Prevalence Rate of Cryptosporidium Parasite among Pregnant Women in Medical Military Hospital, Khartoum State-Sudan

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Abstract: This study aimed to determine the prevalence rate of Cryptosporidium in pregnant women in Medical Military Hospital-Khartoum State. A cross sectional study was conducted in the period between May-December 2016. The study was conducted on 150 stool samples collected from all the study subjects. The age ranged between 16-45 years old. The mean age was 26 ± 6 years old. Stool samples were taken from all subjects included in the study, in addition to the clinical and parasitological data were obtained and recorded. The results showed that prevalence of cryptosporidiosis among pregnant women was 23(15.3%) when using Modified Ziehl-Neelsen (MZN) stain for deposit obtained by Formal-ether concentration technique (F.E.C.T) while 1 sample (0.66%) was detected by using stained direct smear. The results demonstrated that the prevalence of infection was higher (10%) in the age group 21-29 years. Also the prevalence rate of Cryptosporidium was high (8.0%) in the third trimester according to different gestational age, while in first and second trimester, the prevalence was 2.7% and 4.6% respectively. The results were showed that prevalence of cryptosporidiosis according to drinking water source was (14.1%) from tap water while from other sources was 33.3%. The difference in rates was found to be statistically insignificant with p value=0.122. The study revealed that the prevalence of cryptosporidiosis according to diarrheal sign was 22.6% with p value=0.039. The study indicated that the prevalence rate of Cryptosporidiosis according to diarrheal sign was 22.6% with p value=0.039. The study indicated that the prevalence rate of Cryptosporidiosis according to diarrheal sign was 22.6% with p value=0.039. The study indicated that the prevalence rate of Cryptosporidious according to diarrheal sign was 22.6%.

Keywords— Prevalence; Cryptosporidium; Pregnant women; Medical Military Hospital

1. INTRODUCTION

Human cryptosporidiosis is caused by infection with apicomplexan protozoans of the genus Cryptosporidium. Human illness was formerly thought to be caused by a single species, but molecular studies have demonstrated that it is caused by at least 15 different species. Among the more common species are Cryptosporidium hominis, for which humans are the only natural host, and C.parvum, which infects bovines as well as humans [1]. Outbreaks of cryptosporidiosis should be detected by vigilant observation for increased case numbers at primary and public health care levels. The genus Cryptosporidium consists of a group of protozoan parasites within the protist subphylum There are more Apicomplexa. than 26 known Cryptosporidium species, as recognized by host specificity, morphology, and molecular biology studies [2]. Besides humans, the parasite can infect many other species of animals, such as mammals, birds, and reptiles, and is pathogenic to immunocompetent and immunocompromised hosts. Cryptosporidium species are able to infect and reproduce in the epithelial cell lining of the gastrointestinal and respiratory tracts without causing cytopathic effects. C. hominis and C parvum cause most human infections. In immunocompetent individuals, the organisms are primarily localized to the distal small intestines, whereas in immunocompromised hosts, the parasites have been identified throughout the gut, biliary tract, and respiratory tract [1]. The main objectives of the study were to determine prevalence of *Cryptosporidium* among pregnant women in Medical Military Hospital-Khartoum state, to demonstrate the occurrence of cryptosporidiosis among pregnant women according to age groups and gestation age, to investigate the prevalence of cryptosporidiosis according to source of drinking water, diarrheal sign and to the contact with animals and to compare between stained direct smear and stained formal ether concentration technique for detecting oocyst of *Cryptosporidium*.

2. Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area and study duration:

The study was conducted in Medical Military Hospital Khartoum state, during the period between May-December 2016. Khartoum State lies between longitude 31.5-34 east and latitude 15-16 north in an area about 28.165 square kilometres. It is bordered on the north and the east sides by the River Nile State, on the north-western side by the

Northern State and on the eastern and southern sides by Kassala, Gedaref and Gezira States [3].

2.3 Study population:

The study was carried out on pregnant women who attended in pregnancy clinic for follow up.

2.4 Sample size:

The sample size was obtained according to the following equation:

 $N = (t^2 * P(1-p)/M^2)$

N = Sample size

t = the normal standard deviate (t = 1.96)

P = the frequency of occurrence of *cryptosporidium* (1.1%)

M = degree of precision (0.05)

N = 1.96*1.96*0.11*(1-0.11)/0.05*0.05 = 150

According to the above finding, the study was conducted on 150 pregnant women.

2.5 Sample collection:

One hundred and fifty stool samples were collected from all participants. After the direct saline preparation and direct smear were prepared, specimens were fixed with 10% formal saline for further concentration and staining techniques.

2.6 Data collection:

150 designed questionnaires were filled by participants.

3. Methods

3.1 Direct wet preparation:

The technique was used to detect the parasites. All specimens were initially subjected to direct saline preparation and then examined.

3.1.1 Procedure:

One drop of 0.8 5% sodium chloride (physiological saline), were placed on a clean slide. A small amount of stool specimen (2 mg) was taken to make smooth thin preparation. A cover glass was placed on the suspension. Each suspension was systematically scanned with the low-power objective (x10) then high-power objective (x40) was used for detailed study.

3.2 Direct stool smear:

A small amount of stool specimen was taken to make a thin smear. The smears were allowed to air dry then examined after staining steps were done.

3.3 Formal-ether concentration technique (F.E.C.T):

This is recommended for use in district laboratories because it is rapid and can be used to concentrate a wide range of faecal parasites from fresh or preserved faeces [4].

3.3.1 Procedure:

The specimen was preserved in 10% formalin then the preservative mixture was stirred. Depending on the amount and viscosity of the specimen, a sufficient quantity of stool were sieved through wet gauze (no more than two layers) into a conical 15-ml centrifuge tube to give the desired amount of sediment (0.5 to 1 ml) for later steps. Approximately 3 to 4 ml of the preserved-stool mixture was 23 used. 10% formalin almost to the top of the tube was added, and for 10 min at 500 rpm was centrifuged. The

amount of sediment obtained approximately 0.5 to 1 ml. The supernatant fluid was discarded, and the sediment in formalin was resuspended, formalin almost to the top of the tube was added and again for 10 min at 500 rpm was centrifuged. The supernatant fluid discarded, and the sediment on the bottom of the tube in 10% formalin was resuspended. The tube half full only was filled. 4 to 5 ml of diethyl ether were added. The tube with stopper was closed and the suspension vigorously for at least 30 s was mixed. After a 15s to 30s waited, carefully the stopper removed. For 10 min at 500 rpm was centrifuged. For four layers was achieved, a small amount of sediment (containing the parasites) in the bottom of the tube, a layer of formalin; a plug of faecal debris on top of the formalin layer and a layer of diethyl ether the top. The plug debris freed by the plug with an applicator stick was ringed, all of the supernatant fluid was discarded. After proper decanted a drop or two of fluid remained on the side of the tube down into the sediment. The fluid was mixed with the sediment. A small amount of mixure were added to a slide, to make thin smear was spread it, then left it to air dry and after staining steps done was examined.

3.4 Modified Ziehl-Neelsen (M-Zn) stain:

3.4.1. Reagents:

- Carbol fuchsin stain
- Malachite green stain
- Acid alcohol 1% v/v
- Methanol

3.4.2 Procedure:

After the direct smear from stool specimens was prepared and a smear from the sediment obtained by the formal ether concentration technique. The smear was fixed with methanol for 2-3 minutes. With carbol fuchsine for 15 minutes (cold method) was stained. The stain was washed with water. 1% acid alcohol was used decolorized for 10-15 seconds, then was washed by water. Then a malachite green stain was used as counter stain for 30 seconds, the slide was washed water and the slide were stood in a draining rack to dry. The smear was examined microscopically for oocyst, a low power magnification was used to detect the oocyst and the oil immersion objective was used identified them [4].

3.5 Data analysis:

Results were analysed using the computerized program of statistical package of social science (SPSS) version 16. Then data were presented in tables.

3.6 Sensitivity and specificity of the techniques used:

Sensitivity and specificity was calculated according to the formula (table below) [5]:

Sensitivity = TP/(TP+FN)x 100%

Specificity = $TN/(TN+FP) \times 100\%$

- TP= True positive TN= True negative
- FP= False positive
- FN= False negative

The	e table showed the Formula of sensitivity : specificity			
Technique	+ve	-ve	Total	
+ve	TP	FP	TP+FP	
-ve	FN	TN	FN+TN	
Total	TP+FN	FP+TN	TP+FN+ FP+TN	

3.7 Ethical consideration:

Approval was taken from the College of Medical Laboratory Science-Sudan University of Science and Technology and hospital management. The pregnant women were informed for the purpose of the study before collection of the specimen and verbal concept was taken from them.

4. Results

The study was conducted on 150 subjects. Study subjects were divided into 4 age groups as follows :< 20, 20-29, 30-39 and 40-49 years old. The frequency of each age group was 26 (17.3%), 86 (57.4%), 36 (24.1%) and 2 (1.3%) respectively (table 1). The age ranged between 16-43 years old. The mean age was 26 10 years old. Studied subjects were classified to gestation age as follows: 23 (15%) for first trimester, 66 (44%) for second trimester and 61(41%) for third trimester (table 2). Out of 150 stool samples, 1 (0.66%) was positive for *Cryptosporidium* by using stained direct smear and 23 (15.3%) were positive for *Cryptosporidium* by using stained FECT smear. The difference in rate was found

to be statistically significant at p value=0.018 (table 3). According to the formula described in materials and methods, sensitivity and specificity of stained direct smear method was 4% and 100% respectively (table 4). Out of 150 stool samples, the prevalence rates of cryptosporidiosis were 5 (3.3%), 15 (10.0%), 3(2.0%) and 0(0%) in age groups <20, 20-29, 30-39 and 40-49 years old respectively. The difference in rates was found to be statistically insignificant at p value=0.509 (table 5). Out of the 23 positive samples, 4 (17.3%) were in the first trimester, 7 (10.6%) were in the second trimester and 12 (19.6%) were third trimester. The difference in rates was found to be statistically insignificant at p value=0.351 (table 6). Out of 150 subjects, 141were used tap water as source of drinking water. Out of them 20 (14.1%) were positive, while 9 who used other sources, 3 (33.3%) were positive. The difference in rates was found to be statistically insignificant at p value=0.122 (table 7). Out of 150 subjects, 62 had a diarrheal sign. Out of them 14 (22.6%) were positive for cryptosporidiosis while 9 (10.2%) were positive but had no diarrheal sign. The difference in rates was found to be statistically significant at p=0.039 (table 8). Out of 150 stool samples 17 (11.3%) were positive for Giardia lamblia and 5 (3.3%) were positive for Entamoeba histolytica by using wet preparation, while 4 (2.7%) were positive for Cyclospora.spp using stained FECT smear (table 10). From 23 positive cases for Cryptosporidium 3(13%), 2(8.7%) and 4(17.4%) were positive for co-infection Cryptosporidium/ Giardia lambila, *Cryptosporidium*/ Entamoeba histolytica and Cryptosporidium/ Cyclospora spp respectively (table 11).

Age Groups (Years)	Frequency	Percentage (%)
<20	26	17.3
20-29	86	57.4
30-39	36	24
40-49	2	1.3
Total	150	100

Table 1: Frequency of study subjects according to age groups

Fable 2: 1	Frequency	of study	subjects	according t	to gestation	age
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Gestational age	Frequency	Percentage (%)
First trimester	23	15.3
Second trimester	66	44.0
Third trimester	61	40.7
Total	150	100

Table 3: Prevalence of cryptosporidiosis by using M-Zn stain for direct smear and FECT smear prepared technique

Technique	Percentage (%)
Direct smear	0.66
FECT	15.3
p=0.018	

=0	0.0	1	8		

Table 4: Sensitivity and specificity of stained direct smear

Technique			FECT
		+ve	-ve
Direct smear	+ve	1	0
	-ve	22	127

Table 5: Prevalence of cryptosporidiosis according to age groups using M-Zn stain FECT smear

Age groups (years)	ars) FECT		Total
+ve -ve	-ve		
<20	5	21	26
21-29	15	71	86
30-39	3	33	36
40-49	0	2	2
Total	23	127	150

p=0.509

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Table 6: Prevalence of cryptosporidiosis according to gestation age

Gestational age	M-ZN stain		Total
	+ve	-ve	
First trimester	4	19	33
Second trimester	7	59	60
Third trimester	12	49	61
Total	23	127	150

p=0.351

Table 7: Prevalence of cryptosporidiosis according to source of drinking water

Source of drinking water	Cryptosporidiosis		Total
	+ve	-ve	
Tap water	20	121	141
Others	3	6	9
Total	23	127	150
0.100			

p=0.122

Table 8: Prevalence of cryptosporidiosis according to diarrheal sign

Diarrheal sign	Cryptosporidiosis		Total
	+ve	-ve	
Yes	14	48	62
No	9	79	88
Total	23	127	150

p=0.039

Contact with animals	Cryptosporidiosis		Total
	+ve	-ve	
Yes	3	19	22
No	20	108	128
Total	23	127	150

Table 10: Prevalence of other parasites among pregnant women using wet preparation and stained FECT smear

Parasites	G.lamblia	E.histolytica	Cyclospora spp.
Total			
Frequency	17	5	4
Percentage (%)	11.3	3.3	2.7

Table 11: Prevalence of Co-infection of Cryptosporidium and other parasites

Parasites	G.lamblia	E.histolytica	Cyclospora spp.
Total			
Frequency	3	2	4
Percentage (%)	13	8.7	17.4

5. Discussion

The present study was carried out on 150 stool samples collected from pregnant women who attended the Medical Military Hospital-Khartoum State. The results showed that the overall prevalence rate of cryptosporidiosis was 15.3%

using stained FECT smear. This finding agreed with the finding obtained by Sim et al. (2015) [6] in the White Nile State. They found that the prevalence rate was 13.3% and was also similar with the finding obtained by Adam et al. (2007) [7] in Nyala medical laboratory, who found that the prevalence was 15.3%. The results showed the prevalence of cryptosporidiosis in the study subjects according to gestation age was higher (19.6%) in the third trimester, compared to other gestation ages due to their lack of efficient immunological response against the infection. The present study showed a strong relationship between the source of drinking water and prevalence of Cryptosporidium infection. This finding was inagreement with Sim et al. (2015) [6], Adamska (2015) [8] and Shanan et al. (2015) [9]. The results showed that from the 62 subjects who had diarrhoea, 14 (22.6%) were positive for cryptosporidiosis. This difference in rate was found to be statistically significant at p value=0.039, while there is insignificant difference between prevalence of cryptosporidiosis and contact with animals at p value=0.811. This finding was in agreement with Sim et al.(2014). Out of 150 stool samples, 23(15.3%) were positive cases for cryptosporidiosis using stained FECT smear while only one sample (0.66%) was positive using stained direct smear. From this result, stained FECT smear is more accurate than stained direct smear. The study concluded that the prevalence of co-infection of *Cryptosporidium* with other parasites was detected among pregnant women as *Cryptosporidium/ Giardia lambila* (13%), *Cryptosporidium/ Entamoeba histolytica* (8.7%) and *Cryptosporidium/ Cyclospora spp* (17.4%)

6. Conclusion

The study concluded that the prevalence rate of cryptosporidiosis among pregnant women was 15.3%. The prevalence was high among age group 20-29 years. Also, the prevalence was high in third trimester according to gestation age. M-Zn stain FECT smear was more accurate than direct smear in the diagnosis of *Cryptosporidium* parasite.

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