

CO-INFECTION OF *GIARDIA LAMBLIA* AND *HELICOBACTER PYLORI* INFECTION AMONG CHRONIC KIDNEY DISEASED PATIENTS UNDERGOING HEMODIALYSIS IN BENI-SUEF UNIVERSITY HOSPITALS

By

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Abstract

Giardia lamblia and *Helicobacter pylori* are two microorganisms that grow in duodenum and stomach; and sharing the same mode of infection. Chronic kidney disease (CKD) is an end stage disease causing uremia that requires hemodialysis (HD). The association of *Giardia lamblia* and *H. pylori* infection has been known to be common and hemodialysis may play an important role on this co-infection. This study evaluated the interrelation of *Giardia lamblia* and *H. pylori* in patients of CKD treated with hemodialysis.

A case-control study performed on two hundred stool samples collected from patients attending Beni-Suef University Hospital suffering from diarrhea and other GIT symptoms. One hundred patients suffering from CKD and treated with hemodialysis and a hundred control group with normal kidney functions of both genders. Both groups were subjected to copro-parasitological examination and fecal immuno-assays.

The results showed that *Giardia* in 13 CKD patients with a mean age of 45.24±14.52 and in 22 cross-matched control patients. Males showed prevalent of (66%), who were from rural areas (66.5%) and using tap water (83.5%). *H. pylori* infection was in 22 patients CKD and in 27 control patients. Co-infection was found in 10 CKD patients and 19 of control.

Keywords: Egypt, Patients, *Giardia lamblia*, *H. pylori*, Co-infection, CKD, Hemodialysis.

Introduction

Enteric protozoa are a diverse group of unicellular microparasites inhabiting the intestinal tract of high vertebrates including man (Cama and Mathison, 2015). Infections occur by ingestion of cysts/oocysts contaminating food and/or water (Torgerson *et al*, 2014). Diarrhea is a symptom for protozoan infections, and asymptomatic colonization also common (Cama and Mathison, 2015). Attributing diarrhea to an exact parasite identified in a patient's feces is not certain for all protozoa. While other intestinal protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, and *Isospora belli* caused humans' diarrhea (Agholi *et al*, 2013). Diarrhea is mild and self-limited in immunocompetent persons, but severe in immunosuppressed ones (Marcos and Gotuzzo, 2013).

Generally, chronic renal failure (CRF) is the end stage in kidney disease with marked

decline in glomerular filtration rate and uremia that required kidney replacement or dialysis (Eknoyan and Levin, 2002), and, high susceptible to secondary diarrhea (Anders *et al*, 2013). Diarrhea usually caused by viruses, bacteria, and/or protozoa (Manesh *et al*, 2014), a risky factor for morbidity and mortality (Tonelli *et al*, 2006). A strong correlation of *G. lamblia* and *H. pylori*, with proved with *Giardia* assemblage B (El-Badry *et al*, 2017). So, in *H. pylori*, screening for *G. lamblia* was indicated in patients with upper gastrointestinal symptoms (Seid *et al*, 2018).

The study aimed to focus on diagnosis of *Giardia lamblia* and *H. pylori* co-infection in patients suffering from chronic kidney disease (CKD) and treated with hemodialysis with variables duration attending Beni-Suef University Hospitals.

Patients and methods

This case-control study was carried out on one hundred patients suffering from CKD

and treated with hemodialysis, at Beni-Suef University Hospitals, and suffered from diarrhea with GIT symptoms episodes. Patients who were received hemodialysis treatment for at least 1 year were included. Controls were one hundred cross-matched healthy individuals attended Outpatient Internal Medicine Clinics with diarrhea and GIT symptoms. All participants signed a consent form and filled a standardized clinical questionnaire about medical history and demographic characteristics, signs, symptoms...etc.

Inclusion criteria were patients of both sexes, and all ages with a history of CKD and treated with hemodialysis.

Stool analysis: Three morning stool samples were collected labeled plastic containers, on three consecutive days with one-day intervals. Stool samples were divided into two parts.

Copro-diagnosis: First fecal part was examined macroscopically, microscopically using saline and Lugol's iodine stained smear, formal-ether concentration method by using modified iron hematoxylin stain.

Copro-immunoassays: Second part was frozen at -20°C for immunodiagnostic processing using *G. lamblia* ELISA Kit to determine *Giardia* specific antigens (Catalogue No. MBS495070, Bio-Source, San Diego, CA, USA) following the manufacturer's recommendations and *H. pylori* was diagnosed by monoclonal enzyme immunoassay antigen test.

Statistical analysis: Data were analyzed by Statistical Package of Social Science (SPSS) software version 25 for windows 10. Simple descriptive analysis was in numbers and percentages of qualitative data and arithmetic means as a central tendency measurement, standard deviations as a measure of quantitative parametric data. For quantitative parametric data t-test was used to compare between two groups. Chi square test compared between more than two qualitative data. P-value < 0.05 was significant.

Results

The study involved CKD patients of both

sexes 34% females and 66% males, with ages ranged between 16 & 75 years old, mean (45.24±14.48) without significant difference as to sex and/or age ($P > 0.05$). Residence, water for human consumption, and occupation did not show significant differences.

Clinical symptoms as vomiting and fatigue were significantly higher among control as compared with CKD patients ($P = 0.023$ & 0.013) respectively; but bowel habit changes and dyspepsia were higher significantly in CKD patients ($P = 0.028$ & 0.001) respectively. Others GIT symptoms were nearly without significant differences ($P > 0.05$).

As to chronic diseases; diabetes mellitus was significantly more among CKD patients (53%) compared to control (40%, $P=0.044$), and hypertension was (76 %, $P= 0.001$). Associated parasites were higher among control (25%) as compared to CKD (15%, $P = 0.055$), commonest were *Blastocystis hominis* (35%) and *Entamoeba coli* (30%) with significant difference ($P = 0.021$).

Giardia was detected in 11%, 11%, 9% & 13% samples in CKD patients, and in 20%, 19%, 15% & 22% samples respectively in control, but more prevalent among control as compared with CKD patients, but without significant difference ($P > 0.05$).

H. pylori was more prevalent among controls (27%) as compared to CKD patients (22%), without significant difference ($P= 0.256$). *G. lamblia* and *H. pylori* co-infection was more among control (19%) as compared to CKD patients (10%), but without significant difference ($P = 0.107$).

CKD with positive co-infection was significantly among young patients as compared to CKD with negative co-infection ($P = 0.006$), co-infection was significantly higher among ages 20 -< 35 ($P= 0.043$). Positive co-infection was more prevalent in rural male CKD patients. Water for consumption positive and negative co-infected patients was without significant difference.

Flatulence and loss of appetite were more prevalent among cases with positive co-infection; but without significant ($P= 0.171$,

0.108), respectively. Other GIT symptoms were without significant differences. Those with chronic disease history didn't show significant co-infection (P >0.05).

Association between *H. pylori* & *G. lamblia* co-infection with duration of hemodialy-

sis, urea and creatinine level in CKD patients showed that positive co-infection had shorter duration of hemodialysis, higher urea level and higher creatinine level as compared to patients with negative co-infections.

Details were given in tables (from 1 to 16)

Table 1: Basic characteristics of participants (N=200)

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Age (Years)	≥20 - <35	20 (20.0)	38 (38.0)	58 (29.0)	<0.999
	≥35 - <50	40 (40.0)	22 (22.0)	62 (31.0)	
	≥50 - <65	32 (32.0)	34 (34.0)	66 (33.0)	
	≥65	8 (8.00)	6 (6.00)	14 (7.0)	
	Mean ±SD	45.24 ±14.52	45.24 ±14.52	45.24 ±14.48	<0.999
Sex	Female	34 (34.0)	44 (44.0)	78 (44.0)	<0.999
	Male	66 (66.0)	56 (56.0)	122 (61.0)	
Residence	Rural	60 (60.0)	73 (73.0)	133 (66.5)	0.072
	Urban	40 (40.0)	27 (27.0)	67 (33.5)	
Consumption water	Tap	80 (80.0)	87 (87.0)	167 (83.5)	0.126
	Filtered	20 (20.0)	13 (13.00)	33 (16.5)	
Occupation	Not Working	41 (41.0)	34 (34.0)	75 (37.5)	0.190
	Working	59 (59.0)	66 (66.0)	125 (62.5)	

*P-value ≤0.05 significant.

Table 2: Comparison of associated GIT symptoms among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Vomiting	No	76 (76.0)	62 (62.0)	138 (69.0)	0.023*
	Yes	24 (24.0)	38 (38.0)	62 (31.0)	
Nausea	No	42 (42.0)	43 (43.0)	85 (42.5)	0.500
	Yes	58 (58.0)	57 (57.0)	115 (57.5)	
Abdominal pain	No	24 (24.0)	28 (28.0)	52 (26.0)	0.314
	Yes	76 (76.0)	72 (72.0)	148 (74.0)	
Bowel habit	Normal	26 (26.0)	31 (31.0)	57 (31.3)	0.028*
	Diarrhea	68 (68.0)	51 (51.0)	119 (65.4)	
	Constipation	6 (6.0)	0 (0.00)	6 (3.3)	
Flatulence	No	59 (59.0)	65 (65.0)	124 (62.0)	0.233
	Yes	41 (41.0)	35 (35.0)	76 (38.0)	
Fatigue	No	44 (44.0)	28 (28.0)	72 (36.0)	0.013*
	Yes	56 (56.0)	72 (72.0)	128 (64.0)	
Loss of appetite	No	54 (54.0)	54 (54.0)	108 (54.0)	0.556
	Yes	46 (46.0)	46 (46.0)	92 (46.0)	
Dyspepsia	No	50 (50.0)	72 (72.0)	122 (61.0)	0.001*
	Yes	50 (50.0)	28 (28.0)	78 (39.0)	

Table 3: Chronic diseases among participants (N=200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Diabetes mellitus	No	47(47.0)	60 (60.0)	107 (53.5)	0.044*
	Yes	53 (53.0)	40 (40.0)	93 (46.5)	
Hypertension	No	24 (24.0)	62 (62.0)	86 (43.0)	0.001*
	Yes	76 (76.0)	38 (38.0)	114 (57.0)	
Edema	No	42 (42.0)	100 (100.0)	142 (71.0)	<0.001*
	Yes	58 (58.0)	0 (0.00)	58 (29.0)	
Dyspnea	No	72 (72.0)	100 (100.0)	172 (86.0)	<0.001*
	Yes	28 (28.0)	0 (0.00)	28 (14.0)	

Table 4: Duration of haemodialysis, urea and creatinine level among CKD patients (N= 100):

Variable items	No.	Minimum	Maximum	Mean	Std. Deviation
Hemodialysis (years)	100	3.00	10.00	5.30	2.25
Urea (mg/dl)	100	56.00	241.00	115.74	41.71
Creatinine (mg/dl)	100	4.10	9.80	6.87	1.42

Table 5: Associated parasitic infection among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Associated Parasitic Infection	No	85	75	160 (80.0)	0.055
	Yes	15	25	40 (20.0)	
	<i>Blastocystis</i>	5 (33.3)	9 (36.0)	14 (35.0)	0.021*
	<i>E. coli</i> cyst	8 (53.3)	4 (16.0)	12 (30.0)	
	<i>E. histolytica</i> cyst	2 (13.3)	0 (0.00)	2 (5.0)	
	<i>H. nana</i> egg	0 (0.00)	5 (20.0)	5 (12.5)	
	<i>Schistosoma mansoni</i> egg	0 (0.00)	1 (4.00)	1 (2.5)	
	<i>Ancylostoma</i> egg	0 (0.00)	2 (8.00)	2 (5.0)	
	<i>Taenia</i> egg	0 (0.00)	4 (16.0)	4 (10.0)	
	TOTAL	15 (100.0)	25 (100.0)	40 (100.0)	

Table 6: Detection of *Giardia lamblia* by direct wet mount among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
<i>Giardia lamblia</i>	Negative	89 (89.0)	80 (80.0)	169 (84.5)	0.059
	Positive	11 (11.0)	20 (20.0)	31 (15.5)	

Table 7: Detection of *Giardia lamblia* by concentrated fecal material among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
<i>Giardia lamblia</i>	Negative	89 (89.0)	81 (81.0)	170 (85.0)	0.082
	Positive	11 (11.0)	19 (19.0)	30 (15.0)	

Table 8: Detection of *Giardia lamblia* stained by iron haematoxylin among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Iron haematoxylin stain	Negative	91	85	176 (88.0)	0.138
	Positive	9	15	24 (12.0)	

Table 9: Detection of *Giardia lamblia* by ELISA among participants (N= 200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
<i>Giardia lamblia</i>	Negative	87	78	165 (82.5)	0.068
	Positive	13	22	35 (17.5)	

Table 10: Sensitivity and specificity of *G. lamblia* methods as compared with *Giardia* ELISA copro-antigen (N= 200):

	ELISA		Total N=200	Accuracy Measures				
	Positive (N= 35)	Negative (N= 165)		Variable	%	OR	95% CI	P-value
Direct Wet Mount								
Positive	27 (77.14)	4 (2.42)	31 (15.5)	-Sensitivity	77.14	135.8	38.2 – 482.5	<0.001*
				-Specificity	97.57			
				-PPV	77.14			
				-NPV	97.57			
Negative	8 (22.86)	161 (97.58)	169 (84.5)	-Accuracy	94			
				-LR+	31.87			
				-LR-	1.02			
Examination of concentrated fecal samples								
Positive	27 (77.1)	3 (1.8)	30 (15.0)	-Sensitivity	77.14	182.3	45.5 – 730.3	<0.001*
				-Specificity	98.19			
				-PPV	90			
				-NPV	95.29			
Negative	8 (22.9)	162 (98.2)	170 (85.0)	-Accuracy	94.5			
				-LR+	42.38			
				-LR-	0.23			
Stained by iron hematoxylin stain								
Positive	23 (65.7)	1 (0.6)	24 (12.0)	Sensitivity	65.71	314.33	39 – 2531.6	<0.001*
				-Specificity	99.39			
				-PPV	95.83			
				-NPV	93.18			
Negative	12 (34.3)	164 (99.4)	176 (88.0)	-Accuracy	93.5			
				-LR+	0.9			
				-LR-	0.3			

Table 11: Detection of *Helicobacter pylori* by immunoassay among participants (N=200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
<i>H. pylori</i>	Negative	78	73	151 (75.5)	0.256
	Positive	22	27	49 (24.5)	

Table 12: *Giardia lamblia* and *Helicobacter pylori* co-Infection among participants (N=200):

Variable items		CKD Patients (N= 100)	Control (N= 100)	Total (N= 200)	P-value
Co-Infection	Negative	90 (90.0)	81 (81.0)	171 (85.5)	0.107
	Positive	10 (10.0)	19 (19.0)	29 (14.5)	

Table 13: Relation between *H. pylori* & *G. lamblia* co-infection with characteristics among CKD patients (N=100):

Variable items		Negative (N=90)		Positive (N= 10)		total		P-value
		No.	%	No.	%	No.	%	
Age	20 - <35	22	24.4	6	60.0	28	28.0	0.043*
	≥35 - <50	30	33.3	2	20.0	32	32.0	
	≥50 - <65	30	33.3	2	20.0	32	32.0	
	≥65	8	8.9	0	0.0	8	8.0	
Mean ±SD		46.56 ±14.05		33.40 ±13.9		45.24 ±14.5		0.006*
Sex	Female	42	46.7	2	20.0	44	44.0	0.179
	Male	48	53.3	8	80.0	56	56.0	
Residence	Rural	53	58.9	7	70.0	60	60.0	0.736
	Urban	37	41.1	3	30.0	40	40.0	
Consumption water	Tap	72	80.0	8	80.0	80	80.0	0.999
	Filtered	18	20.0	2	20.0	20	20.0	
Occupation	Not Working	40	44.4	1	10.0	41	41.0	0.044*
	Working	50	55.6	9	90.0	59	59.0	

Table 14: Relation between *H. pylori* & *G. lamblia* co-infection with GIT symptoms among CKD patients (N=100):

Variable items		Negative (N=90)		Positive (N= 10)		total		P-value
		N	%	N	%	N	%	
Vomiting	No	68	75.6	8	80.0	76	76.0	0.999
	Yes	22	24.4	2	20.0	24	24.0	
Nausea	No	37	41.1	5	50.0	42	42.0	0.738
	Yes	53	58.9	5	50.0	58	58.0	
Abdominal Pain	No	22	24.4	2	20.0	24	24.0	0.999
	Yes	68	75.6	8	80.0	76	76.0	
Bowel Habit	No.	25	27.8	1	10.0	26	26.0	0.444
	Diarrhea	60	66.7	8	80.0	68	68.0	
	Constipation	5	5.6	1	10.0	6	6.0	
Flatulence	No	55	61.1	4	40.0	59	59.0	0.171
	Yes	35	38.9	6	60.0	41	41.0	
Fatigue	No	40	44.4	4	40.0	44	44.0	0.099
	Yes	50	55.6	6	60.0	56	56.0	
Loss of Appetite	No	51	56.7	3	30.0	54	54.0	0.108
	Yes	39	43.3	7	70.0	46	46.0	
Dyspepsia	No	49	54.4	1	10.0	50	50.0	0.016*
	Yes	41	45.6	9	90.0	50	50.0	

Table 15: Relation between *H. pylori* & *G. lamblia* co-infection with chronic disease history among CKD patients (N=100):

Variable items		Negative (N=90)		Positive (N= 10)		total		P-value
		No.	%	No.	%	No.	%	
DM	No	52	57.8	8	80.0	60	60.0	0.721
	Yes	38	42.2	2	20.0	40	40.0	
HTN	No	20	22.2	4	40.0	24	24.0	0.308
	Yes	70	77.8	6	60.0	76	76.0	
Edema	No	37	41.1	5	50.0	42	42.0	0.246
	Yes	53	58.9	5	50.0	58	58.0	
Dyspnea	No	64	71.1	8	80.0	72	72.0	0.738
	Yes	26	28.9	2	20.0	28	28.0	

Table 16: Co-infection with haemodialysis duration, urea and creatinine level among CKD patients (N=100):

Variable items		No.	Mean	SD	Minimum	Maximum	p-value
Hemodialysis (years)	Negative	90	5.51	2.3	3.00	10.00	0.005*
	Positive	10	3.40	0.7	3.00	5.00	
Urea (mg/dl)	Negative	90	111.56	37.9	56.00	241.00	0.002*
	Positive	10	153.30	56.1	98.00	241.00	
Creatinine (mg/dl)	Negative	90	6.75	1.4	4.10	9.00	0.014*
	Positive	10	7.90	0.9	5.80	9.80	

Discussion

Generally speaking, zoonotic giardiasis is a common diarrhea-genic parasite mainly infecting especially children with different genotypes significantly associated with the residence area, animal contact, and hand-washing habits (Elhadad *et al.*, 2021). Meanwhile, *Helicobacter pylori* is considered as a public health problem, especially in developing countries, infection rate in Egyptian patients with dyspepsia was high and gastritis was the most revealed finding upon endoscopy (Diab *et al.*, 2018). Frequency of co-infections added to the complexity of understanding disease, as different organisms have potentially synergistic or antagonistic interactions, impacting treatment, clinical outcomes, and susceptibility to other diseases (Chard *et al.*, 2019). Eldash *et al.* (2013) in Egypt reported that *H. pylori* and *Giardia intestinalis* (10-20%) were reported among organic causes of recurrent abdominal pain, with different prevalence as common association diseases causing agents. Because of dysfunction of the immune response, CKD patients on hemodialysis were more susceptible to opportunistic bacterial, viral and/or parasitic infections (Chonchol, 2006). Chronic uremia gave some symptoms associated with intestinal parasitosis since some of most frequent symptoms in patients suffered from both conditions were similar (Gil *et al.*, 2013).

In the present study, the age groups were (35 to 65) years in CKD patients (40%) and (20 to 34) years (38%) in controls, while elderly individuals above 65 years were the least presented (0.8%) and (0.6%) in all participants, but without significant differences. This agreed with Júlio *et al.* (2012) who didn't find significance differences in sexes and ages among their patients.

In the current study, patients were rural residents (66.5%) while (33.5%) were urban ones without significant difference between both ($P > 0.05$). This agreed with others who reported higher prevalence of CKD among patients in rural community and low-to-middle income countries; regardless ages and se-

xes (Sumaili *et al.*, 2009; Varma *et al.*, 2010; Salve *et al.*, 2012; Stanifer *et al.*, 2014). Kaze *et al.* (2015) explained that this might be related to lower awareness of chronic renal disease patients to the related risk factors.

In the current study, the majority of patients used tap water (83.5%), and (16.5%) of them used filtered water, but without significant differences. Choy *et al.* (2014) and Al-Mekhlafi *et al.* (2017) found that patients preferred drinking unfiltered water had a remarkable higher prevalence of giardiasis as compared to those who used filtered water. Globally, there was a strong relation between diarrheal epidemics and consumption of unfiltered water, due to viability of cysts, which remain viable up to three months in cold water (Hedayati *et al.*, 2008), and being strongly resistant to ozone and chlorine, and thus filtered water was better for consumption (Lane and Lloyd *et al.*, 2002).

In the current study, vomiting and fatigue were significantly higher among controls; while bowel habit changes were significantly higher among CKD patients. All other GIT symptoms were nearly similar among participants without significant differences ($P > 0.05$). Among CKD patients the commonest bowel habit symptom was dyspepsia (92.3%) followed by diarrhea and abdominal pain (84.6%), fatigue (69.2%), loss of appetite and flatulence (61.5%), nausea (38.5%) and vomiting (15.4%). The signs and symptoms agreed with others who reported that CKD patients often have gastrointestinal symptoms due to high urea levels, decline of gastrointestinal motility, amyloid protein deposition and decreased sensory disturbance (Schoonjans *et al.*, 2002). The quality of life in CKD patients was usually poor, which affects the nutrition status leading to the development of malnutrition with a potent factor of morbidity and mortality (Strid *et al.*, 2004). Also, they have higher risks of gastric mucosal damages compared to persons with normal renal function due to systemic and local chronic circulatory failure (Block *et al.*, 2007). Ankarklev *et al.* (2010) rep-

orted that *G. lamblia* might be manifested with a wide range from asymptomatic to life threatening ones affected by various factors as to numbers, virulence and host immune system, which diagnosis was proved with stool analysis than clinical pictures (Quihui *et al.*, 2010). Heyworth (2014) reported that laboratory diagnosis was better due to clinical similarity between giardiasis and cryptosporidiosis, amoebiasis, strongyloidiasis, Crohn's disease and irritable bowel disease.

In the current study, high prevalence of *G. lamblia* was among both healthy control and hemodialysis patients. Controls showed higher giardiasis than renal patients, but without significance differences Microscopic by direct wet mount, *Giardia* was detected in 11% of hemodialysis patients versus 20% of controls. Formol-ether concentrated fecal samples showed 11% *Giardia* positive in patients versus 19% in controls. Iron hematoxylin stain showed *Giardia* in 9% of CKD patients versus 15% of controls and serological assay showed *Giardia* coproantigen positive in 13% of patients and 22% of controls. This agreed with Gil *et al.* (2013), who reported in chronic renal disease patients, giardiasis in 0.9% & in controls 2.3%. Also, the prevalence of *E. histolytica* and *G. lamblia* in Egyptian immunocompetent and immunosuppressed patients was 24.6% vs. 6% & 17.6% vs. 4.8% respectively and infection in immunosuppressed patients caused some gut structure changes (Abel-Hafeez *et al.*, 2012).

As to giardiasis in renal-free healthy individuals, Ghieth *et al.* (2016) in Beni-Suef Governorate, Sadek *et al.* (2013), Fahmy *et al.* (2015) in Menoufiya Governorate and El-Tantawy and Taman (2014) in Dakahlia Governorate reported a higher prevalence (27.9%, 35%, 33.1%, & 30%, respectively). But, El Beshbishi *et al.* (2005) and El-Naggar *et al.* (2006) in Dakahlia Governorate reported lower frequency (2.3%, & 7.9%). These differences in giardiasis frequency in different Egyptian Governorates may be due to differences in the personal hygiene, type of studied population, social habits, water

sources, rural or urban areas and may be due to the variable sensitivity of used diagnostic methods (Asher *et al.*, 2014).

In the present study, copro-antigen detection of *Giardia* had the highest technique accuracy. This agreed with the fact that *G. lamblia* antigen in stool was more accurate in patients with chronic gastrointestinal-complaints (Mabehr *et al.*, 1996). Microscopy was less accurate and less valuable that need trained technician for *Giardia* diagnosis, but it was accepted as the gold standard for new developed tests (Koneman *et al.*, 1992). Shetty and Prabhu, (1988) reported that iron hematoxylin stained 61% of cases as they proved that trophozoites of *Giardia lamblia* stained the best with iron hematoxylin than with Trichrome. Also, Ferreira *et al.* (2003) and Garcia-Torres *et al.* (2016) preferred iron hematoxylin stain for preserving most of the intestinal protozoa, and for preparation of permanently stained slide for *Giardia*.

In the current study, *H. pylori* was more prevalent among controls (27/100) as compared to CKD patients (22/100), but without significant differences (P= 0.256). The low prevalence of *H. pylori* in hemodialysis patients was explained by Hwang *et al.* (2002) who declared that patients on dialysis have higher levels of pro-inflammatory cytokines from activated inflammatory cells infiltrating the gastric epithelium, including IL-1 β , IL-6, IL-8 & tumor necrosis factor- α , and so, gastric atrophy progresses, accompanied by increased pH, and finally *H. pylori* was unable to colonize in gastric mucosa (Wesdorp *et al.*, 1981). Besides, it was speculated that most dialysis patients have higher chances of bacterial infection, so antibiotics were commonly used and that cured *H. pylori* as well (Gladziwa *et al.*, 1993). But, Min *et al.* (2013) showed that *H. pylori* infection rate in hemodialysis was (54.5%) slightly higher than in controls (45.9%). This may be due to elevated blood urea and urea nitrogen levels in gastric juice during renal failure, which predisposed to *H. pylori* growth in the stom-

ach (Shousha *et al*, 1990).

In the present study, co-infection of *G. lamblia* and *H. pylori* by immunoassay methods was more prevalent among controls (19%) as compared to CKD patients (10%). Abou Holw *et al*. (2009) in Alexandria found that one of the commonest intestinal protozoa associated with *H. pylori* was giardiasis. Shafie *et al*. (2009) in Iran reported a higher rate of *Giardia* and *H. pylori* co-infection; all *H. pylori* positive patients were infected with *G. lamblia*. Moreover, El-Badry *et al*. (2017) in Cairo reported that *H. pylori* patients (52.5%) were positive giardiasis supporting the theory that conditions for *H. pylori* bacterium survival were heightened by *G. lamblia* infection.

Infection with giardiasis and *H. pylori* is a direct reflection of socio-environmental levels (Patterson *et al*, 2012). There was higher colonization level in developing countries than developed ones (Hasosah *et al*, 2015). However, studies have different theories in explaining which of the two organisms against in the presence of each ones. Increased urease production by *H. pylori* converts urea of the stomach wall to ammonia leading to increase in stomach pH and facilitating the crossing of intestinal parasites to intestine. In addition, the fecal–oral transmission routes of intestinal parasites and *H. pylori* may clear the observed high prevalence of co-infection (Boyanova *et al*, 2011). Oberhuber *et al*. (1997) noted that antral mucosa colonized by *G. lamblia* is coinfecting with *H. pylori* in a large number of cases.

Prevalence of other associated parasitic infections among CKD patients was (15%) and among controls was (25%). The prevalence of intestinal parasites in patients with CKD was from 11 % to 51 % in different studies (Ali *et al*, 2000; Kulik *et al*, 2008; Omrani *et al*, 2015). Gil *et al*. (2013) showed higher prevalence of protozoa infection in hemodialysis patients (61.6%) compared to controls (51.3%). Karadag *et al*. (2013) reported that patients may spend up to 20 years undergoing hemodialysis that increased

the risk of acquiring nosocomial parasitic infections (Abdel-Motagaly *et al*, 2017).

In the present study, the commonest intestinal parasites in CKD patients were *B. hominis* (35%) and *E. coli* (30%) without a significant difference ($P = 0.021$). *Blastocystis hominis*, cryptosporidiosis, and *Endolimax nana* were the most common protozoa (Ali *et al*, 2000; Kulik *et al*, 2008; Karadag *et al*, 2013; Omrani *et al*, 2015).

In the present study, *Blastocystis* was higher than that in Iran as Seyrafiyan *et al*. (2006) found *Blastocystis* spp. (8%), *E. coli* (5.6%), & *Endolimax nana* (4.2%) in hemodialysis patients. Kulik *et al*. (2008) reported *Blastocystis* were 20.9%, Karadag *et al*. (2013) in Turkey found (23.9 %) and Gil *et al*. (2013) in Brazil found (24.5 %).

Despite that, there was controversy whether *Blastocystis* is pathogenic for man or just a normal flora (Kulik *et al*, 2008), lysis of intestinal mucosa and release of toxins causing diarrhea apparently occurs, particularly in immunocompromised patients (Graczyk *et al*, 2005). Omrani *et al*. (2015) reported that *Cryptosporidium* spp. (11.5%) was one of the commonest parasitic infections detected in ESRD patients. Moreover, Ali *et al*. (2000) detected *C. parvum* in 15% and *Microsporidia* in 8.3% in hemodialysis patients and controls with diarrhea. El Sayad *et al*. (2020) in Alexandria reported that *Microsporidia* was the most common bacteria followed by *B. hominis*. Difference in prevalence may be due to different behavior, nutritional status, socioeconomic, seasonal factors, sample size and methods of diagnosis.

In the current study, showed that diarrhea and abdominal pain were more frequent in patients with co-infection (84.6%, & 84.6%, respectively), while vomiting was the least (15.4%). This agreed with that of El-Badry *et al*. (2017), who found that diarrhea and abdominal pain were more common (73.8%, & 47.6%, respectively) and vomiting was less (9.5%). However, there were no clinical symptoms specific for co-infection. Zeyrek *et al*. (2008) in Turkey reported that the as-

sociation between *G. lamblia* and *H. pylori* co-infection, and clinical symptoms were controversy with an impact on patients with frequent abdominal pain. Kader *et al.* (1998) examined 30 patients with symptoms related to peptic ulcers or gastritis; only three (10%) cases of gastric giardiasis with *H. pylori* were in all cases. They concluded that there was a relation between the *Giardia* and *H. pylori* infection.

In the present study, renal patients with positive co-infection results had shorter duration of hemodialysis as compared with patients without this co-infection. Sugimoto *et al.* (2009) declared that the prevalence of *H. pylori* in individuals with normal renal function is similar with patients receiving hemodialysis treatment for less than one-year period. These data suggest that hemodialysis, but not uremia, plays a role in the lower prevalence of *H. pylori* infection. Many previous studies reported that there was no significant correlation between the prevalence of intestinal parasitic infections and the duration of hemodialysis in various different geographic populations irrespective to gastric symptoms (Block *et al.*, 2007 and Sugimoto and Yamaoka *et al.*, 2011). Whereas, one study in Iran reported that hemodialysis patients (63.0%) and CKD patients (66.2%) had significantly higher prevalence of *H. pylori* infection compared with normal individuals (27.5%) (Khedmat *et al.*, 2007). However, since the prevalence of *H. pylori* infection in Iranian population is reported to be more than 60%; further studies are required to clarify this variation (Fabbian *et al.*, 2002).

Diabetes mellitus is the second common cause of chronic renal disease (El-Tawdy *et al.*, 2016). It affects the immune system and leads to simultaneous impairment of other organs (Gil *et al.*, 2013). This study showed that diabetes mellitus was significantly more prevalent (53%) among hemodialysis group compared with healthy controls (40%); *p*-value= 0.044. Hypertension, edema and dyspnea; all of them were significantly high-

er among studied CKD. Similar studies revealed that diabetes, hypertension and long lasting use of herbal medications were significantly related to the high prevalence and incidence of CKD (Stanifer *et al.*, 2014 and Levey *et al.*, 2016). Weisbord *et al.* (2005) and Murtagh *et al.* (2007) reported the prevalence of dyspnea between 20 to 60% among CKD patients.

Conclusion

Giardia/Helicobacter was more prevalent in rural young male patients. CKD patients were risky group. Diagnosis and treatment of co-infected causes were indicated

ELISA assay for *Giardia* copro-antigen showed the advantages of the rapid screening and detection of the parasite.

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