

Original paper

Cryptosporidium infection among hemodialysis patients attending to the dialysis center at Kosti Teaching Hospital, Sudan

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ABSTRACT. Cryptosporidiosis is an illness caused by a protozoan parasite *Cryptosporidium*. *Cryptosporidium* species are an opportunistic pathogens cause a diarrheal disease worldwide, and can be more severe in immunocompromized patients. Until now, a little data have been available on its prevalence rate among haemodialysis patients in Sudan. Therefore, this article was designed to examine the prevalence of *Cryptosporidium* among hemodialysis Sudanese patients attending hemodialysis center at Kosti Teaching Hospital. A case-control study including one-hundred and twelve hemodialysis patients between November 2016 and January 2017 have been conducted. For the control group, we include one-hundred and twelve normal population. A total of two-hundred and twenty-four stool samples were collected. The stool samples were processed and examined using the modified Ziehl-Neelsen (ZN) staining method. High *Cryptosporidium* prevalence of 14/112 (12.5%) was detected in hemodialysis patients compare to the normal individuals 3/112 (2.7%). There was no correlation between the prevalence of *Cryptosporidium* infection with the age, sex, and the duration of dialysis ($P>0.05$). Therefore, an early detection and prompt treatment of *Cryptosporidium* infected hemodialysis patients is crucial.

Keywords: prevalence, cryptosporidiosis, hemodialysis patients, Kosti, Sudan

Introduction

Cryptosporidium species are protozoan coccidian parasites that cause cryptosporidiosis characterize by watery diarrhoea, especially among immunocompromised patients [1–4]. Contaminated untreated water (not boiled or filtered), as well as contaminated recreational water sources including lakes, rivers, swimming pools, ponds, and streams [2,5–8].

Globally, the disease caused by the intestinal coccidian parasite *Cryptosporidium*, that infects humans. In immunocompetent individuals usually a self-resolving from the symptoms can occur.

Hemodialysis patients are at more danger and life-threatening from dehydration which resulting from severe diarrhoea [9–12]. Recently, the awareness about *Cryptosporidium* infection was increasing especially among organ transplantation, renal dialysis patients, HIV, and malignancies.

In fact, many molecular and immunological assays have been tested for the sensitive diagnosis of cryptosporidiosis, but still, the microscopic detection of the parasite oocysts in stool samples smears stained by modified acid stain is considered as a reliable, sensitive and specific technique that widely used in many clinical diagnostic laboratories. The *Cryptosporidium* oocysts in unconcentrated stool

smears can be easily detected by phenol–auramine and/or acid-fast staining techniques [1,2,13–17].

In the low- and middle-income countries, the parasite was gradually linked with malnutrition and death caused by diarrhea and dehydration [5,6,18–22]. Cryptosporidiosis is of great health concern because of its economic losses association and the public health importance significance in humans. *Cryptosporidium* spp. in low resource developing countries are the second cause of diarrheal diseases and life-threatening in immunocompromised patients, and until now, no effective treatment is available [7,9,10,23–29].

Hemodialysis patients are immunocompromised and at high risk of acquiring cryptosporidiosis compare to other patient hospitalize individual. Usually, hemodialysis patients suffer from prolong diarrhea and dehydration episode. During treatment and follow up of *Cryptosporidium* infection the disease is neglected among these patients. These patients are suffering from chronic renal failure, to resolve the case transplantation is best since the usage of immunosuppressive drugs for long time is the must. Chronic renal failure patients are associated with uremic toxin which is significant factor responsible for the high mortality and morbidity rates. There are few published or available data on the prevalence rate of *Cryptosporidium* infection among hemodialysis patients in Sudan. In the current study, we aim to determine the prevalence rate of *Cryptosporidium* infection among hemodialysis patients attending to the dialysis center, in Kosti Teaching Hospital, Sudan.

Materials and Methods

Study area

The study was conducted at Abbas Ibrahim Center for Dialysis and the clinical laboratory section of Kosti Teaching Hospital, Sudan. This Hospital serves large customers from Kosti town and its surrounding areas or cities in the White Nile State. Kosti town is one of Sudan cities which lies south of the capital Khartoum, and sits opposite to Rabak city at the western area of the White Nile river where there is connected with a bridge. Kosti city health services: one teaching hospital, three rural hospitals, thirty-four health units, twenty health centers, and four medical clinics. The most essential or main water resource is rainwater and the White Nile river. Most activities are agriculture,

fishing, trade and grazing [15].

Study design

This study is a hospital-based case-control study conducted at the dialysis center in Kosti Teaching Hospital between November, 2016 and January, 2017. A total of 112 participants in the dialysis center were included in the study. For the control group, we include one-hundred and twelve normal individuals. A consecutive sampling method was performed, and the duration of dialysis was recorded. A total of two-hundred and twenty-four stool samples were collected. All stool samples were examined and analyzed using the modified Ziehl-Neelsen (ZN) staining method and then examined for *Cryptosporidium* infection microscopically.

Stool samples collection, handling and storage

All participants were given labelled or marked stool containers to collect or gather one stool sample at the time of collection. With a suitable gloves the stool containers were handled. The samples were examined or checked firstly macroscopically. Fresh stool samples were stored in the refrigerator at the clinical lab, of Kosti Teaching Hospital until used.

Direct wet preparation method

The detection of *Cryptosporidium* parasite in stool specimen was done according to described previously [12] in which in a clean microscopic slide, a one drop of physiological saline was placed and by using a wooden stick, a suitable size of sample (2 mg), was added and mixed to prepare thin preparation, then the cover glass was used to cover the preparation, and microscopically examined using 10× and 40× objectives.

Formol-ether concentration method

The method was performed as described previously [12], in which about 1 g of stool sample placed in a clean container; about 5 ml of 10% formol saline was added, and then sieved. The sieved stool sample was centrifuged after the sample transferred to centrifuge tube, then diethyl ether added, gently shaken for seconds, and centrifuged, the faecal debris removed, and the supernatant discarded. The remaining sediment mixed and transferred to a clean microscopic slide, covered with cover glass and examined microscopically.

Table 1. Overall prevalence of cryptosporidiosis among hemodialysis Sudanese patients and the control group using different parasitological techniques

Groups	Methods	Direct wet preparation method +ve/n (%)	Formol-ether concentration method +ve/n (%)	Modified ZN staining method +ve/n (%)
Hemodialysis patients		06/112 (5.4)	09/112 (8.04)	14/112 (12.5)
Control group		00	01/112 (0.9)	03/112 (2.7)

Table 2. *Cryptosporidium* infection among hemodialysis Sudanese patients and the control group using mZN staining method

Groups	Positively infected/n (%)	<i>P</i> -value
Hemodialysis patients	14/112 (12.5)	0.006
Control group	03/112 (2.7)	

Table 3. *Cryptosporidium* infection among hemodialysis Sudanese patients and the control group according to the age

Age (years)	Hemodialysis patients +ve/n (%)	Control group +ve/n (%)	<i>P</i> -value
<20	2/10 (20)	0/9 (0)	
21–40	3/28 (10.7)	0/30 (0)	
41–60	4/33 (12.1)	1/29 (3.4)	0.238
>60	5/41(12.2)	2/44 (4.5)	
Total	14/112 (12.5)	3/112 (2.7)	

Modified Ziehl-Neelsen (mZN) staining method

The stool samples were handled from the fridge, thawed at room temperature, smeared on microscope slides and were air dried. All smears were stained according to the method previously described [1].

Statistical analysis

Data was registered and analyzed using the Chi-square test, Student's *t* tests, and ANOVA by a statistical package for social science (SPSS version 21) program. $P < 0.05$ was considered significant for all statistical analyses.

Ethical considerations

The study was approved by the Institutional Ethics Committee of Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi. Informed consent from both patients and control groups was obtained.

Results

A total of 224 participants were enrolled in this study in which 112 of them were dialysis patients with the mean age of 42.2 (SD=13.6) years, and the other 112 participants were apparently healthy as control group with the mean age of 40.9 (SD=12.5) years. Seventy-two (64.3%) of dialysis patients, and seventy-six (67.9%) of control groups were males. Also, a total of 40 (35.7%) of dialysis patients, and 36 (32.1%) of control group were females. The duration of dialysis mean was 21.5 months.

The overall prevalence rate of cryptosporidiosis among hemodialysis Sudanese patients was, 06/112 (5.4%), 09/112 (8.04%), and 14/112 (12.5%), using direct wet preparation, formol-ether concentration, mZN staining methods respectively and the prevalence rate of cryptosporidiosis among normal individuals was 0, 01/112 (0.9%), and 03/112 (2.7%) using direct wet preparation, formol-ether concentration, mZN staining methods respectively, as shown in table 1. The differences between the

Table 4. *Cryptosporidium* infection among hemodialysis Sudanese patients and the control group according to the gender

Gender	Hemodialysis patients +ve/n (%)	Control group +ve/n (%)	P-value
Male	10/72 (13.9)	2/76 (2.6)	0.157
Female	4/40 (10)	1/36 (2.8)	
Total	14/112 (12.5)	3/112 (2.7)	

Table 5. *Cryptosporidium* infection among hemodialysis Sudanese patients according to the duration of dialysis

Duration of dialysis (months (n))	Positive/n (%)	P-value
<12	6/63 (9.5)	0.372
13–24	1/10 (10)	
25–36	2/5 (40)	
37–48	1/12 (8.3)	
49–60	1/8 (12.5)	
>60	3/14 (21.4)	

two groups in the prevalence rates of *Cryptosporidium* infection were statistically significant ($P < 0.05$), as explained in table 2.

In the data presented in table 3 and table 4 show there is no association or correlation between the presence of infection with *Cryptosporidium*, age, and sex among hemodialysis Sudanese patients and the control group. The prevalence of cryptosporidiosis among hemodialysis Sudanese patients according to the duration of dialysis was presented in table 5. There was no correlation between the presence of *Cryptosporidium* infection and the duration of dialysis among hemodialysis Sudanese patients ($P > 0.05$).

Discussion

Cryptosporidiosis is an illness caused by a protozoan parasite *Cryptosporidium*. *Cryptosporidium* species are opportunistic pathogens cause a diarrheal disease worldwide, and can be more severe in immunocompromized patients. *Cryptosporidium* is recognized as a highly and more infectious pathogen with mainly faecal-oral route transmission [1,2]. This present study aimed to examine and determine the prevalence rate of *Cryptosporidium* infection among hemodialysis patients attending the dialysis center at Kosti Teaching Hospital, Kosti city, White Nile State, Sudan. Total 224 stool samples were collected and

examined using direct wet preparation and formal ether concentration technique firstly and then, examined using modified ZN staining technique.

In the current results, the overall prevalence rate of cryptosporidiosis among hemodialysis Sudanese patients was 14/112 (12.5%) attending the dialysis center (Abbas Ibrahim Center for Dialysis) at Kosti Teaching Hospital using the modified Ziehl-Neelsen technique. This result explains a higher prevalence rate compared to the findings reported among hemodialysis compare to control group in Turkey, Guilan province in Iran, and in Isfahan, central Iran [3,6,10] with the prevalence of (2.1% vs 0.0%), (0.0% vs 0.0%), and (3.0% vs 0.7%), respectively. A high prevalence rate have been reported in African countries with high and more rainfall such as Egypt [5], with 40.0%. Our finding is in line with the result conducted in Tabriz, Isfahan, in Iran, and in Irbid city in Jordan, with prevalence rate 11.5%, 11.5%, and 11.0%, respectively, and near to that findings gained in Turkey, and Brazil, 20.27%, 26.4%, respectively [4,7]. In Kosti city there is an improved access to drinkable water, with less faecal contamination rates, and uses of latrine compared to other cities in Sudan these factors may justify this prevalence rates, therefore, limiting and reducing the occurrence of parasitic infections generally and in particularly cryptosporidiosis. Additionally, this low prevalence rate may be expressed by using microscopy in the unavailability of molecular

methods.

In the present findings, a high *Cryptosporidium* oocyst was detected among elder age group (more than 60) 5/14 (35.7%) than the other age groups among hemodialysis patients. This finding or result is in agreement with study carried out in Egypt, with (33.33%) [5], where a higher prevalence rate was observed among hemodialysis patients of the elder ages than youngest. In this age, the high prevalence rate is may be associated with the danger of diarrhoea caused by *Cryptosporidium*, and also, for the reason that may be related to the weak immune system of their body [1,12].

Our relevant investigation expressed that the prevalence rate of cryptosporidiosis among hemodialysis Sudanese patients and control group was a higher increase in males (13.9% vs 2.6%) than in females (10% vs 2.8%) respectively. The reason for these variations is not obvious since they are similar in situation as immunocompromised patients, but probably due to the less degree of the females' activity and they are mostly at home. This result is comparable to the results obtained in Egypt, and Iran [5,15].

Further investigations explained that there is no significant correlation between the prevalence rate of *Cryptosporidium* and the duration of the dialysis ($P>0.05$). This finding is in the line with the study reported in Egypt, and Iran [5,15].

The overall prevalence of cryptosporidiosis among hemodialysis Sudanese patients attending to the dialysis center at Kosti Teaching Hospital was 12.5%. *Cryptosporidium* is an opportunistic and essential causative agent of diarrhea among immunocompromised patient such as hemodialysis patients, therefore, it is necessary to examine all hemodialysis patients attending to the dialysis center for *Cryptosporidium*. The early detection and investigation of cryptosporidiosis among hemodialysis patients may expect their health level or status, resulting in a better investigation, therapy, and, thus, public health status improvement. Continuous programs of personal hygiene and health education must be held to learn the population how to avoid cryptosporidium infection.

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