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OPPORTUNISTIC PROTOZOA COINFECTED WITH *HELICOBACTER PYLORI* AMONG EGYPTIAN DIABETIC PATIENTS

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Abstract

Diabetes mellitus (DM) is a hyperglycemic state wherein the patient has a high level of blood glucose. People with diabetes are vulnerable to infection with opportunistic intestinal parasites as it is considered a state of immune suppression.

The study evaluated the opportunistic protozoa co-infected with *Helicobacter pylori* among 156 DM patients and estimated the risk factors. Early morning stool samples were obtained from each participant and examined for *H. pylori* and intestinal protozoa. The overall rate of protozoa was 79.5% (124/156), which were *Blastocystis hominis, Cryptosporidium parvum* and *Isospora belli* were detected in 13.5%, 29.5%, &16.7% respectively. *Giardia intestinalis* and *Entamoeba histolytica/dispar* were in 9% & 8.3% respectively. The overall rate of *H. pylori* was 19.9%, and its co-infection with *I. belli*, *C. parvum, E. histolytica/dispar*, *G. intestinalis* was 6.4%, 32.2%, 12.9% & 9.6% respectively. A significant relation was between *H. pylori* and diarrhea (P <0.001), *C. parvum* and abdominal pain (P=0.001), diarrhea (P <0.001), and animal contact (P <0.001), but *I. belli* showed a significant association with diarrhea (P = 0.002), flatulence (P = 0.027), and animal contact (P = 0.002).

Keywords: Diabetes mellitus, *H. pylori*, opportunistic intestinal protozoa

Introduction

Zoonotic parasites are one of the causes of gastrointestinal troubles, growth retardation, malnutrition, and increasing metabolic disorders in immunocompromised persons as diabetic patients (Zibaei *et al,* 2023). Meanwhile, both diabetes type 1 & 2 were encountered in Egypt (El-Tawdy *et al,* 2017). The diabetes alters several immune responses causing suppression in innate and acquired immunity ending in an immune-suppression state (Waly *et al,* 2021). Opportunistic parasites are accounted for a severe illness in immunosuppressed patients (Morsy *et al,* 2022), with high mortality risk (Jalan *et al,* 2013). Also, *H. pylori* can cause duodenal and gastric ulcers and/or cancer (Mutaz and Carmen, 2015).

The protozoa and *H. pylori* are widely distributed human pathogens (Ghallab and Morsy, 2020). *H. pylori*, a gram-negative bacterium colonizes in human stomach, is one of the most common bacteria infecting more than 50% of the global populations (Ali and Al Hussaini, 2024).

H. pylori produces some enzymes such as urease enzyme, that damage stomach epithelial lining (Cameron *et al,* 2001), and alters its urea into ammonia causing gastric PH (Isaeva and Efimova, 2010). The urease produced by *H. pylori* helps intestinal parasites and bacteria to easily cross the stomach's acid environment (David *et al,* 2006). Parasites and *H. pylori* may share numerous clinical manifestations such as dyspepsia, diarrhea, abdominal distention, dysentery, and vomiting (Ahmed *et al,* 2018).

This study aimed to evaluate the frequency of opportunistic intestinal protozoa co-infected with *H. pylori* in diabetic patients.

Materials and Methods

Study design: This cross-sectional study included 156 stool samples collected from

diabetic patients attending the outpatient clinics of Kafrelsheikh University's Hospitals. The study was accomplished from October 2022 till October 2023.

Study population: Participants were collected from diabetic patients of both sexes (89 females & 67 males) aged between 15 and 60 years old and divided into 3 age groups; 16-30, 31-45, & 45-60 years suffered from gastrointestinal disorders, such as abdominal pain, and/or bloating, diarrhea, nausea, and vomiting. Exclusion criteria were those on diarrheal treatment. Cases were requested for verbal consent.

Stool examination: Morning stool samples were collected in labeled carton containers. Each sample was examined macroscopically for consistency, any gravid segments, blood, or mucous. Smears were microscopically examined as direct smear with Lugol's iodine stain; modified Ziehl-Neelsen and/or Trichrome stain (El-Shazly *et al,* 2006). The negative samples were re-examined after formalin-ethyl acetate sedimentation concentration (CDC, 2016). *H. pylori* antigen in stool was identified using ICT (ACON Laboratories Inc., San Diego, USA) according to the manufacturer's instructions.

Statistical analysis: Data were coded and analyzed using version 28 of the statistical package for the Social Sciences (SPSS, NY, USA). They were reviewed using frequency (count) and relative frequency (percentage). Chi-square test compared categorical data. When the expected frequency was less than 5, an exact test was performed instead. P value less than 0.05 was considered significant (Chan, 2003).

Ethical considerations**:** The study was approved by the Ethical Committee, Faculty of Medicine, Kafr El-Sheikh University; Number KFSIRB200-63 that agreed with Helsinki declaration (2013)**.**

Results

In the present study, enteric protozoa were detected with an overall detection rate of 79.5% (124/156), where, *C. parvum* was detected in 29.5% (46/156), *I. belli* in 16.7% (26/156)*, B. hominis* in 13.5% (21/156), *G. intestinalis* in 9% (14/156) and *E. histolytica/dispar* in 8.3% (13/156). Also, *H. nana* was detected in 2.6% (4/156).

H. pylori was detected in 19.9% (31/156), and co-infected with *C. parvum, I, belli, E. histolytica and G. intestinalis* in 32.2%, 6.4%, 12.9%, & 9.6% respectively. Highly infected age group was 45-60. Gastrointestinal troubles; abdominal pain, flatulence, diarrhea, nausea and vomiting were 86.5%, 61.5%, 71.2%, 50.6%, & 37.8% respectively. *H. pylori* was significant associated with diarrhea, *C. parvum* with abdominal pain, diarrhea, and animal contact and *I. belli* was significantly associated with diarrhea, flatulence, and animal contact. Details were given in tables (1, 2, & 3).

Table 1: *H. pylori* clinical manifestations in different age groups of both sexes

Variations		Positive		Negative		P value
		No.	Percentage	No.	Percentage	
Age groups	16-30	7	22.6%	35	28.0%	0.105
	31-45	2	6.5%	25	20.0%	
	45-60	22	71.0%	65	52.0%	
Abdominal pain	Yes	28	90.3%	107	85.6%	0.769
	No	3	9.7%	18	14.4%	
Nausea	Yes	12	38.7%	67	53.6%	0.138
	No	19	61.3%	58	46.4%	
Vomiting	Yes	11	35.5%	48	38.4%	0.764
	No	20	64.5%	77	61.6%	
Diarrhea	Yes	13	41.9%	98	78.4%	< 0.001
	No	18	58.1%	27	21.6%	
Flatulence	Yes	16	51.6%	80	64.0%	0.204
	No	15	48.4%	45	36.0%	
Animal contact	Yes	14	45.2%	53	42.4%	0.781
	No	17	54.8%	72	57.6%	

Table 2: *C. parvum* clinical manifestations in different age groups of both sexes.

Variations		Positive		Negative		P value
		No.	Percentage	No.	Percentage	
Age groups	16-30	12	26.1%	30	27.3%	0.890
	31-45	9	19.6%	18	16.4%	
	45-60	25	54.3%	62	56.4%	
Abdominal pain	Yes	46	100.0%	89	80.9%	0.001
	No	0	0.0%	21	19.1%	
Nausea	Yes	28	60.9%	51	46.4%	0.098
	No	18	39.1%	59	53.6%	
Vomiting	Yes	20	43.5%	39	35.5%	0.346
	No	26	56.5%	71	64.5%	
Diarrhea	Yes	46	100.0%	65	59.1%	< 0.001
	No	0	0.0%	45	40.9%	
Flatulence	Yes	28	60.9%	68	61.8%	0.912
	No	18	39.1%	42	38.2%	
Animal contact	Yes	42	91.3%	25	22.7%	< 0.001
	No	4	8.7%	85	77.3%	

Table 3: *I. belli* clinical manifestations in different age groups of both sexes.

Variations		Positive		Negative		P value
		No.	Percentage	No.	Percentage	
Age groups	16-30	4	15.4%	38	29.2%	0.360
	31-45	5	19.2%	22	16.9%	
	45-60	17	65.4%	70	53.8%	
Abdominal pain	Yes	19	73.1%	116	89.2%	0.052
	No	7	26.9%	14	10.8%	
Nausea	Yes	14	53.8%	65	50.0%	0.720
	No	12	46.2%	65	50.0%	
Vomiting	Yes	11	42.3%	48	36.9%	0.605
	No	15	57.7%	82	63.1%	
Diarrhea	Yes	12	46.2%	99	76.2%	0.002
	No	14	53.8%	31	23.8%	
Flatulence	Yes	11	42.3%	85	65.4%	0.027
	No	15	57.7%	45	34.6%	
Animal contact	Yes	4	15.4%	63	48.5%	0.002
	No	22	84.6%	67	51.5%	

Discussion

Opportunistic intestinal parasites have obtained rising recognition as crucial pathogens that have clinical importance in immunocompromised patients such as diabetes **(**Khanna *et al*, 2022). The cause of immune depression condition could be a broad scale of illnesses including diabetes as they are linked to the suppression of both innate and acquired immunity throughout the chronic course of this disorder (Ibrahim *et al,* 2022). In the present study, enteric protozoa were detected with an overall detection rate of 79.5%, where, *Cryptosporidium* spp. was detected in 29.5%, *I. belli* was detected in 16.7%, *Blastocystis* was detected in 13.5%, *G. intestinalis* was detected in 9%, and *E. histolytica/dispar* was detected in 8.3%. However, intestinal helminthes such as *H. nana* were detected in 2.6%. Akinbo *et al,* (2013), observed an overall occurrence of 18.7% of intestinal parasites among diabetic patients. They detected *Entamoeba histolytica, Ascaris lumbricoides*, and hookworm parasites in diabetic patients. Also, Eldash *et al* (2013), in Egypt correlated between *H. pyloyi* and giardiasis among Egyptian children, El Nadi *et al.,* (2015), found that out of 100 diabetic patients, 25% were infected with intestinal parasites. They recorded that *G. lamblia*, *E. histolytica/dispar, Cryptosporidium* spp., and *H. nana* were detected in 22%, 7%, 5%, and 5% respectively. Also, Ali *et al.* (2018), found that 14.8 % of diabetic patients were infected with *B. hominis*, *G. lamblia*, *Entamoeba histolytica/dispar*, and, *C. parvum* in 35.48%, 25.8%, 17.74%, and 12.9% respectively. However, *Hymeno-*

lepis nana was reported in 4.84% and *Strongyloides stercoralis* was found in 1.61% of the diabetic patients.

El Drawany *et al.* (2019) detected an overall rate of (27%), which were *C. parvum*, *B. hominis, G. lamblia, and H. nana*, and added that opportunistic protozoa was significantly greater than helminthes (P<0.001). Moreover, Rady *et al.,* (2019), recorded that the overall parasitic detection rate was 45.2%. Parasites were *E. histolytica/dispar* (7.43%), *G. lamblia* (10.29%), *B. hominis* (9.14%), *H. nana* (3.43%), and *C. parvum* (9.71%). But, Ibrahim *et al.* (2020) detected *Blastocystis* in 87% of diabetic patients. They added that diabetes mellitus must be considered as a risk factor for opportunistic parasitic infections. In Egypt, the rate of *Blastocystis hominis* was 67.4% in Alexandria (Eassa *et al.* 2016), and up to 82% in Minia (Gabr *et al.* 2018). Waly *et al.* (2021) in diabetic patients reported *B. hominis*, followed by *C. parvum* (12%), *G. lamblia* (7%), *E. histolytica* (2%), and *H. nana* (2%). There were many debates about the *Blastocystis* pathogenicity (Paboriboune *et al,* 2014). But, Fathy (2016) in Egypt reported that *B. hominis* could be considered pathogenic mainly when present alone in large numbers in symptomatic patients. Also, Deng *et al.* (2022) in Singapore, found *Blastocystis* ST7 (pathogenic subtype) was associated with lower bacterial diversity altered microbial structure in diarrheal patients. Kumarasamy *et al.* (2023) in Malaysia found that *Blastocystis* sp. reduced the efficacy of 5-fluorouracil as a colorectal cancer chemotherapeutic treatment.

In the present study, *H. pylori* was detected in 19.9% (31/156). However, co-infection between *H. pylori* and *C. parvun, I. belli, E. histolytica/dispar* and *G. intestinalis* was detected in 32.2%, 6.4%, 12.9%, & 9.6% respectively. Rady *et al.* (2019) reported that *H. pylori*-positive children was (65.22%), with an elevated association with *G. lamblia* (10.56%), followed by *B. hominis* (9.32%), *E. histolytica/dispar* (6.21%), *Cryptosporidium* (5.59%), and finally *H. nana* (1.24%). Also, Nami *et al.* (2022) in Libya, reported that parasitic infection and gastrointestinal symptoms with *H. pylori* among diabetic patients was 72% with a highly significant difference (p=0.000).

In the present study, of the 156 diabetic patients 42.9% were males & 57.1% were females with ages ranged from 15-60 years, showed parasites more in the age group 45-60. This agreed with both Ali *et al*. (2018) and Waly *et al*. (2021), they reported that the highest percentage of intestinal parasites was in diabetic patients over 40 years old. They explained that the weak immune system that accompanies aging linked to diabetic status increased the parasitic complications. Also, Khanna *et al*. (2022) in India reported that intestinal parasites were more among diabetic patients in age group above 40 years. But, Tangi *et al.* (2016) in Cameroon reported that the intestinal parasites prevalence in diabetics was 10% in the middle age. They added that diabetic patients must be screened routinely for intestinal parasites especially protozoa

In the present study, all patients suffered from gastrointestinal manifestations such as abdominal pain, nausea, vomiting, diarrhea, and abdominal flatulence were represented by 86.5%, 50.6%, 37.8%, 71.2%, & 61.5% respectively. Significant association was by *C. parvum* and both abdominal pain and diarrhea and by *I. belli* and diarrhea and flatulence. El Drawany *et al.* (2019 in Egypt, reported that diabetic patients suffered from diarrhea only had (48%) of *Cryptosporidium* followed by B. *hominis, G. lamblia* and *H. nana* than those suffered from diarrhea and abdominal pain (18%). They added that protoza were more than helminthic infections.
Also, Ghallab *et al.* (2020) in Egypt showed that gastrointestinal pictures were nausea, abdominal pain, vomiting, bloating, and diarrhea, the significant difference between diarrhea and *H. pylori* were in 160/240 associated protozoa diarrhea (P=0.015) were *G. intestinalis* (P=0.002), *E. histolytica/dispar* (P=0.041), *C. parvum* (P=0.018) and *B. ho-*

minis (P=0.040). Moreover, Castillo-Montoyaa *et al.* (2017) in Mexico, among children found a statistical difference between *H. pylori* (28%) and protozoa causing epigastric pain (P<0.001), recurrent peri-umbilical pain (P<0.001), bloating (P=0.016), heartburn (P=0.0007), nausea (P=0.0061), diarrhea (P= 0.0389), and constipation (p=0.0019). Besides, Waly *et al.* (2021) reported that all their diabetes participants suffered from diarrhea, abdominal pain, vomiting, nausea, flatulence, anorexia, and/or constipation with rates of 61%, 51.5%, 25.5%, 43%, 38%, 37.5%, &16.5% respectively. They explained that the higher prevalence of intestinal parasites in their study was due to the presence of patients complaining only of several gastrointestinal complaints that increased the probability of the discovery of intestinal parasites. Moreover, Yilmaz (2022) in Turkey, reported that 46.2% of the diabetic patients had gastrointestinal complaints, of which 19 had dyspepsia and the other patients had diarrhea and/or constipation. Nevertheless, Tangi *et al.* (2016) reported that the asymptomatic diabetic patients were more infected with intestinal parasites. The difference may be due to different climatic environments.

In the present study, there was significant association between *C. parvum* and *I. belli* with the animal contact. This agreed with Ibrahim *et al.* (2023), who found significant association between animal contact and *C. parvum* (*P*=0.007). Meanwhile, Tanih and Ndip (2013) in South Africa reported significant association between animal contact and *H. pylori* on one hand and both *C. parvum* and *I. belli* on the other hand.

Conclusion

Opportunistic zoonotic parasites especially protozoa are risky problem for dietetic patients. Regular examination and proper treatment is a must to minimize complications.

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