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 activation (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-compromised 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
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 kidney 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.

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-
significant 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

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>Positive cases (n=12)</th>
<th>Negative cases (n=78)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel habits: constipation diarrhea</td>
<td>0 (0.0%)</td>
<td>20 (25.6%)</td>
<td>0.047*</td>
</tr>
<tr>
<td>Abdominal pain: Yes</td>
<td>12 (100.0%)</td>
<td>57 (73.1%)</td>
<td>0.040*</td>
</tr>
<tr>
<td>No</td>
<td>0 (0.0%)</td>
<td>21 (26.9%)</td>
<td></td>
</tr>
<tr>
<td>Nausea: Yes</td>
<td>8 (66.7%)</td>
<td>41 (52.6%)</td>
<td>0.361</td>
</tr>
<tr>
<td>No</td>
<td>4 (33.3%)</td>
<td>37 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>Vomiting: Yes</td>
<td>5 (41.7%)</td>
<td>26 (33.3%)</td>
<td>0.572</td>
</tr>
<tr>
<td>No</td>
<td>7 (58.3%)</td>
<td>52 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant ($p<0.05$)

Table 2: Comparison between Cryptosporidium infection and chronic diseases

<table>
<thead>
<tr>
<th>Items</th>
<th>Positive cases</th>
<th>Negative cases</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension: Yes</td>
<td>11 (91.7%)</td>
<td>42 (53.8%)</td>
<td>0.013*</td>
</tr>
<tr>
<td>No</td>
<td>1 (8.3%)</td>
<td>36 (46.2%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus: Yes</td>
<td>7 (58.3%)</td>
<td>52 (66.7%)</td>
<td>0.572</td>
</tr>
<tr>
<td>No</td>
<td>5 (41.7%)</td>
<td>26 (33.3%)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant ($p<0.05$)

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

<table>
<thead>
<tr>
<th>Variable items</th>
<th>Negative</th>
<th>Positive</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemodialysis (years)</td>
<td></td>
<td></td>
<td>0.005*</td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>4.51</td>
<td>3.2</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>3.40</td>
<td>0.7</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td></td>
<td></td>
<td>0.003*</td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>116.56</td>
<td>38.9</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>163.30</td>
<td>57.1</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td></td>
<td>0.015*</td>
</tr>
<tr>
<td>Negative</td>
<td>78</td>
<td>7.75</td>
<td>1.6</td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>8.90</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*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). Diabet es 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.
References


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Explanation of figure

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