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PARASITES AND *HELICOBACTER PYLORI* IN EGYPTIAN CHILDREN WITH OR WITHOUT DIABETES WITH GASTROINTESTINAL MANIFESTATIONS AND HIGH CALPROTECTIN LEVEL

By

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Abstract

This study highlighted the prevalence of parasitic infection in type-1 diabetes mellitus children and evaluated the effect of intestinal parasites on fecal calprotectin as a part of innate mucosal immunity. A total of 431 children with gastrointestinal manifestations attended the outpatients clinics, Aboul-Reesh Pediatric Teaching Hospital, Cairo University were randomly selected. Medical sheets were filled out on each patient. Stool samples were collected in labeled covered cartoon boxes and macroscopically examined for adult worms and segments, and microscopically for ova and protozoa by Lugol's iodine1% smears and formol-ether concentration method, also, MZN was used for *Cryptosporidium* oocysts. Besides, stool samples were examined for *Helicobacter pylori.*

The results showed that 171/431 (39.67%) were type-1DM (p=0.000) and 260 (60.32%) were non-diabetic used as positive control. The overall intestinal parasitosis was (45.26%), *H. pylori* was (39.47%), irritable bowel syndrome was (25.78%), inflammatory bowel disease was (3.96%). Treatment of the causative agents diminished the gastrointestinal troubles.

Keywords: Children, diabetes mellitus type 1, Intestinal parasitosis, *Helicobacter pylori*, Irritable bowel syndrome, Calprotectin.

Introduction

Diabetes mellitus is the most prevalent metabolic disorder worldwide (WHO, 2016), and the same encountered in Egypt (El-Tawdy *et al*, 2016). However, gastrointestinal parasites create benign diseases, and induce complications with high morbidity and mortality in the immunocompromised, including diabetic patients (El-nadi *et al*, 2015). Also, diabetes mellitus type 1 was associated with an increased prevalence of *H. pylori*, which contributed to autoimmune thyroiditis pathogenesis (Zekry and Abd El-wahid, 2013), strong association in diabetic retinopathy, neuropathy and nephronpathy (Agrawal *et al*, 2010) as well as gastrointestinal diseases (Tsay and Hsu, 2018).

Berni *et al.* (2004) reported that the fecal calprotectin in diagnosis and assessment of IBD was not fully defined. The positive fecal calprotectin test supported diagnosis or confirmed relapse of inflammatory in pediatric patients, but a negative one did not exclude bowel disease (Kostakis *et al*, 2013).

The study aimed to evaluate the role of the intestinal parasites with or without *Heliobacter pylori* in children with or without diabetes and gastrointestinal disorders.

Materials and Methods

Total of 413 children (215 males & 245 females) were with diabetes mellitus Type 1 (190) and 260 without diabetes. All children were outpatients attending the clinics at Aboul-Reesh Pediatric Teaching Cairo University Hospital from May 2016 to December 2017.

Patients' ages ranged from 4 to 12 years. They suffered from gastro-intestinal disorders (GIDs) in the form of abdominal colic, fatigues, loss of weight, diarrhea altered with constipations. Some patients in Gastroenterology outpatient clinic were diagnosed as *H. pylori* infection, irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD). Diarrhea was defined by three or more loose or watery stools within 24hrs. Demographic and clinical data were collected by questionnaires and patients' files. Children with *Salmonella, Shigella,* fungus, and /or endemic viral infections was excluded.

Routine macroscopic and microscopic examination was done for three successive stool samples. Microscopic screening included direct wet smear stained with Lugol's iodine 1% and formol-ether concentration method. Modified ZN stain was used for *Cryptosporidium* oocysts.

The one site rapid test cassette (flow chromatographic immunoassay) of Cikbiotech detected the specific coproantigen for *Helicobacter* bacteria in stool samples.

Stool specimen was stabbed in at least different sites to collect 50mg of feces, and shacked to mix the specimen and the extraction buffer. Samples of *Helicobacter* antigen was developed two lines, for test and control (Montalto *et al.* 2010).

Fecal calprotectin was measured quantitatively (Radin *et al*, 2014). DRG: HYBRiD-XL Calprotectin Kit, which is a solid phase sandwich ELISA (Calprest1, Eurospital, Trieste, Italy). Wells of reagent cartridges were antibody-coated ones. Murine monoclonal antibody captured an exclusive antigenic site in the chemical composition of the calprotectin molecule. Aliquots from the collected samples and enzyme conjugate were incubated together in the coated well. The used enzyme conjugate was a monoclonal anti-calprotectin antibody impregnated with horse-radish peroxidase enzyme. Wells were washed off to remove unbound conjugate. Bound peroxidase conjugate amount was measured by color intensity. Calprotectin concentration measure higher than 200µg/g was considered positive.

The Ethical Committee of Cairo University was followed and written informed agreement was obtained from the parents of the participated patients who joined.

Results

The *E. histolytica/dispar, G. lamblia, B. hominis, C. parvum* infections significantly differed among two groups. *H. pylori* was only significant with *G. lamblia* ($P<0.05$). Among DM patients the intestinal parasitosis was 45.26%, but *H. pylori* were 39.47%. DM status was significantly associated with increased prevalence of intestinal parasitic infections. The fecal calprotectin was significantly associated ($p<0.05$) with *C. parvum* 10 (52.63%), *G. lamblia* 10 (45.45%) and *H. pylori* infection 40(21.62%). Analysis showed strong significant relationship between the gastrointestinal disorders and *E. histolytica/dispar, H. nana, C. parvum* infections, and *H. pylori* ($P<0.05$). Details were given in tables (1, 2, 3, 4 & 5).

Table 1: Intestinal parasites among diabetic and non-diabetic patients

Type of parasite	Positive		DM		Non-DM		P value
	Count	%	count	%	count	%	
Entamoeba histolytica/dispar	20	4.84	13	7.43	7	2.94	0.035
Giardia lamblia	22	5.33	18	10.29	4	1.68	< 0.001
Blastocystis hominis	35	8.47	16	9.14	9	3.78	0.023
Hymenolepis nana	10	2.42	6	3.43	4	1.68	0.335
Ascaris lumbricoides	4	0.97	1	0.57	4	1.68	0.402
Cryptosporidium parvum	19	4.60	17	9.71	2	0.84	< 0.001
Parasite free	303	73.37	104	59.43	208	87.39	< 0.001
Total	413	100.00	175	100	238	100	---

Table 2: *Helicobacter pylori* positive cases among patients with intestinal parasites

Type of parasite	Positive		*H. pylori* +ve		P value
E. histolytica /dispar	20	4.84%	10	6.21%	0.409
G. lamblia	22	5.33%	17	10.56%	< 0.001
B. hominis	35	8.47%	15	9.32%	0.827
H. nana	10	2.42%	2	1.24%	0.209
A. lumbricoides	4	0.97%	3	1.86%	0.310
C. parvum	19	4.60%	9	5.59%	0.571
Parasite free	303	73.37%	105	65.22%	< 0.001
Total	413	100.00	161	41.11	---

Table 3: Relation between diabetes mellitus with or without parasitic infection

Infective agent	Positive		DM		Non DM		P value	OR	95% CI	
	No.	%	No.	%	No.	%			Lower	Upper
With parasites	147	32.67	86	45.26	52	20.00	<0.001	3.308	2.180	5.019
No parasites	303	67.33	104	54.74	208	80.00				
H. pylori	185	41.11	75	39.47	110	42.31	0.546	0.889	0.608	1.302
No *H. pylori*	265	58.89	115	60.53	150	57.69				

Table 4: Relation between fecal calprotectin and parasitic infection

Infective agent	Total	Fecal calprotectin positive (above 200µg/g)		P value
E. histolytica /dispar	20	3	15.00%	0.736
G. lamblia	22	10	45.45%	< 0.001
B. hominis	35	2	5.71%	0.293
H. nana	10	1	10.00%	1
A. lumbricoides	4	0	0.00%	1
C. parvum	19	10	52.63%	< 0.001
Parasite free	266	30	11.28%	0.004
H. pylori	185	40	21.62%	< 0.001
No *H. pylori*	265	19	7.17%	

Table 5: Frequencies of protozoan parasites and gastro-intestinal disorders

Type of parasite	Total		IBS		IBD		Normal or other		P value
	No.	%	No.	%	No.	%	No.	%	
E. histolytica/dispar	20	4.84	5	25.00	2	10	13	65.00	< 0.001
G. lamblia	22	5.33	15	68.18	3	13.64	4	18.18	< 0.001
B. hominis	35	8.47	13	37.14	3	8.57	19	54.29	0.272
H. nana	10	2.42	4	40.00	4	40	2	20.00	0.003
A. lumbricoides	4	0.97	3	75.00	0	0	1	25.00	0.106
C. parvum	19	4.60	0	0.00	10	52.63	9	47.37	< 0.001
Parasite free	303	73.37	70	23.10	12	3.96	221	72.94	< 0.001
Total	413	100	110	26.63	34	8.23	269	65.13	---
H. pylori	185	41.11	66	35.68	25	13.51	94	50.81	< 0.001
No *H. pylori*	265	58.89	50	18.87	20	7.55	195	73.58	
Total	413	100	110	26.63	34	8.23	289	64.22	---

Discussion

In the present study, comprehensive fecal examination among children with gastrointestinal manifestations showed that the overall intestinal parasites in diabetic children was (45.26%) compared to (20%) in non-diabetic ones. In the diabetic children (type 1), *E. histolytica/dispar* (7.43%), *G. lamblia* (10.29%) *B. hominis* (9.14%) *H. nana* (3.43%) *A. lumbricoides* (0.57%) and *C. parvum (*9.71%) compared to 2.94%, 1.68%, 3.78%, 1.68%, 1.68%, & 0.84% respectively. The overall *H. pylori* positive cases were (65.22%), with high associated with *G. lamblia* (10.56%), followed by *B. hominis* (9.32%), *E. histolytica/dispar* (6.21%), *C. parvum* (5.59%), *A. lumbricoides* (1.86%). and lastly *H. nana* (1.24%). Association between DM-type1 and parasitosis was due to tendency of diabetes to exacerbate anemic by diminishing the erythropoietin production (Mohtashamipour *et al*, 2015), particularly when associated with the low personal hygiene (Khan and Tisman, 2010). Consequently, the innate and adaptive immune responses together with the reduced intestinal motility in diabetic patients fail to resolve intestinal parasitosis (Knapp, 2013). Establishment of a regulatory network contributes to control of overt immune responses to allow longer survival of the parasite while restricting inflammation that might otherwise lead to pathology (Elliott and Weinstock, 2017). Alterations in the host immune state might influence and be affected by other concomitant disease(s) (Liu *et al*,

2010). Fecal calprotectin and inflammatory biomarker respond to any unspecific nature to various gastrointestinal conditions, high values were recorded in *C. parvum* and *G. lamblia* infections (Radin *et al,* 2014). But, helminthes as *H. nana* and *Ascaris* recorded high calprotectin levels that described by the masterful immune regulation exerted by regulatory T cells, alternatively activated by macrophages in luminal helminthes (Cordeiro-da-Silva, *et al,* 2014). No doubt, undiagnosed intestinal parasitic infections led to confliction bet- ween functional (IBS) and organic bowel (IBD) disorders (Pohl *et al.* 2013). *Ascaris lumbricoides* produce anti-enzymes, which stand beyond increased excretion of fats, nitrogenous compounds and lactose (Duque *et al.* 1972), *G. lamblia* trophozoites damage the upper villus of small intestine (Solomons, 1982), where lactase enzyme is highly expressed (Vahedi, *et al,* 2012). Moreover, *G. lamblia* were associated with lower ferritin levels in anemic children without significant associations as to residence and/or body mass index (Atwa and Thabet, 2016). Cryptosporidiosis was the cause of risky diarrhea in children (Shalaby and Shalaby, 2015)

In the present study, 185 patients (41.11%) were *H. pylori* positive; (42.31%) among non-diabetic children compared to (39.47%) in diabetic children. Diagnosis of *H. pylori* antigen by immune-chromatography proved highly dependable and sensitive (Andersen *et al,* 1995). Also, *H. pylori* and *G. intestinalis* were reported among organic causes of recurrent abdominal pain, with different prevalence mainly in developing countries as common associated diseases causing agents (Eldash *et al,* 2013). Consequently, the early diagnosis helped patients to escape chronic gastritis complications (Sigthorsson *et al.* 2001)**,** and progression into painful stomach ulcers due to excessive release of gastrin hormone and loss of blood **(**Gulcelik *et al.* 2005). No doubt, the poor glycemic control, autonomic neuropathy and impaired cellular and humoral immunity supported *H. pylori* colonization (Bener *et al,* 2007). Mohammad *et al*. (2008) reported that not only that the *H. pylori* infection was extremely higher among Egyptian schoolchildren, but also its' adverse effects was far beyond the stomach.

Thus, treatment of the inflammatory bowel disorder with steroids as anti-inflammatory agents, expose these children to the side effects of the drug. Drawbacks encompass bad control of glucose homeostasis and aggravation of the immunity weakness (Asseldonk *et al.* 2012). Steroids exert immunomodulation on T & B cells; affect the size of lymphoid organs and lymphocyte cell death (Coutinho and Chapman, 2011). This action worsens both the child diabetic status of and aggravates the opportunity of some parasites (Dave *et al.* 2014). Moreover, diabetes mellitus is a metabolic disorder with abnormally high level of blood glucose makes diabetics to be considered as immune-compromised individuals. Two types of intestinal parasites are helminthes and protozoa are important causes of infections in immuno-compromised individuals (Nazligul *et al,* 2001).

The inflammation that can be observed as (IBD) be either organic or inorganic type (Kaser *et al,* 2010). Additionally, irritable bowel syndrome (IBS) resembles somehow clinically IBD which is also considered as the second health problem to GIT. IBS is a highly prevalent gastrointestinal disorder of the unknown origin (Wilson and Crabtree, 2007). IBD is a disease of unknown cause associated with diarrhea and colonic lesions that are identified by endoscopy (Kaya ***et al,*** 2005). The etiology of each one is varying and may involve some microbial agents such as invasive *Entamoeba histolytica* that causes ulceration of the mucosa of the large intestine. (Friedman and Blumberg, 2008)

In diabetic infants the hyperglycemic environment, gastrointestinal dysmotility and immune dysfunction including neutrophil damage, depression of the antioxidant system and humoral immunity favor gastrointestinal infections. Intestinal parasitic infections play a crucial deceiving role by being

the intermittent in their nature of excretion (Munns *et al*, 2016).

Conclusion

Intestinal parasitic infections were high in diabetic children than in non-diabetic ones.

H. pylori co-infection with intestinal parasites was highly prevalent. Pathogenicity of intestinal parasites induce symptoms mimic irritable bowel syndrome. Intestinal protozoans induce fecal calprotectin while intestinal helminthes evade innate mucosal immunity. Parasites infection and *H. pylori* must be considered with low hygiene style and impaired immunity as diabetic children. Symptoms related to some parasites mimic irritable bowel syndrome due to their intercalated pathogenesis. Protozoan infections and *H. pylori* may induce high calprotectin values. The low values of fecal calprotectin in intestinal helminthes were a model for immune evasion. Confusing parasitic causes that induce high calprotectin values with IBD may expose children to immune compromising treatment, especially those with type-1 DM. Children treatment will be published in due time by the first author.

Recommendations

Generally, Intestinal parasites usually create benign diseases, though they may induce complications with high morbidity and mortality to the immune-compromised patients, particularly diabetic and handicapped children. Children with or without diabetes who have gastrointestinal disorders may be presented with varying behavioral manifestations.

Thus, the proper diagnosis and specific treatment of them **a**re indicated particularly in diabetic ones to avoid risky complications

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