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CRYPTOSPORIDIOSIS PREVALENCE ASSOCIATED WITH GASTROINTESTINAL MANIFESTATIONS AMONG HEMODIALYSIS PATIENTS WITH CHRONIC RENAL DISEASE IN BENI-SUEF UNIVERSITY HOSPITALS, BENI-SUEF, EGYPT

By

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Abstract

Cryptosporidium spp. has an elevated incidence rate particularly in immune-compromised patients including chronic renal disease (CRD) patients. This study evaluated cryptosporidiosis among patients with chronic renal disease undergoing hemodialysis attending outpatient clinics of Internal Medicine Department, Faculty of Medicine, Beni-Suef University Hospitals. Stool samples were collected from 90 patients and examined for *Cryptosporidium* oocysts stained with cold acid fast stain. The results showed 13.3% were infected with *Cryptosporidium* oocysts but without significant difference as to age, sex, and residence. The most frequent symptoms were diarrhea and abdominal pain with significant difference, but without significance with other clinical symptoms. Hypertension and duration of hemodialysis showed significant difference with the prevalence rate of cryptosporidiosis, without significant difference with diabetes.

Key words: Egypt, *Cryptosporidium,* Chronic renal disease, and Hemodialysis.

Introduction

Chronic renal disease (CRD) is a progressive and irreversible loss of renal functions with uremic toxins conservation naturally excreted in urine (Glorieux *et al*, 2013). Accumulation of these toxins in the body results in the immune system functions defects owing to production of antibodies, impairment of leukocyte functions, and T-cell activiation (Chonchol, 2006; Ocak and Eskiocak, 2008). These defects in the immune system are linked to high susceptibility to various viral and parasitic infections that are considerably responsible for mortality and morbidity in patients suffering from chronic renal failure (Tonelli *et al,* 2006). Intestinal opportunistic parasites particularly *Cryptosporidium* spp. have a high incidence rate in developing countries mainly in immuno-commpromised patients such as chronic renal disease patients underwent hemodialysis (Karadag *et al,* 2013). Hemodialysis patients usually experience immune suppression, subsequently, they were at increased susceptibility to opportunistic *cryptosporidium* spp. infections (El-Kady *et al,* 2018). They always suffered from numerous gastrointestinal troubles with repeated episodes of diarrhea and thus suspected to shed hidden cryptosporidiosis that might be unexpected by the treating physician as the disease is neglected among hemodialysis patients who were not investigated before (Hijjawi *et al,* 2017).

Cryptosporidium species is an intracellular apicomplexan protozoan infecting a wide variety of hosts including humans (Ryan *et al,* 2014; Parghi *et al,* 2014). Infections were recognized as an important zoonotic parasitosis responsible for global waterborne diarrheal outbreaks (Checkley *et al,* 2015; Wang *et al,* 2018). *Cryptosporidium* spp. is predominantly transmitted to new hosts upon the consumption of the infective oocysts in contaminated food and/or water or via the fecal-oral route (Pielok *et al,* 2019). Infection is a self-limiting disease in immuno-competent individuals, and might produce life-threatening in immunocompromised patients (Parghi

et al, 2014). The disease might cause watery or mucoid diarrhea and abdominal pain that may continue for a few days or long (Hunter and Nichols, 2002; Mohaghegh *et al,* 2017). Cryptosporidiosis showed elevated mortality and morbidity in immunocompromised patients with AIDS (Mohaghegh *et al,* 2017), hemodialysis, and cancer patients (Wang *et al*, 2018). Microscopic examination by using acid-fast stain (Mahgoub *et al,* 2004), with or without concentration was the most extensively used screening test to diagnose cryptosporidiosis as being available and cheap particularly in rural areas, with elevated sensitivity compared to other techniques (Parghi *et al,* 2014; Hijjawi *et al,* 2017).

The present study aimed to assess *Cryptosporidium* spp. prevalence by using cold Acid Fast stain among patients undergoing hemodialysis in Beni-Suef University Hospitals.

Materials and Methods

This is a cross-sectional study performed on stool samples collected from 90 patients attended the outpatient clinics of Internal Medicine Department, Faculty of Medicine, Beni-Suef University Hospitals. The study was done in the period from May 2019 to April 2020. Patients were complained from chronic renal disease and were undergoing hemodialysis. Demographic data, in addition to risk factors, were documented including age (ranged from 15 to 75 years), sex, residence, duration of dialysis (3-10 years), and kdney function tests (urea and creatinine). Gastrointestinal symptoms such as change in bowel habits, abdominal pain, vomiting, nausea with other disorders as diabetes and hypertension were filled out of medical sheets.

Parasitological diagnosis: Three fecal morning samples were taken from each one (after informed consent was obtained) in clean tight fitted containers labeled with the participant's name and date of sample collection. Stool samples were examined macroscopically for consistency, presence of mucus, blood, gravid segments, and others. Microscopic examination was done by direct smears (mixtures of stool with one drop of iodine) and concentrated by formalin ethyl acetate sedimentation procedure to detect helminths eggs, larvae, and protozoa cysts. Patients infected with other parasites or those received antiparasitic drugs were excluded. The sediments were stained with cold Acid Fast stain to identify *Cryptosporidium* oocysts, which was a commercially accessible readily prepared (Kinyoun Kit cat no 25765-1, Polysciences, Germany).

A thin fecal smear was prepared, allowed to dry in air, fixed with absolute methanol and then stained by cold AF stain. Then, smears were examined with light microscopy at 1000× for the *Cryptosporidium* oocysts. Oocysts appeared as rounded, oval, pink to red to deep purple-stained, with size 4-5µm, against a blue background (Garcia, 2007).

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 percent of qualitative data and arithmetic means as a central tendency measurement. T-test compared between 2 groups and Chi square test compared between more than 2 groups. *P*-value < 0.05 was considered significant.

Results

Out of 90 patients suffered from CRD and undergoing hemodialysis, 12 (13.3%) were infected with *Cryptosporidium* spp. as identified by cold acid-fast stain. Ages ranged from 15 to 75 years (45.5±13.9) of both sexes; 34.4% females and 65.6% males. Higher infection rate was in males aged ≥45 years. As to residence, 43.3% were from urban areas and 56.7% were from rural ones, without significant difference as to age (p=0.34), sex (p=0.572), and/or residence (p=0.617). Most *Cryptosporidium*-positive patients displayed at least one of the characteristic gastrointestinal symptoms; diarrhea (100%), abdominal pain (100%), nausea (66.7%), and vomiting (58.3%), frequency of diarrhea and abdominal pain was without significant (p>0.05). As to chronic diseases; 65.6% were diabetic, 58.9% hypertensive, and 36% were diabetic and hypertensive. Hypertension showed sig-

nificant difference with *Cryptosporidium* infection (P =0.013), but diabetes mellitus did n't show significant difference (P=0.572). The hemodialysis duration ranged from 3 to 10 years with a mean of 3.4 years in cryptosporidiosis positive patients, with significant difference ($p<0.05$). Details were given in tables (1, 2 & 3) and figure (1).

Table 1: Comparison between *Cryptosporidium* infections as to clinical manifestations

Clinical manifestations	Positive cases (no=12) No. %	Negative cases (n=78) No. %	*P-value*
Bowel habits: constipation	0 (0.0%)	20 (25.6%)	0.047*
diarrhea	12 (100.0%)	58 (74.4%)	
Abdominal pain: Yes	12 (100.0%)	57 (73.1%)	0.040*
No	0 (0.0%)	21 (26.9%)	
Nausea: Yes	8 (66.7%)	41 (52.6%)	0.361
No	4 (33.3%)	37 (47.4%)	
Vomiting: Yes	5 (41.7%)	26 (33.3%)	0.572
No	7 (58.3%)	52 (66.7%)	

*Significant ($p< 0.05$)

Table 2: Comparison between *Cryptosporidium* infection and chronic diseases

Items	Positive cases No. %	negative cases No. %	*P-value*
Hypertension: Yes	11 (91.7%)	42 (53.8%)	0.013*
No	1 (8.3%)	36 (46.2%)	
Diabetes mellitus: Yes	7 (58.3%)	52 (66.7%)	0.572
No	5 (41.7%)	26 (33.3%)	

*Significant ($p< 0.05$)

Table 3: Comparison between *Cryptosporidium* infection as to hemodialysis duration and kidney function tests

Variable items		No.	Mean	SD	Minimum	Maximum	*P-value*
Hemodialysis (years)	Negative	78	4.51	3.2	5.00	10.00	0.005*
	Positive	12	3.40	0.7	3.00	5.00	
Urea (mg/dl)	Negative	78	116.56	38.9	66.00	245.00	0.003*
	Positive	12	163.30	57.1	99.00	245.00	
Creatinine (mg/dl)	Negative	78	7.75	1.6	5.10	9.03	0.015*
	Positive	12	8.90	0.9	6.80	9.90	

*Significant ($p< 0.05$)

Discussion

In the present study, patients underwent hemodialysis, 13.3% (90) were cryptosporidiosis infected. Ali *et al.* (2000) in Zagazig reported 15% (120) *Cryptosporidium* spp. among hemodialysis patients. In Egypt, El Nadi and Taha (2004) in Sohag reported cryptosporidiosis (48%) among hemodialysis patients. El-Kady *et al.* (2018) in Qena reported high protozoa prevalence mainly *Cryptosporidium* (40%) among hemodialysis cases. El-Badawy *et al.* (2021) in Benha reported a low cryptosporidiosis rate (7%) in the CRD patients.

Abroad, Kulik *et al*. (2008) in Brazil and Karadag *et al*. (2013) in Turkey reported rates of 4.7% (86) & 2.1% (142) among patients respectively. Gill *et al.* (2013) in Brazil reported that 51.6% of CRD patients were intestinal parasites infected mainly *Cryptosporidium* (24.5%) in CRD patients. Omrani *et al.* (2015) in Iran found *Cryptosporidium* infection rate of 11.5% (85) in CRD patients. Hawash *et al.* (2015) in Saudi Arabia detected cryptosporidiosis in CRD-patients versus controls was 8% & 6% respectively. Tamomh *et al.* (2021) in Sudan reported cryptosporidiosis among 12.5% (112) hemodialysis patients. Differences of prevalence results might be due to difference in the climatic conditions, hygienic factors, and/or sample sizes (Zueter *et al,* 2019).

In the present study, ages ranged from 15 to 75 years (45.5±13.9) of both sexes; 34.4% females and 65.6% males, without significant difference among hemodialysis patients, with a higher rate infection in males ≥45 years. Higher prevalence rates in males might be due to outdoors hand working activities. This agreed with Turkcapar *et al*. (2002), and Omrani *et al.* (2015). Besides, El-Kady *et al*. (2018) reported that age and sex were

not significance among CRD patients. In the present study, 43.3% of patients were from urban areas and 56.7 % were from rural ones without significant difference. This agreed with El-Kady *et al*. (2018) reported that residence didn't show significant difference as to infection prevalence among CRD patients. However, high infection prevalence was among CRD patients in the rural community and low-to-middle countries; regardless age and/or sex was reported (Sumaili *et al,* 2009; Varma *et al,* 2010; Salve *et al,* 2012; Stanifer *et al,* 2014). Kaze *et al.* (2015) explained that this may be due to less awareness of CRD patients to communities' risk factors.

In the current study, the common symptoms were diarrhea and abdominal pain with significant difference for both, but there was no significant association for nausea and vomiting. This agreed with Mohaghegh *et al.* (2017) who reported that hemodialysis patients infected with *Cryptosporidium* suffered from diarrhea and abdominal pain, but without significant difference as to vomiting, and/or nausea. El-Kady *et al.* (2018) found a statistical significance between the cryptosporidiosis and diarrhea in dialysis patients. El-Badawy *et al.* (2021) reported that diarrhea was present in 40.3% of hemodialysis patients without significant difference and that intestinal parasite was more abundant in them than in controls. Zueter *et al.* (2019) found that gastrointestinal symptoms were in most *Cryptosporidium*-positive hemodialysis patients, including nausea (24%), abdominal pain (23%), and diarrhea (15%) without significant association ($P>0.05$). Besides, Shehata *et al.* (2019) didn't find significant association between diarrhea and cryptosporidiosis. Hawash *et al.* (2015) reported that the main symptoms in hemodialysis patients were abdominal pain (100%), nausea/vomiting (60%), but without significant differences. Kulik *et al.* (2008) didn't find association between parasites and diarrhea among hemodialysis patients. Differences in gastrointestinal manifestations could be due to differences in immunity level, uremic toxins concentrations, other metabolic comorbidity, drugs and psychosocial problems that led to symptoms as diarrheal severity (Strid *et al,* 2002; Dong and Guo, 2010).

In the present study, there was a significant difference in prevalence of *Cryptosporidium* and hypertension but not with diabetes. Hypertension with edema and dyspnea were significantly high among CRD patients (Stanifer *et al,* 2014). Hypertension and diabetes were significantly high in prevalence and incidence of CRD (Levey *et al,* 2016). Diabetes cause chronic renal disease affecting immune system and led to other organs simultaneous impairment (El-Tawdy *et al,* 2016). El-Askary *et al*. (2021) showed that diabetes was significantly more prevalent (53%) in hemodialysis patients compared to controls ($P= 0.044$). But, Amin *et al.* (2021) didn't find significance between cryptosporidiosis and diabetic patients.

In this study, hemodialysis duration showed significant difference ($p<0.05$), all *Cryptosporidium* positive cases underwent hemodialysis within 3-5 years (±3.4 years). Sharaf *et al.* (2021) on pediatric patients underwent hemodialysis reported dialysis duration (±5.2 years) in *C. parvum* positive cases. Mohaghegh *et al.* (2017) and El-Kady *et al.* (2018) reported that hemodialysis patients infected with *Cryptosporidium* didn't correlate with dialysis duration.

The present results showed that CRD patients were infected with *Cryptosporidium* showed higher serum levels of urea and creatinine compared to non-infected ones with significant difference. Increased blood urea was due to immune system more susceptibility to infections with increased incidence of co-morbidity and mortality (Kato *et al,* 2008).

Conclusion

Cryptosporidium causes high prevalence of diarrhea in immunosuppressed CRD patients. Treatment of diarrhea among the CRD patients is a must to avoid life threatening.

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Explanation of figure

Fig. 1: *Cryptosporidium* oocysts by cold acid-fast stain (X1000).