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CYCLOSPORA INFECTION IN RENAL TRANSPLANT RECIPIENT

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Abstract

Cyclospora cayetanensis (C. *cayetanensis*) can cause serious diarrheal illness in immunocompromised patients. The present work aimed to detect C. *cayetanensis* infection among patients with renal transplantation attending the nephrology unit of Kasr Al-Aini, Faculty of Medicine, Cairo University. A total of 50 stool samples were collected and subjected to direct microscopy to screen for parasitic stages. A modified acid fast staining technique (Kinyoun's method) was used to detect C. *cayetanensis* oocysts. *Cyclospora* oocysts were revealed in 5 (10%) of the stool samples examined. Other parasites detected among the patients included *Cryptosporidium parvum* 5 (10%) and *Blastocystis* 15 (30%). 30% of the patients were suffering from diarrhea and or colic. All C. *cayetanensis* positive cases were presenting with diarrhea.

Keywords: *Cyclospora cayetanensis*, renal transplant recipients, modified aid fast

Introduction

Intestinal protozoan infections are detected more commonly in immunocompromised hosts, especially those with disturbed T-cell function. The immune response to these infections is complex and pathogenesis in humans is poorly understood (Marcos and Gotuzzo, 2013). C. *cayetanensis* is an obligate intracellular sporulating coccidian protozoan parasite inhabits epithelial cells of upper small intestine (Li *et al*, 2019). Cyclosporiasis occurs most commonly in tropical and subtropical regions (Hall *et al*, 2011). The parasite produces environmentally resistant unsporulated oocysts, which are shed in feces of the infected persons and can take several weeks to become fully sporulated and infectious. So, person to person transmission is unlikely (Mansfield and Gajadhar, 2004).

The main risk factors for acquiring the infection are linked to the consumption of oocysts in contaminated water and food produce. The fresh fruits, herbs and vegetables (blackberries, raspberries, basil, and lettuce) are foods most commonly identified as a source of human infection (Herwaldt and Beach, 1999; Mansfield and Gajadhar, 2004, Hoang *et al*, 2005; Abanyie *et al*, 2015).

Cyclosporiasis is manifested by profuse watery diarrhea, vomiting, nausea, anorexia, fatigue, weight loss, flatulence, and abdominal cramping. Cyclosporiasis respond well to trimethoprim and sulfamethoxazole treatment, whereas, untreated cases can have remitting relapsing disease for several weeks and months (Ortega and Sanchez, 2010)

Host susceptibility has been suggested as the most important factor that influences the course of cyclosporiasis A prolonged sever course of infection has been reported in immunocompromised individuals (Mathur *et al*, 2013; Bednarska *et al*, 2015)

The present study aimed to detect *Cyclospora* infection in stool samples from patients with renal transplantation.

Materials and methods

Study setting and sampling: This work is a cross sectional study performed in the period from October 2018 to July 2019 on 50 subjects of both sexes with transplanted kidney attending the Nephrology Unit of Kasr Al-Aini, Faculty of Medicine, Cairo University. A total of 50 stool samples were collected in labeled, leak-proof, dry and clean plastic stool containers then brought to the laboratory immediately. A data collection sheet was obtained with each sample. Data and sample collection were performed after obtaining their consent. The study was done in the Medical Parasitology Department, Faculty of Medicine, Cairo University. Stool samples were subjected to the following:

Direct wet smear and concentration techni-

que: Stool samples examined using direct wet smear and formalin-ethyl actate sedimentation methods for ova and other parasitic stages (Garcia, 2007).

Permanent staining technique: Modified acid fast staining technique (Kinyoun's method) was conducted for the detection of *C. cayetanensis* oocysts. Briefly, thin stool smears were prepared and dried. Smears were fixed with absolute methanol for 1 min and then flooded with Kinyoun's carbol fuchsin stain for 5min, and rinsed briefly with tap water. Stained smears were decolorized with 0.5% acid-alcohol for 2 min, counterstained with 1% methylene blue for 1 min, washed and air dried. Slides were examined for *Cyclospora* oocysts and other coccidian parasites by light microscopy using oil immersion lens for at least 10min (Weber *et al,* 1992).

Statistical analysis: Data were analyzed by using statistical package SPSS version 23. Numerical data were summarized as mean & standard deviation. Qualitative data were summarized using frequency and percentage

Results

A total 50 fecal samples were collected from renal transplant recipients with a mean age of 42 years. Of these 29 (58%) were males and 21 (42%) were females (Tab. 1). Difference was non-significant (*P* value > 0.05). Creatinine level ranged from 2 to 8 with a mean of 3.2.

Table 1: Age distribution in relation to sex in studied group

Sex	no	%	Mean of age	Std deviation
Male	29	58%	42.96	15.88
Female	21	42%	41.95	16.24
Total	50	100%	42.54	15.87

Out of 50 renal transplant recipients, 15 (30%) suffered from diarrhea, 15 (30%) from colic, and 5 (10%) from fever. Out of 15 diarrheic patients, 5 had chronic diarrhea (Tab. 2). All *Cyclospora* positive cases have diarrhea.

Table 2: Clinical manifestations in studied group

Symptoms	Total number (n= 50)	Cyclospora (n=5)
	Frequency [n. (%)]	Frequency [n. (%)]
Diarrhea	15 (30%)	5 (100%)
Colic	15 (30%)	0 (0%)
Fever	5(10%)	0 (0%)

Macroscopic examination showed formed consistency in (60%), soft in (30%) and loose in (10%). Microscopic examination detected *Blastocystis* forms in 15 (30%); *Cyclospora* oocysts in 5 (10%) and *Cryptosporidium* oocysts in 5 (10%). Among 5 *Cyclospora* positive stool samples, 2 had co-infection with *Blastocystis* (Tab. 3, Fig, 1) .

Table 3: Intestinal parasites among renal transplant recipients

Protozoa parasites	Frequency [n. (%)]
Blastocystis	15 (30%)
Cyclospora oocysts	5 (10%)
Cryptosporidium oocysts	5 (10%)

Discussion

Protozoa are major pathogens that cause intestinal infection in immunocompromised patients due to their impaired cellular immunity, including transplant patients (Ferreira and Borges, 2002; Azmi *et al*, 2010). *Cyclospora* infection is among a group of renal transplant recipients as one of the major sectors of organ recipients in Egypt (Saadi *et al,* 2016). cyclosporiasis is often difficult to diagnose and may be overlooked as a cause of diarrhea in such patients.

The present study revealed that *Cyclospora* was responsible for diarrhea in 5 cases (10%), with neither colic nor fever in these patients. Also, two of them had coinfection with *Blastocystis,* the latter being the most prevalent protozoan infections (15%).

In the current study, cryptosporidiosis had the same frequency as cyclosporiasis (10%). *Cryptosporidium* is one of the most common protozoa infections in transplant recipients in general, with worldwide incidences up to 38% (Udgiri *et al*, 2004; Raja *et al*, 2014; Yadav *et al*, 2016). Concurrent infection of both *Cryptosporidium* and *Cyclospora* is quite common (Ortega and Sanchez, 2010; Bhandari *et al*, 2015). The presence of multiple protozoa infections in those patients highlights the results of several previous reports which stated that intestinal parasitic infections in immunocompromised patients was related to prevalence of intestinal parasitism in their respective localities (Meamar *et al*, 2007; Valar *et al*, 2007, Azmi *et al*, 2010). No relation was found between sex or kidney functions infected with *Cyclospora*.

Little data on the prevalence of cyclosporiasis in renal transplant recipients is available as reports on this association are generally sparse. However, the present study showed that *Cyclospora* is a relatively common pathogen among recipients of kidney transplants. Kilbas (2009) in Turkey described the detection of *Cyclospora* in one renal transplant recipient. Also, Azmi *et al.* (2010) stated that none of the investigated transplant patients with diarrhea had cyclosporiasis in Iran. But, the parasite itself was rarely distributed there (Rezaian *et al*, 2000). One case of cyclosporiasis was reported in the USA in a renal transplant recipient, who was originally from the Dominican Republic (Visvesvar *et al*, 2013). Bednarska *et al.* (2015) reported a case of cyclosporiasis in a renal transplant recipient that was acquired while traveling in Asia. Yadav *et al.* (2016) in India reported cyclosporiasis infection rate of 5% among transplant patients. Undoubtedly, cyclosporiasis has a less global prevalence in the immunosuppressed compared to other coccidian protozoa (Kulkami *et al*, 2009).

It's noteworthy that latest diagnostic recommended the use of microscopy for stained slides. The auto-fluorescence and gastrointestinal multiplex molecular assays were recommended that reveal even a higher infection rate in transplant patients, and others (La Honz *et al*, 2019).

Conclusion

Opportunistic protozoa infections including cyclosporiasis appear to be high in renal transplant recipients in Egypt. It is advised to perform stained stool analysis using for proper rapid detection and management, thereby helping with decreased postoperative morbidity and achieving quick recovery. It is recommended to use immunodiagnostic and molecular diagnostic techniques when available.

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Explanation of figure

Fig. 1: Parasites detected in stool samples of the renal transplanted patients: A: *Cyclospora* oocysts stained with modified acid fast stain (x 1000). Oocysts between 8 to 10µm, with variable staining characteristics from light pink to deep red, appeared round and wrinkled. B: *Cryptosporidium* oocysts stained with modified acid fast (x 1000), C*: Blastocystis* by direct smear microscopy (x 400)