**+ve**	**N**	3	5	24.290	0.001*
		%	4.3%	7.1%		
	-ve	**N**	0	62		
		%	.0%	88.6%		
***Cryptosporidium* spp.**	**+ve**	**N**	0	6	0.294	0.588
		%	.0%	8.6%		
	-ve	**N**	3	61		
		%	4.3%	87.1%		
C. cayetanensis	**+ve**	**N**	0	1	0.045	0.831
		%	.0%	1.4%		
	-ve	**N**	3	66		
		%	4.3%	94.3%		
E. histolytica	**+ve**	**N**	0	1	0.045	0.831
		%	.0%	1.4%		
	-ve	**N**	3	66		
		%	4.3%	94.3%		

* Significant (*P* value < 0.05)

Table.5 Findings of iodine stain examination

			Iodine		X^2	*P* value
			+ve	-ve		
G. duodenalis	**+ve**	**N**	8	0	61.219	0.001*
		%	11.4%	.0%		
	-ve	**N**	1	61		
		%	1.4%	87.1%		
E. histolytica	**+ve**	**N**	1	0	6.879	0.009*
		%	1.4%	.0%		
	-ve	**N**	8	61		
		%	11.4%	87.1%		

* Significant (*P* value < 0.05)

Table.6 Intestinal coccidian infections by modified Z.N. stain

			Z.N.		X^2	*P* value
			+ve	-ve		
***Cryptosporidium* spp.**	**+ve**	**N**	6	0	70.001	0.001*
		%	8.6%	.0%		
	-ve	**N**	0	64		
		%	.0%	91.4%		
C. cayetanensis	**+ve**	**N**	1	0	10.821	0.001*
		%	1.4%	.0%		
	-ve	**N**	5	64		
		%	7.1%	91.4%		

Table.7 Immunoassay technique for antigen detection

			Immunoassay		X^2	*P* value
			+ve	-ve		
G. duodenalis	**+ve**	**N**	4	3	19.213	0.001*
		%	5.7%	4.3%		
	-ve	**N**	3	60		
		%	4.3%	85.7%		
***Cryptosporidium* spp.**	**+ve**	**N**	2	3	5.379	0.020*
		%	2.9%	4.3%		
	-ve	**N**	5	60		
		%	7.1%	85.7%		
***G. duodenalis* & *Cryptosporidium* spp.**	**+ve**	**N**	1	3	1.058	0.303
		%	1.4%	4.3%		
	-ve	**N**	6	60		
		%	8.6%	85.7%		

Table.8 Histopathological examination of biopsy samples

			Biopsy		X^2	*P* value
			+ve	-ve		
G. duodenalis	**+ve**	**N**	8	0	39.603	0.001*
		%	11.4%	.0%		
	-ve	**N**	5	57		
		%	7.1%	81.4%		
***Cryptosporidium* spp.**	**+ve**	**N**	6	0	28.774	0.001*
		%	8.6%	.0%		
	-ve	**N**	7	57		
		%	10.0%	81.4%		
C. cayetanensis	**+ve**	**N**	0	1	0.231	0.631
		%	.0%	1.4%		
	-ve	**N**	13	56		
		%	18.6%	80.0%		
E. histolytica	**+ve**	**N**	0	1	0.231	0.631
		%	.0%	1.4%		
	-ve	**N**	13	56		
		%	18.6%	80.0%		

Table.9 Association between symptomatology and parasite-positive cases

			Follow-up		Gastrointestinal bleeding		Pyrosis and epigastric pain		Other indication	
			Yes (n=21)	No (n=49)	Yes (n=6)	No (n=64)	Yes (n=27)	No (n=43)	Yes (n=16)	No (n=54)
G. duodenalis	**+ve**	**N**	3	5	2	6	1	7	2	6
	(n=8)	**%**	14.3%	10.2%	33.3%	9.4%	3.7%	16.3%	12.5%	11.1%
***Cryptosporidium* spp.**	**+ve**	**N**	4	2	1	5	1	5	0	6
	(n=6)	**%**	19.0%	4.1%	16.7%	7.8%	3.7%	11.6%	.0%	11.1%
C. cayetanensis	**+ve**	**N**	0	1	0	1	1	0	0	1
	(n=1)	**%**	.0%	2.0%	.0%	1.6%	3.7%	.0%	.0%	1.9%
E. histolytica	**+ve**	**N**	0	1	0	1	1	0	0	1
	(n=1)	**%**	.0%	2.0%	.0%	1.6%	3.7%	.0%	.0%	1.9%
A. lumbercoides	**+ve**	**N**	0	1	0	1	1	0	0	1
	(n=1)	**%**	0%	2.0%	0%	1.6%	3.7%	0%	0%	1.9%
					%		6.7%			

Figure.1 *Ascaris lumbricoides* detected directly by endoscopy

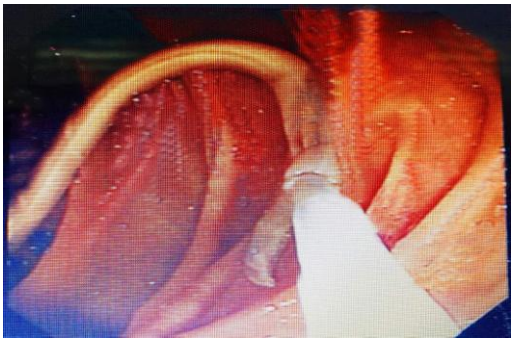


Figure.2 Iodine-stained duodenal aspirate sample showing *G. duodenalis* cysts (arrows) (× 1000)

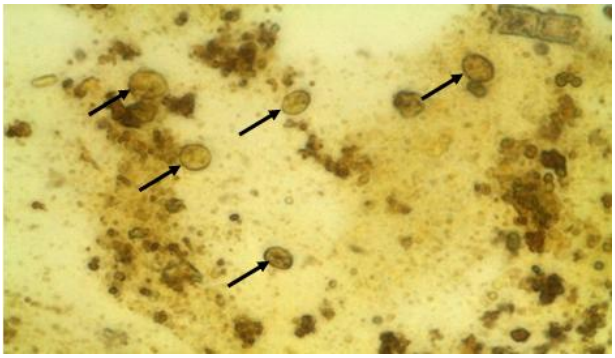


Figure.3 Duodenal aspirate sample showing *Cryptosporidium* oocysts (arrows) with modified Z.N. stain (× 1000)

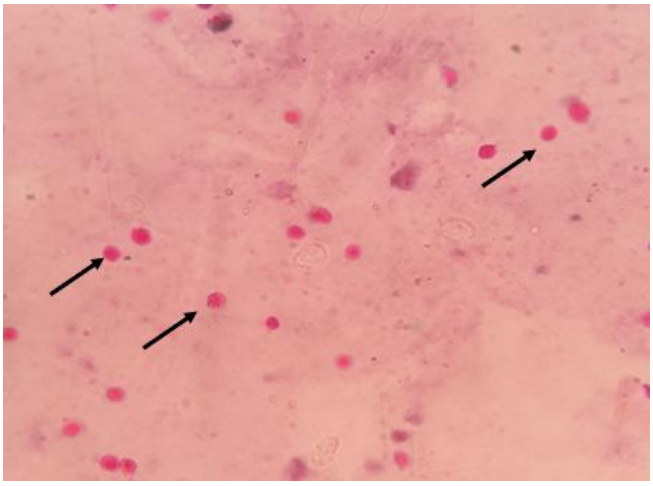


Figure.4 Duodenal aspirate sample showing *C. cayetanensis* oocysts (arrows) by modified Z.N. stain (× 1000)

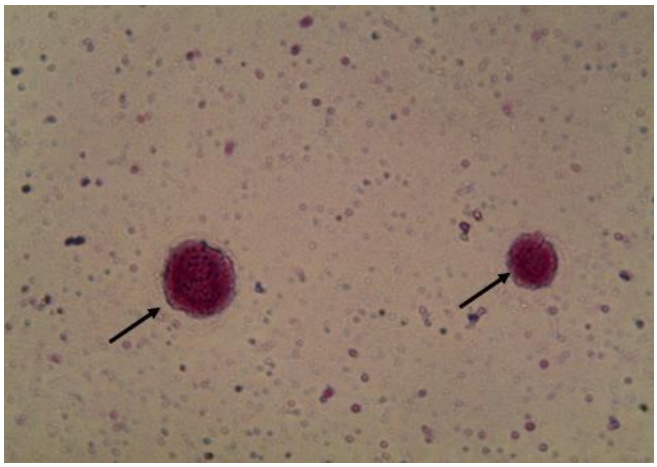


Figure.5 Immunochromatographic strips (*Cryptosporidium*/ *Giardia* Combi), (1) positive for both *C. parvum* and *G. duodenalis*, (2) positive for *C. parvum* and (3) positive for *G. duodenalis*

(C): *Cryptosporidium parvum;* (G): *Giardia duodenalis;* (P): Positive control

Figure.6 Micrograph of a duodenal mucosal biopsy illustrating the presence of pear-shaped *G. duodenalis* trophozoites overlying the epithelium (black arrows), chronic inflammatory cells in the core of flattened villi (yellow arrow) as well as goblet cell hyperplasia (red arrows) (H&E × 400)

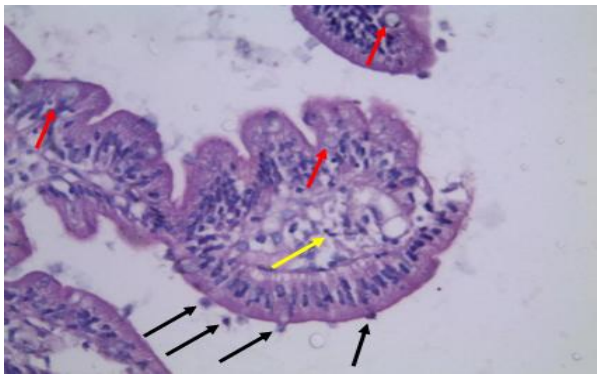


Figure.7 Micrograph of a duodenal mucosal biopsy showing *G. duodenalis* trophozoites (black arrows) as well as chronic inflammatory cells and eosinophils (yellow arrow) in the core of villi (H&E × 400)

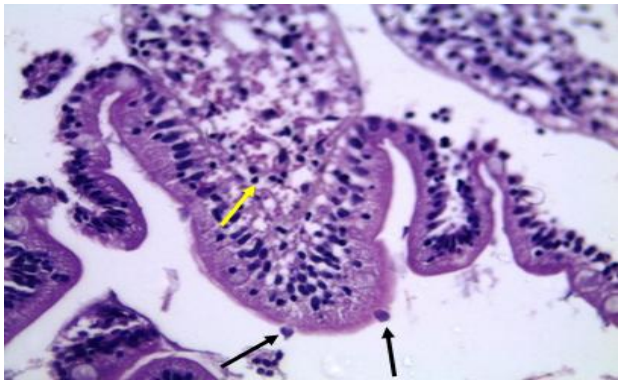


Figure.8 Micrograph of a duodenal mucosal biopsy in *Cryptosporidium*-positive case showing fused flattened villi (black arrow) as well as goblet cell hyperplasia (red arrow) (H&E × 100)

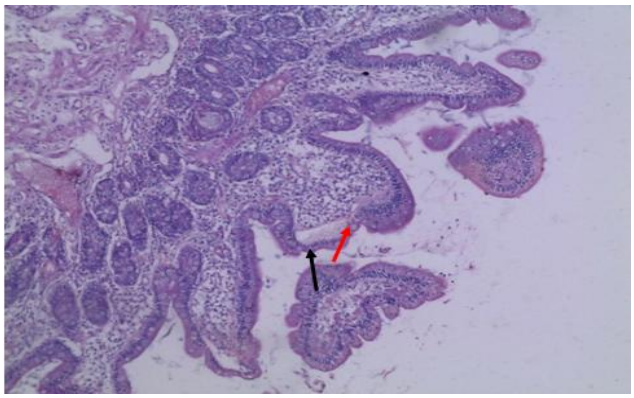
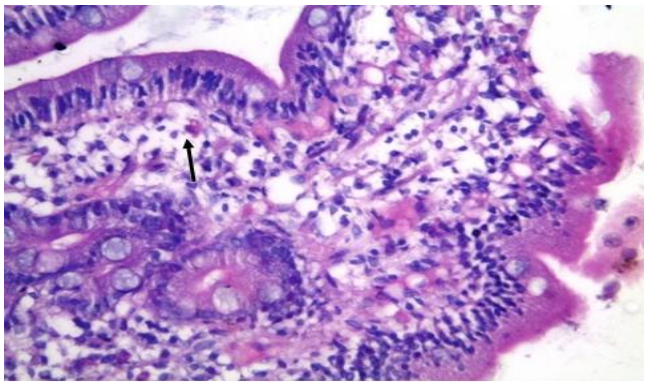


Figure.9 Micrograph of a duodenal mucosal biopsy in *Cryptosporidium*-positive case showing infiltration by chronic inflammatory cells and eosinophils (arrow) (H&E × 400)



Parasitic diseases caused by helminths and protozoan parasites constitute significant but neglected public health problems in the developing countries and marginalized communities (Devleesschauwer *et al*., 2014). Diagnosis of gastrointestinal parasitic infections is commonly based on coprology for detection of eggs or other parasitic stages in the stools and/or elevated count of eosinophils in blood picture (Mohamed *et al*., 2000). Thus, false negative cases are possible when the aforementioned findings are missed. Serological assays also are available for the diagnosis of parasitic infections but cross reactivity limits their sensitivity and specificity. Therefore, histopathological examination may be a reliable way to diagnose parasitic diseases by detecting the characteristic morphology of parasites. However, failure to demonstrate the parasite does not exclude the possibility of infection where inflammatory reaction in the surrounding tissue could give a clue about parasitic infestations (Mohamed *et al*., 1997; Rosenblatt *et al*., 2009).

In the current study, there is a considerable range of reported prevalence of parasitic infections in duodenal aspiration and biopsy by upper gastrointestinal endoscopy, which likely reflects differences in demographic factors, parasite endemicity, and differences in the performance of diagnostic techniques used.

As regards our study, the ratio of parasitic infections was higher (60%) in the age group ranging from (40−60) years. This finding is consistent with a study involving clinical, endoscopic and histopathological profile of parasitic duodenitis done by Santos *et al.,* (2011) who found that the mean age for detected *Strongyloides stercoralis* and *Cryptosporidium*-positive cases was 32.4 and 43.8 years, respectively.

Concerning gender in this study, the incidence of parasitic infections was generally higher in males (73.3%) than females (26.7%) and this coincides with a retrospective study on detection of parasitic infections during upper gastrointestinal endoscopic procedures by Zaher *et al.,* (2012), where 62.5% of detected cases were males and 37.5% were females. Similarly, Santos *et al.,* (2011) reported that 54.5% of detected cases were males. This may be related to the higher risk of exposure in males than females.

This study was conducted in Gharbia Governorate, which is considered a rural-urban area (El-Khoby *et al*., 2000). Unsurprisingly, most of detected parasites in this work are found in rural areas, where 93.3% of positive cases were of rural origin. This coincides with Zaher *et al.,* (2012), where all cases of parasitic infections were of rural origin. Likewise, many studies had reported higher incidence of parasitic infections in rural communities in comparison to urban communities (Ikeh *et al*., 2006; Ngrenngarmlert *et al*., 2007). This may be explained by lack of health education, lack of proper sewage disposal systems, poor hygiene as well as exposure to soil and farm animals.

In the current research, parasitic infections were reported in 15 patients out of 70 studied cases (21.4%). One patient was diagnosed by direct observation of *Ascaris lumbricoides* by endoscopy and 14 patients were diagnosed by examination of duodenal aspiration and biopsy samples. Thus, upper gastrointestinal endoscopy may have an important role in the diagnosis of parasitic infections.

Along the same line of evidence, a study by Carswell *et al.,* (1973) on giardiasis and celiac disease, found that 50% of *Giardia*-positive cases were missed by stool examination alone. Moreover, Ament *et al.,* (1973) had studied the structure and function of gastrointestinal tract in primary immunodeficiency syndromes in 39 patients by examination of stools and small intestinal biopsies for *Giardia* cyst or trophozoite. They mentioned that only 30% of their cases of giardiasis were detected by stool examination alone, the remainder being identified by small bowel biopsy. Mahdi and Taha, (2002) revealed the presence of *G. lamblia* trophozoites in duodenal aspirates taken from 15 patients, five of whom had positive stool examination. Also, *C. parvum* was recovered from four patients, compared to only two cases discovered by stool examination. They mentioned that rapid examination of duodenal aspirate besides its clear liquid nature considerably facilitates the recognition of trophozoites.

Moreover, Roberts *et al.,* (1989) reported an unusually high prevalence of cryptosporidiosis (12.7%) in duodenal aspirates from immunocompetent patients during routine upper endoscopy, while stool samples were positive in less than 50% of those adults with positive duodenal aspirates. In addition, in a study by Mohamed *et al.,* (2000), gastroscopy showed multiple *Ascaris lumbricoides* worms in one patient presented with abdominal pain and vomiting.

However, Nair *et al.,* (1977) and Grazioli *et al.,* (2006) showed that stool examination is as accurate as the direct smear examination of the duodenal samples in diagnosing *G.*

lamblia. On the contrary to the high percentage of parasitic infections in our study, Zaher *et al.,* (2012) reported lower incidence where only 0.16% of their participants were incidentally diagnosed with parasitic infection during upper endoscopic examination for other reasons. This may be explained by different method of diagnosis where all cases were diagnosed by macroscopic detection of adult worm by endoscopy and not by microscopic examination of duodenal aspirate or biopsy samples. Furthermore, Hanevik *et al.,* (2007), on studying persisting symptoms and duodenal inflammation related to *G. duodenalis* infection, found *Giardia* trophozoites in duodenal biopsies in only 4 (10%) of the 40 cases with *Giardia*-positive faecal samples while duodenal aspirate microscopy showed no positive results. The explanation for the poor outcome may be due to the procedure of duodenal aspiration where NaCl-instillation then suction of fluid rather than direct suction or may be due to long standing or subsiding infection and previous treatment.

Regarding direct smear examination, our study had detected three positive cases for *G.duodenalis* with a rate of 4.3%, while no positive cases for *Cryptosporidium* spp., *C. cayetanensis* or *E. histolytica* were detected by direct smear examination. Sensitivity of direct smear in diagnosis of *G. duodenalis* was found to be 37.5%, whereas specificity for *G.duodenalis, Cryptosporidium* spp., *C. cayetanensis* and *E. histolytica* was found to be 100%, 95.3%, 95.7% and 95.7%, respectively.

Upon considering results of iodine and modified Z.N. staining of duodenal aspirate as the gold standard tests, iodine staining was highly sensitive in the diagnosis of *G. duodenalis* and *E. histolytica* (100% for both) and highly specific (98.4% and 88.4%, respectively). Modified Z.N. staining was highly sensitive for diagnosing *Cryptosporidium* spp. and *C. cayetanensis* (100% for both parasites) and highly specific (100% and 92.8%, respectively). In a similar study by Kerlin *et al.,* (1978) for the detection of *Giardia* by upper endoscopy showed that the prevalence of *Giardia* was (2.1%) in both duodenal aspirate and mucosal immersion smears. Furthermore, El-Hady *et al.,* (2018) studied the parasites in duodenal aspirate using direct wet and modified acid-fast stained smears in Sohag, Egypt. *Cryptosporidium* and *Giardia* cases were detected in duodenal aspirate samples with ratio 44% and 8%, respectively. *Cyclospora* cases were detected in 11% of duodenal aspirate samples. In addition, *Blastocystis hominis* was detected in 21% of patients.

However, Chakarova (2010) detected *G. duodenalis* and *Cryptosporidium* in human stool and duodenal aspirate by direct smear examination where sensitivity was 97.9% and 86.5%, respectively. In addition, Elsafi *et al.,* (2013) reported that microscopic examination of iodine-stained stool samples and Kinyoun's acid-fast technique for detection of *G. lamblia* and *C. parvum*, respectively were less sensitive and less specific than ImmunoCard STAT® and real-time PCR analysis.

Concerning the use ofrapid immunodiagnostic techniques (RDT), they are rapid to perform and do not require experienced staff or special technical equipment and could be adopted as a screening test especially for smaller and less well-equipped labs (Regnath *et al*., 2006). The present study had detected seven cases by immunoassay technique (Rida quick *Cryptosporidium*/*Giardia* Combi): four cases (5.7%) positive for *G. duodenalis*, two cases (2.9%) positive for *Cryptosporidium* spp.,and one case (1.4%) positive for both infections. Sensitivity of antigen detection in the diagnosis of *G. duodenalis* and *Cryptosporidium* spp. is moderate (62.5% and

50%, respectively) and specificity is high (96.8% and 93.8%, respectively), but still not valid test for diagnosis as sensitivity <80%).

Johnston *et al.,* (2003) showed comparable results for *Giardia* and *Cryptosporidium* using the ImmunoCard STAT rapid assay for the detection of *Giardia* and *Cryptosporidium* in stool with sensitivity (81.3% and 67.6%) and specificity (99.5% and 99.0%), respectively. On the contrary, Goudal *et al.,* (2019) showed that copro-antigen detection in giardiasis and cryptosporidiosis using RDT was 89.2% sensitive and 99.3% specific for the diagnosis of *G. intestinalis* as it detected 33 out of the 37 cases detected by the microscopic examination of stool. While for *Cryptosporidium* spp., it was 86.7% sensitive and 100% specific as it detected 26 out of the 30 samples detected by the microscopic examination of stool. Similarly, Regnath *et al.,* (2006) found that all microscopically positive specimens were also positive by both the RidaQuick *Giardia* and RidaQuick *Crypto/Giardia* Combi assay. Thus, the sensitivity values of the RidaQuick single species and Combi assays were 100% for both parasites compared with microscopy. The specificity was 99% for *G. lamblia* in the single species and Combi assays, while it was 100% for *Cryptosporidia* in both assays. These differences can be explained by the low burden of parasites in our samples.

This study included histopathological examination of duodenal mucosal specimens from all 70 patients. *G. duodenalis* trophozoites were detected in eight cases (11.4%) and *Cryptosporidium* spp. intracellular stages were also detected in six cases (8.6%). These specimens also showed histopathological features of parasitic infections as infiltration by chronic inflammatory cells and eosinophils as well as villous atrophy.

Various studies were done to correlate histopathological changes in parasitic infections via endoscopic biopsies and had showed variable results. Similar to our findings, Mohamed *et al.,* (2000) studied the histological diagnosis of parasitic infections from endoscopic biopsies and surgical specimens. *G. lamblia* was isolated from duodenal biopsies in eight patients. Villous atrophy was detected in a patient with malabsorption. Other findings were non-specific and showed only inflammation, erosions, or whitish nodules. In four of those patients, repeated stool examinations did not show *G. lamblia*. They mentioned that the diagnosis of gastrointestinal parasites is not only made by stool examination but can be detected by histopathological examination of endoscopic biopsies or surgical specimens. Moreover, Hanevik *et al.,* (2007) reported that duodenal biopsies in 57 patients (47.1%) showed inflammation with oedema and infiltration of leukocytes, increased number of plasma cells in the lamina propria as well as architectural distortion with shortening and blunting of intestinal villi. *Giardia* trophozoites were visible in duodenal biopsies in only 4 (10%) of the 40 cases with *Giardia*-positive faecal samples.

Furthermore, in a retrospective analysis of parasitic diseases diagnosed by tissue biopsy, giardiasis was seen only in three cases (2%). Pear-shaped trophozoites were found in duodenal mucosa. All cases showed normal villous to crypt ratio with only increase in intraepithelial lymphocytes. They revealed that giardiasis is associated with various histological changes, which range from minimal to severe enterocyte damage, villous atrophy and crypt hyperplasia (Manandhar *et al*., 2018).

However, Santos *et al.,* (2011) detected parasites in only 1% of duodenal biopsies performed and concluded that no endoscopic

or histopathological features were characteristic to parasitic duodenitis. Moreover, Doganci *et al.,* (2002) found only one positive duodenal aspirate sample for *C. parvum* while duodenal biopsies were negative in all samples. This wide range of variable results may be explained by different virulence of detected strains, host factors, or differences in the duration and severity of infection. On the contrary to our results, Zafar *et al.,* (1991) investigated the frequency of *G. lamblia* in patients admitted for upper endoscopy for various causes and revealed that *G. lamblia* has been obtained in 9% of duodenal aspirates and in only 1.8% of biopsy specimens. They mentioned three factors which may be responsible for the low yield. Firstly, the size of the endoscopic biopsy, secondly, the staining technique and lastly, the third factor is the excessive use of antiprotozoal drugs and antibiotics in their country.

Furthermore, Grazioli *et al.,* (2006) reported that histological examination of duodenal biopsies for *G.lamblia* was unsuitable due to an unacceptable rate of false negative results (e.g., 22.2% sensitivity). Manandhar *et al.,* (2018) detected amoebiasis in 2% of total positive cases where histopathology showed round trophozoite with ingested erythrocytes in the cytoplasm. However, *E. histolytica* trophozoites were not detected in our biopsies, which may be explained by different biopsy site where in our study biopsy was taken from duodenum while in their study biopsies were taken from colon.

This study included 70 patients admitted for upper gastrointestinal endoscopy unit for various indications. *G. duodenalis* and *Cryptosporidium* spp. were detected in patients admitted for haematemsis, pyrosis or epigastric pain, persistent vomiting or dysphagia and follow-up of chronic liver diseases, liver cirrhosis or esophageal varices. *Cyclospora cayetanensis* and *E. histolytica* were detected in patients with pyrosis or epigastric pain. *Ascaris lumbricoides* adult was detected in patient manifested by recurrent epigastric pain.

Also, Zafar *et al.,* (1991) found that 33.3% of patient with *G. lamblia* in duodenal aspirate were follow-up cases of duodenal ulcer. Jahani *et al.,* (2008) studied a group of 130 dyspeptic patients who had undergone upper gastrointestinal endoscopy and *Giardia* was detected in two (1.5%) patients. Moreover, Doganci *et al.,* (2002) reported that dyspepsia was the main indication of upper endoscopy in the *Cryptosporidium*-positive case found in their study.

Furthermore, McHenry *et al.,* (1987) on studying the yield of routine duodenal aspiration for *G. lamblia* during esophagogastroduodenoscopy, reported that the main indications for routine upper gastrointestinal endoscopy were abdominal pain53%, gastroesophageal reflux symptoms 8%, nausea/vomiting 6%, gastrointestinal bleeding 6%, diarrhea 6%, ulcer follow-up 4%, weight loss 3% and miscellaneous causes 14%.

Similarly, Zaher *et al.,* (2012) detected one case of *A. lumbricoides* in a patient manifested by epigastric pain. However, they correlated some other parasites diagnosed by upper endoscopy with other clinical presentations where *Ancylostoma duodenale* parasite was detected in five cases. *Strongyloides stercoralis* and *Fasciola hepatica* each was detected in one separate case.

All cases of *Ancylostoma* had anemia, four cases had hemoglobin level <10 gm %, *Strongyloides* case had mild anemia and epigastric pain while *Fasciola* presented with obstructive jaundice.

In conclusion, our study highlighted the value of duodenal aspirate and mucosal biopsies examination for detection of intestinal parasites. Microscopic examination of the samples either by direct wet mount or by stained preparations, showed relatively accepted results. Antigen detection was found to be easy but still need further evaluation in more cases. Histopathological examination proved helpful in the diagnosis as it allowed the detection of both the parasite and its pathology.

Recommendations

It is recommended to collect and examine duodenal contents for parasitic infections as routine procedures during upper gastrointestinal endoscopy. Further studies should be done on larger number of patients and comparative studies between duodenal aspirate and stool examination for search of parasites are recommended. Elaboration of effective and practical techniques for evaluation of the impact of intestinal parasitic infections upon the pathology of the gastrointestinal tract is recommended.

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