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Full Length Research Paper

Prevalence of Cryptosporidiosis and Intestinal Parasites among Immunocompromised Patients in Khartoum State- Sudan

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This cross-sectional study was carried out in Khartoum state, Sudan during the period from September 2013 to August 2016. The study conducted on 384 patients, 179 were males and 205 were females, with the mean age was 36±12yeas old. Stool samples were taken from all immunocompromised patients; all samples were examined for *Cryptosporidium* spp using wet preparation and Formal ether concentration technique. The study showed that the overall prevalence of *Cryptosporidium spp* was (19.8%). The prevalence of *Cryptosporidium spp* increased gradually with age reaching (8.9%) among the age group 15- 30 years old followed by (5.7%)among age group 31- 45 years old and (5.2%)among age group 46 - 60 years old. From the study, 7 species of intestinal parasites were detected in 266 (69.2%) stool samples, *G. lamblia* were 134 (34.9%), *E. histolytica* were 54 (14.1%), *H. nana*were38 (9.9%), *S. mansoni* were12 (3.1%), *Taenia spp*. was21(5.5%), *T. trichiura* were 1 (0.3%) and *S. stercoralis* were 6 (1.6%).

Keywords: Cryptosporidiosis, Intestinal parasites, Immunocompromised.

INTRODUCTION

Cryptosporidiosis is zoonotic disease caused by protozoan parasite of genus *Cryptosporidium*, human cryptosporidiosis caused mainly by *C. hominis, C. parvum*, other species as *C. meleagridis, C. felis* or *C. canis* also are reported, in 2004 WHO defined *Cryptosporidium* as

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neglected disease (Julius et al., 2015; Vohra et al.,2012). Transmitted through oral- fecal route, in immunocompetent individual may be a symptomatic or self-limited, but it's fetal in immunocompromised patients as human immunodeficiency virus (HIV), patients with hepatitis B virus (HBV), malignancy and tuberculosis patients (Curry et al., 1991; Yang et al., 2017).

Cryptosporidiosis is worldwide distributed especially in developing countries which prevalent about (5% -10 %) (Vohra et al., 2012). About 500 million people affected annually and most of them in Africa (Julius et al., 2015). Cryptosporidiosis has three major clinical presentations in both immunocompetent and immunocompromised patients, asymptomatic carrier, acute diarrhea, and persistent diarrhea, in people infected with (HIV), people with malignancies and patients with solid-organ transplants and hemodialysis patients *Cryptosporidium* spp. may cause severe clinical gastrointestinal disorders (Curry et al., 1991). Its prevalence has been reported to be 5-50% among patients with AIDS and it is also an important cause of AIDS-associated deaths due to severe diarrhea (Rafiei et al., 2014).

MATERIALS AND METHODS

This was cross sectional study carried out in Khartoum State, Sudan. The samples were collected from different hospitals and health centers, during the period from September 2013 to August 2016. Immunocompromised patients, including HIV, TB, Cancer (under immunosuppressive chemotherapy) and renal transplant patients who were laboratory and clinically diagnosed were enrolled in this study, apparently healthy and immunocompromised people who their ages were less than 5 years or more than 60 years were excluded from this study.

Ethical consideration

The study was approved by the ethical committee of Medical Laboratory Science- Alzaiem Alazhari University. The participants were informed about the purpose of the study before sample collection and verbal or sign agreement was taken from them.

Sampling technique and Sample size

Non probability sampling technique, the study were based on case study, so the samples was calculated according the following formula:

$N = (z^2 \times p \times q) / d^2$ d: degree of accuracy according to the expected prevalence = (0.05)

N: Sample size. z: stander deviation (1.96). q=1-p

p: prevalence among target group (taken as 50 %). $N= (1.96)^2 \times 0.5(1 - 0.5) / (0.05)^2 = 384$ samples.

Data collection

The relative information relates to study such as age, gender, stool consistency and contact with animals were collected by a structured questionnaire.

Stool collection and processing

A single fresh stool sample was collected with leak proof and tightly cupped and labeled from each patient, some of stool was preserved with 10% formal saline and the remaining part was processed using the following methods:

Direct Microscopy (Wet mount)

A direct wet mount with normal saline (0.85% NaCl solution) was prepared from each sample and examined for the presence of motile intestinal parasites and trophozoites under light microscope using 10x and 40x magnification. Lugol's iodine staining was also used to observe cysts of intestinal parasites (Wegayehu et al., 2013).

Formalin-Ether concentration technique

Using an applicator stick, about ~5g of preserved stool sample was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin. The sample was mixed thoroughly with the applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. After adding 3 ml of diethyl ether to the mixture and shaking, the content was centrifuged at 500 x g for 10 min. The supernatant was discarded and iodine stain preparation was made using the sediment and examined under a microscope using 10x and 40x magnifications. Microscopic examinations were done. Stool samples was screened for co-infection with other intestinal parasites (Adamu and Petros, 2009).

Data analysis

Statistical analysis was done using the SPSS (version 21.0 statistical software). Data were summarized using frequency tables. Contingency tables were used and the strength of association was measured using the chi-square and its associated *P*-value. Values were considered to be statistically significant when the *P*-value obtained was less than 0.05.

Table (1): Distribution of the study population (n=384) according to gender.

Gender	Immunocompromised group				Total
	HIV	**TB**	**Ca**	**RT**	
Male	60	43	45	31	179
Female	70	47	45	43	205
Total	130	90	90	74	384

Table (2): distribution of the study population (n=384) according to age group, gender and Immunocompromised group.

Agegroup years	Gender		Immunocompromised group				Total
	Males	females	HIV	TB	Ca	RT	
15-30	74 (19.3%)	74 (19.3%)	36 (9.4%)	41 (10.7%)	45 (11.7%)	26 (6.8%)	148 (38.5%)
31-45	73 (19%)	72 (18.8%)	48 (12.5%)	32 (8.3%)	31 (8.1%)	34 (9.8%)	145 (37.8%)
46-60	32 (8.3%)	59 (15.4%)	46 (12%)	17 (4.4%)	14 (3.6%)	14 (3.6%)	91 (23.7%)
Total	179 (46.6%)	205 (53.4%)	130 (33.9%)	90 (23.3%)	90 (23.3%)	74 (19.3%)	384 (100%)

RESULTS

Among the patient participants in study, 130 (33.9%) were HIV sero-positive individuals, 90 (23.4%) were confirmed TB patients, 90 (23.4%) were with malignancies, 74 (19.3%) were post renal transplant patients (Table 1), fecal samples were collected from patients with and without diarrhea during the study period. A total of 179 (46.6%) of study papulation were males and 205 (53.4%) were females (Table 1), the mean age of the study participants was 36±12 years (Table 2).

Out of the 130 HIV sero-positive individuals, 60 (46.1%) were males, 70 (53.9%) were females, from 90 confirmed tuberculosis individuals 43(47.8%) were males, 47 (52.2%) were females, out of 90 patients with malignancies (under immunosuppressive chemotherapy) 45 (50%) were males, 45 (50%) were females, out of 74 post renal transplant patients 31 (41.9%) were male and 43 (58.1%) were females. (Table 1)

Frequency of *Cryptosporidium spp*. according stool consistency:

According to stool consistency of the patients was recorded as follows: diarrhea in 49 (12.8%) patients, soft consistency in 160 (41.7%), formed consistency in 175 (45.6%). Among the 76 *Cryptosporidium*-positive 47(12.2%) were had diarrhea, 19 (4.9%) with soft consistency stool, 10 (2.6%) with formed consistency stool. The differences in rate according To *Cryptosporidium* infection was to be statistically significant associated with diarrhea at *P*.value=0.000 (Table 3).

Frequency of *Cryptosporidium species* according to age group:

According to age, oocyst of *Cryptosporidium species* was detected in high in age group 15 – 30 years, 31 - 45 years and 46 – 60 years respectively. The differences in rate was found to be statistically significant with *Cryptosporidium* infection associated with age group at *P*. value>0.05 (Table 5).

Frequency of *Cryptosporidium species* and other intestinal parasites:

According to intestinal parasitological examinations of the stool specimens, 7 species of intestinal parasites were detected in 266 (69.2%) patients. Among the detected intestinal parasites *Giardia lamblia* was the most prevalent. *G. lamblia* 134 (34.9%), *Entamoeba histolytica* 54 (14.1%), *Hymenolepis nana* 38 (9.9%), *Schistosoma mansoni* 12

Table (3): Frequency of *Cryptosporidium spp* according to stool consistency.

Stool consistency	*Cryptosporidium species*		Total
	+ve	-ve	
Diarrhea	47 (12.2%)	2	49
Soft	19 (4.9%)	141	160
Formed	10 (2.6%)	165	175
Total	76 (19.8%)	308	384

Table (4): Frequency of *Cryptossporidium spp* according to gender:

Gender	**Result of *Cryptosporidium spp***		Total
	+ve	**-ve**	
Males	33	146	179
Females	43	162	205
Total	76	308	384

Table (5): Frequency of *Cryptosporidium spp* according to age group:

Age group years	Result of *Cryptosporidium species*		Total
	+ve	**-ve**	
15-30	34	114	148
31-45	22	123	145
46-60	20	71	91
Total	76	308	384

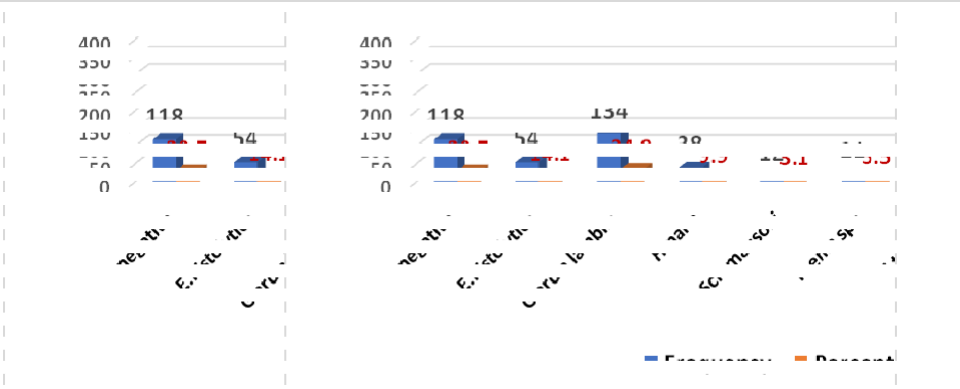

Figure (1): Frequency of otheer intestinal parasite infections among study participants (n= 384)

(3.1%), *Taenia spp*. 21(5.5%), *Trichuris trichhiura* 1 (0.3%) and *Strongyloides stercoralis* 6 (1.6%) (Figuree 1).

Frequency of *Cryptosporidium species* according to animal contact:

Majority of the patients reported no directt and indirect contact with domestic animals. Out of 384 patients, 110 (28.6%) had close contact with animals 25 (32.9%) were positive for *Cryptosporidium.* while274 (71.4%) patients had no contact with the animals, from them 51 (67.1%) were positive for *Cryptosporidium.* The differences in rate was found to be statistically innsignificant *P*.value=0.360 (Table 6).

Table (6): Frequency of *Cryptosporidium species* according to animal contact:

Animal contacted	Result of *Cryptosporidium species*		TOTAL
	+ve	-ve	
Yes	25	85	110
No	51	223	274
Total	76	308	384

P.value = 0.360

DISCUSSION

The overall frequency of *Cryptosporidium* spp. infection detected in this study (19.8%) was higher than that reported from children with diarrhea in White Nile State, Sudan (13.3%) by Sim et al.(2015) and also higher than the rate detected in AIDS patients with chronic diarrhea in Addis Ababa, Ethiopia (7.6%) by Adamu et al. (2010)and also was disagreed with finding obtained by Wanyiri et al (2014)in Kenya who they found that the frequency was (34%).

The high intestinal *Cryptosporidium* spp. frequency within the age group 15 – 30 years in males and in the females was similar to the findings of Adamu et al (2010). The higher prevalence observed in this age group may be partly explained by their frequent consumption of food outside their homes or acquisition of the organism during conducting manual work or contamination with dusty environment.

The study showed that, out of 76 *Cryptosporidium*-positive, 35 (46.1%) were HIV positive, 15 (19.7%) were tuberculosis, 19 (25%) were malignancies and 7 (9.2%) were post- renal transplant. Similar results were reported by Musa et al. (2016) study done in Khartoum State who they found that the highest frequency of infection by the *Cryptosporidium spp.* in the immunocompromised was in the HIV positive patients (96.0%) and the frequency of the positive *C. parvum* in the groups of tuberculosis, renal failure, immunosuppressed patients were 92.5%, 88.3% and 86.6% respectively. Also, these findings disagree with finding obtained by Salehi et al. (2016)in Iran who found that the low prevalence of *Cryptosporidium spp* among HIV patients (0.4%), malignancies patients (1.1%) and renal transplant recipients (0.5%). This may be due to differences in sample size, technique and study area.

Among the 76 *Cryptosporidium*-positive, 43.4% were males and 56.6% were females and this was disagreed with the result of Adamu et al. (2010) in Ethiopia who reported that the C*ryptosporidium*-positive humans were 50.6% in males and 49.4% in females. This may be due to clean of the homestead by females which resulting in inhalation of oocyst of C*ryptosporidium spp.*

The study revealed that, the prevalence of cryptosporidiosis in relation to animal's contact was (32.9%). This finding is not in line with a study made in the United Kingdom by Roy et al*.* (2004)who reported that the different parts of the world that support the association of contact with cattle, this may be due to the risk factors for acquiring cryptosporidiosis was determined to be with other factors.

In this study among the 76 *Cryptosporidium*-positive humans, 47 (61.8%) patients were symptomatic (had diarrhea), these findings disagree with finding obtained by Iqbal et al. (2015) in Malaysia who found that the low frequency (9.3%) in patients with watery diarrhea.

The study was revealed that, the frequency of others intestinal parasites was detected in the study using wet preparation and formal ether concentration technique, these parasites were: *G. lamblia* 134 (34.9%), *E*. *histolytica* 54 (14.1%), *H. nana* 38 (9.9%), *S. mansoni* 12 (3.1%),*T.spp*. 21(5.5%), *T. trichiura* 1 (0.3%) and *S. stercoralis* 6 (1.6%). These findings were found to disagree with the finding obtained by Obateru etal*.* (2017)in Nigeria who found that *G. lamblia* (3.7%), *E. histolytica* (8.4%), *S. mansoni* (0.4%) *T. trichiura* (0.8%) and *S. stercoralis* (1.7%). This may be due to the differences of frequency of parasites according to geographical distribution.

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