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Prevalence Rate of Intestinal Protozoan Parasitic Infections By Using Different Faecal Diagnostic Techniques in Kosti Teaching Hospital, White Nile State- Sudan

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Abstract: *The study was aimed to determine the prevalence of intestinal protozoan parasitic infections in Kosti Teaching Hospital in White Nile State- Sudan. A cross-sectional hospital-based study was carried out from March- December 2015. A total of 150 subjects were included in this study. From them, 63 (42%) were males and 87(58%) were females with age ranging between (1-75) years old of mean age 31±1 years old. Faecal samples were taken from all subjects included in the study, in addition to clinical and parasitological data were obtained and recorded. The results showed that prevalence of intestinal protozoan infection in the study area was 30(20%). When using direct wet preparation (DWP), formal ether concentration technique (FECT) and zinc sulphate floatation technique ($ZnSO_4$ FT) respectively the prevalence of intestinal protozoan parasitic infection were 11 (7%), 26 (17%) and 10 (7%) respectively. From 30 positive cases, 9 (6%), 7 (5%) and 13(9%) respectively were positive for G.lamblia, E.histolytica and E.coli respectively. The most common causative agents of intestinal protozoan infection in the study area were G.lamblia. The study revealed that the prevalence of intestinal protozoan infection was higher in females (20.6%) than in males (19%). The prevalence was higher (20%) in the age group between 1-14 years old (p=0.445).*

Keywords— Intestinal protozoan infections; Faecal diagnostic techniques; Kosti Teaching Hospital; White Nile State

1. INTRODUCTION

Intestinal protozoa are unicellular eukaryotic organism distributed worldwide in the most habitats. They reproduce sexually by fusion of male and female's gametocyte or asexually by binary fission. Most species are free living, but some are pathogenic causing infections range from asymptomatic to life threatening disease. Protozoa varies in size, shape and life style and are classified on the bases of their microscopic morphology. The stage of protozoa that actively feed and multiply is called trophozoites. In some protozoa other terms are used in life cycle and some of it surrounds themselves with protective membranes (forming cyst) during exposure to hard environmental conditions [1]. The World Health Organization (WHO), (2005) [2] ranks diarrheal disease as the second highest cause of morbidity and mortality in children in the developing world. Enteric protozoa are one case of diarrheal disease in children. Intestinal protozoa are transmitted by the fecal-oral route and exhibit life cycles consisting of a cyst stage and a trophozoite stage. The major parasitic causes of gastroenteritis are *Giardia lamblia (G.lamblia), Cryptosporidium parvum (C.parvum)* and *Entamoeba histolytica (E.histolytica), Entamoeba coli (E.coli), Entamoeba hartmani, Endolimax nana, Iodamoeba buetschlii* and *Balantidum coi (B.coli).* Parasites enter the intestine through the mouth from uncooked or unwashed food, contaminated water and hands when organisms are swallowed, they move into intestine where they can reproduce and causes symptoms [3]. The cysts consist of a resistant wall and are excreted in the feces. The cyst wall functions to protect the organism from desiccation in the external environment. Unhygienic conditions promote transmission of most protozoa. Traditionally parasites have been identified by simple microscopy and serologic methods. New approaches include antigen detection and polymerase chains reaction (PCR). The main objectives of study were to study the prevalence of intestinal protozoan parasitic infections in Kosti Teaching Hospital, White Nile State- Sudan, to determine the prevalence of intestinal protozoan parasitic infections by using DWP, FECT and $ZnSO_4$FT, to determine the prevalence of intestinal protozoan parasitic infections

according to gender and age groups in Kosti Teaching Hospital and to identify the species of intestinal protozoan parasitic infections in wards in Kosti Teaching Hospital.

2. Materials and methods

2.1 Study design:
It was cross-sectional descriptive study.
2.2 Study area:
This study was conducted at Kosti Teaching Hospital in Kosti City, White Nile State- Sudan.
2:3 Study period:
This study was conducted in the period between March to December 2015.
2.4 Study population:
The study was carried out on patients admitted in wards in Kosti Teaching Hospital with different ages and gender.
2.5 Sample size:
$N = t^2 * P (1-p)/M^2)$
N = Sample size
t = the normal standard deviate (t = 1.96)
P = the frequency of occurrence of intestine parasite (1.1)
M = degree of precision (0.05%)
According to equation above the sample size was calculated as follow:
N = 1.96x1.96x.11x (1-.11)/.05x.05 =150
The study was conducted on 150 clinically suspected patients.
2.6 Sample collection:
Faecal specimens were collected from all participants. Faecal samples were collected in wide mouth container free from water. The samples were labeled clearly with identifying number. These samples were preserved in 10% formal ether then transferred to laboratory to be examined later.

3. Methods

3.1: Direct wet preparation (DWP):
Faecal examination consists of macroscopical and microscopical examination.
3.1.1 Macroscopical examination:
The faeces were examined for consistency which may be formed, semi-formed and soft. The faeces were examined for the presence of blood and mucus with certain intestinal infection [4].
3.1.2 Microscopical examination:
The faeces were prepared by mixing small amount of faeces with a drop of 0.9% solutions of NaCl on a glass slide and the slide was covered with a glass cover slip and examined for the presence of parasites. The same procedure was mixed with a drop of Lugol's iodine and examined for the presence of cysts of parasites [4].
3.2 Zinc sulphate floatation technique ($ZnSO_4$ FT):
The technique was used as described by Cheesbrough (1987) [5]; zinc suphate solution was added up to one quarter of tube placed in vertical position. The tube has completely smooth rim. About 0.5g of faeces were added using applicator stick and emulsified in solution. The tube covered by clean cover glass, and left to stand for about 30-45 minutes so as to leave cyst and egg to float. After that, the cover glass was taken and placed in a clean slide and examined under microscope.
3.3 Formal ether concentration technique (FECT):
In this technique about 1gram of feces was emulsified in 4ml of 10% formal saline in screw-cap tube. Then, 3-4ml of formal saline was added, and mixed by shaking for 20 second. Feces were sieved in a beaker; suspensions were transferred to centrifuge tube. Then 3-4ml of diethyl ether was added and contents were stoppered, shaken for one minute and then was centrifuged immediately for one minute at 300rpm. After centrifugation the parasites were sedimented at the bottom of the tube. And fecal debris was collected in the layer between the ether and formal saline. The layer of faecal debris was lost from side of tube using stick. The tube was rapidly inverted to discard ether, faecal debris and formal saline and returned back to its upright position to allow the fluid to drain to the bottom. Sediment was mixed by Pasteure pipette and transferred to clean slide, covered with cover glass and examined microscopically [5]. Intensity of parasites was determined by using the criteria described by Cheesbrough (1998) [6] as follows: 1-3 stages in one gram presented as scanty infection, 4-10 stages as few infection, 11-20 stages as moderate infection, 21-41 stages as many infections and over 41 stages as very many infections.
3.4 Data collection:
The primary data were collected by using a questionnaire which has specific design to obtain information that helped in the study.

3.5 Data analysis:
Results obtained were analyzed by the computerized program of statistical package of social science (SPSS) version 11.5. Frequency, mean and Chi-squire test were used. Then data were presented in tables.
3.6 Ethical consideration:
Approval was taken from the College of Medical Laboratory Science- Sudan University of Science and Technology. Consent was taken from all participants or their guardians before being enrolled in the study. All participants were informed on the nature of the study.

4. Results

The study was conducted on 150 patients who admitted in wards in Kosti Teaching Hospital. All age groups were ranged 1-75years and their mean age was 31±1.3 years old. Out of 150 subjects, 63(42%) were males and 87 (58%) were females (Table 1). The study subjects were divided into five age groups as follow: 1-14, 15-30, 31-45, 46-60, 61-75 years old and the frequency of each age group were 50 (32.9%), 30 (19.7%), 33 (21.7%), 24 (15.8%), 13 (8.6%) respectively (Table 2). Out of 150 faecal samples, 30 samples were found

to be positive for intestinal protozoan infections. This constituted an overall prevalence was 20% (Table 3). Out of 150 faecal samples, 11(7.0%), 26 (17%) and 10 (7.0%) were positive for intestinal protozoan infections by using DWP, FECT and $ZnSO_4FT$ respectively (Table 4). Out of 150 faecal samples, 9 (6%), 7 (5%) and 13 (9%) were positive for *G.lamblia*, *E.histolytica* and *E.coli* respectively (Table 5). Out of 7 positive case of *E.histolytica* by FECT, $ZnSO_4FT$ and DWP respectively were found 7 (100%), 3(42.8%) and 3(43%). Out of 9 positive case of *G.lamblia* were found (66.7%), 6(66.7%) and 6(66.7%) by FECT, $ZnSO_4FT$ and DWP respectively. Out of 13 positive case of *E.coli* by FECT, $ZnSO_4FT$ and DWP respectively were found 11 (85%), 4 (31%) and 3(23%) were positive for *E.coli* by FECT, $ZnSO_4FT$ and DWP respectively (Table 6). DWP having significant value with *G.lamblia* (p=0.000), *E. coli* (p=0.014) and *E.histolytica* (p=0.001), FECT having significant value with *G.lamblia* (p=0.000), *E. coli* (p=0.000) and *E.histolytica* (p=0.000) $ZnSO_4FT$ having significant value with *G.lamblia* (p=0.000), *E. coli* (p=0.000) and *E.histolytica* (p=0.000) (Table 6). Out of 13 faecal samples examined by FECT as scanty, 4(30.8%), 6(46.2%) and 6(46.2%) respectively were found to be for *G.lamblia*, *E.histolytica* and *E.coli*, out of 3 faecal samples examined by $ZnSO_4FT$ as scanty, 2(66.7%), 0(0%) and 2(66.7%) respectively were found to be for *G.lamblia*, *E.histolytica* and *E.coli*. Out of 20 faecal samples examined by FECT as few, 3(15%), 1(5%) and 3(15%) respectively were found to be for *G.lamblia*, *E.histolytica* and *E.coli* and out of 4 faecal samples examined by $ZnSO_4FT$ as few, 1(25%), 2(50%) and 1(25%) respectively were found to be for *G.lamblia*, *E.histolytica* and *E.coli* (Table 7) and (Table 8) respectively. Out of 63 males, 12 (19%) were positive for intestinal protozoan infections and form 87 females 18(20.6%) were positive for intestinal protozoan infections (Table 9). Out of 63 males examined, 9(14.3%), 9(14.3%) and 4(6.3%) were found positive by FECT, $ZnSO_4FT$ and DWP respectively and out of the 87 females examined, 17(19.5%), 4 (4.6%) and 7(8%) were found positive by FECT, $ZnSO_4FT$ and DWP respectively (Table 10). Out of 4 faecal samples examined, 3 (75%), 0(0%) and 1 (25%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by wet preparation, out of 9 faecal samples examined, 6(66.7%), 2(22%) and 4(44.4%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by FECT and out of 6 faecal samples examined, 5(83.3%), 2(33.3%) and 2(33.3%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by $ZnSO_4FT$ (Table 11). Out of 7 faecal samples examined, 3 (42.9%), 3(42.9%) and 2 (28.6%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by DWP, out of 17 faecal samples examined, 3(17.6%), 6(35.5%) and 7(41.2%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by FECT and out of 4 faecal samples examined, 1(25%), 1(25%) and 2(50%) respectively were positive for *G.lamblia*, *E.hitsolytica* and *E.coli* by $ZnSO_4FT$ (Table 12). Out of the 26 faecal samples examined by FECT, 9 (34.6%), 6 (23.1%),7(26.9%), 3(11.5%) and 1(3.8%) respectively were positive for age 1-14, 15-30, 31-45, 46-60 and 61-75, out of the 10 fecal samples examined by $ZnSO_4FT$, 5(50%), 0(0%), 3(30%), 2(20%) and 0(0%) respectively were positive for age 1-14, 15-30, 31-45, 46-60 and 61-75 and out of the 11 faecal samples examined by DWP, 2(18.2%), 2(18.2%), 5(45.5%), 1(9.1%) and 1(9.1%) respectively were positive for age 1-14, 15-30, 31-45, 46-60 and 61-75 (Table 13). Out of 50 faecal samples examined, 10(20%) were found positive in age group 1-14, out of 30 faecal samples examined, 5(17%) were found positive in group15-30, out of 33 faecal samples examined, 8(24.2%) were found positive in age group15-30, out of 24 faecal samples examined, 5(20.8%) were found positive in age group 31-45 and out of 12 faecal samples examined, 2(15.4%) were found positive in age group 61-75 respectively (Table 14).

Table 1: Frequency of study subjects according to gender

Gender	Frequency	Percentage (%)
Male	63	42%
Female	87	58%
Total	150	100

Table 2: Frequency of study subjects according to age groups

Age groups (Years)	Frequency	Percentage (%)
1-14	50	32.9%
15-30	30	19.7%
31-45	33	21.7%
46-60	24	15.8%
61-75	13	8.6%
Total	150	100%

Table 3: Overall prevalence of intestinal protozoan infections among patients in the study area

Number examined	Number positive	Percentage (%)
150	30	20%

Table 4: Overall prevalence of intestinal protozoan infections by using DWP, FECT and $ZnSO_4$ FT in the study area

Technique	Number examined	Number positive	Percentage%
DWP	150	11	7%
FECT	150	26	17%
$ZnSO_4$FT	150	10	7%

Table 5: Prevalence of intestinal protozoan infections species in the study area

Species	Number positive	Percentage (%)
G.lamblia	9	6%
E.histolytica	7	5%
E.coli	13	9%
Total	30	20%

Table 6: Overall prevalence of intestinal protozoan infections species by using DWP, FECT and $ZnSO_4$**FT**

Species	Number examined	DWP		FECT		$ZnSO_4$FT	
		Number positive	Percentage	Number Positive	Percentage	Number Positive	Percentage
G.lamblia	9	6	66.7%	6	66.7%	6	66.7%
E.hitolytica	7	3	43%	7	100%	3	42.8%
E.coli	13	3	23%	11	85%	4	31%

Table 7: Detection of intensity of parasites species by using FECT

Species	FECT			FECT		
	Scanty			Few		
	Number examined	Number positive	Percentage	Number examined	Number positive	Percentage
G.lamblia	13	4	30.8%	20	3	15%
E.histolytica	13	6	46.2%	20	1	5%
E.coli	13	6	46.2%	20	3	15%

G.lamblia (p=0.000), *E.histolytica* (p=0.000) and *E.coli* (p=0.000) by FECT

Table 8: Detection of intensity of parasites species by using by $ZnSO_4$FT

Species	$ZnSO_4FT$			$ZnSO_4FT$		
	Scanty			Few		
	Number examined	Number positive	Percentage	Number examined	Number positive	Percentage
G.lamblia	3	2	66.7%	4	1	25%
E.histolytica	3	0	0%	4	2	50%
E.coli	3	2	66.7%	4	1	25%

G.lamblia (p=0.000), *E.histolytica* (p=0.000) and *E.coli* (p=0.000) by $ZnSO_4FT$

Table 9: Prevalence of intestinal protozoan infections according to gender

Gender	Number examined	Number positive	Percentage
Male	63	12	19%
Female	87	18	20.6%
Total	150	30	20%

p=0.626

Table 10: Prevalence of intestinal protozoan infections by using FECT, $ZnSO_4FT$ and DWP according to gender

Techniques	FECT			$ZnSO_4FT$		DWP	
Gender	Number examined	Number positive	(%)	Number positive	(%)	Number positive	(%)
Male	63	9	14.3%	9	14.3%	4	6.3%
Female	87	17	19.5%	4	4.6%	7	8%

FECT (p=0.401), $ZnSO_4FT$ (p=0.233) and DWP (p=0.694)

Table 11: Prevalence of intestinal protozoan infections species using DWP, FECT and $ZnSO_4FT$ in males

Techniques	Number examined	*G.lamblia*		*E.histolytica*		*E.coli*	
		Number positive	(%)	Number examined	(%)	Number examined	(%)
DWP	4	3	75%	0	0%	1	25%
FECT	9	6	66.7%	2	22.2%	4	44.4%
$ZnSO_4FT$	6	5	83.3%	2	33.3%	2	33.3%

Table 12: Prevalence of intestinal protozoan infections species using DWP, FECT and $ZnSO_4FT$ in females

Techniques	Number examined	*G.lamblia*		*E.histolytica*		*E.coli*	
		Number positive	**(%)**	**Number examined**	**(%)**	**Number examine**	**(%)**
DWP	7	3	42.9%	3	42.9%	2	28.6%
FECT	17	3	17.6%	6	35.5%	7	41.2%
$ZnSO_4FT$	4	1	25%	1	25%	2	50%

Table 13: Prevalence of intestinal protozoan infections by using DWP, FECT and $ZnSO_4FT$ among age groups

Techniques		Age groups (years)									
		1-14		15-30		31-45		46-60		61-75	
	Number examined	No. positive	(%)	No. positive	(%)	No. positive	(%)	No. positive	(%)	No. positive	(%)
FECT	26	9	34.6 %	6	23.1 %	7	26.9 %	3	11.5%	1	3.8%
$ZnSO_4FT$	10	5	50%	0	0%	3	30%	2	20%	0	0%
DWP	11	2	18.2 %	2	18.2 %	5	45.5 %	1	9.1%	1	9.1%

FECT (p=0.782), $ZnSO_4FT$ (p=0.782) and DWP (p=0.385)

Table 14: Prevalence of intestinal protozoan infections among age groups

Age (Years)	Number examined	Protozoan parasites	
		Number Positive	Percentage
1-14	50	10	20 %
15-30	30	5	17%
31-45	33	8	24.2%
46-60	24	5	20.8%
61-75	13	2	15.4%
Total	150	30	20%

p=0.445

5. Discussion

From the results showed that the overall prevalence of intestinal protozoan infections in Kosit Teaching Hospital was (20%), however, it was higher than the rate (19.8%) reported by Sandokji *et al.*(2009) [7] in Al-Madinah Al-Munawarh Hospitals and higher than rate (11.1%) which reported by Alrifai *et al.*(2009) [8] in Tikrit Teaching Hospital. The difference in the prevalence is related to many factors such as hygiene practices, type of microorganisms endemic in the area and sanitation level of the hospitals. From the investigation, it was obvious that the rate of infection in female was higher (20.6%) than in males 12(19%). This result was disagreed with Alrifai *et al.* (2009) [8] who found that the prevalence was higher in males (33.5%) than in females (30.8%) in Tikrit Teaching Hospital. In this study, the highest prevalence intestinal protozoan infections 10 (20%) was reported among the 1-14years age groups and lowest rate (15.4%) was reported among the 61-75 age groups. This difference in rate was found to be statistically insignificant at p=0.445. The study was conducted to determine the pathogens of intestinal protozoan infections cases, for these purpose, 150 fecal samples were examined, 9 (6%) were positive for *G.lamblia* cysts, 7(5%) for *E.histolytica* and 13 (9%) for *E.coli*. The study showed that *G.lamblia* and *E.coli* were the most common causative agents of parasitic infections in the study area, *E.coli* don't consider as pathogens. The results showed that the prevalence of intestinal protozoan infections species by using FECT and $ZnSO_4FT$, which were present scanty by using FECT 4(30.8%), 9(69.2%) and 6(46.2%) respectively for *G.lamblia*, *E.histolytica* and *E.coli* respectively while 3(15%), 17(85%), 3(150%) and 0(0%) were found few by using FECT for *G.lamblia*, *E.histolytica* and *E.coli.* respectively. When using $ZnSO_4FT$, intensity as scanty 2(66.7%), 0(0%) and 2(66.7%) for *G.lamblia*, *E.histolytica* and *E.coli.* respectively while 1(25%), 2(50) and 1 (25%) were intensity as few. The most intensity of parasites species by using FECT and $ZnSO_4FT$ between scanty and few infection.

6. Conclusion

The study concluded that the prevalence of spread intestinal protozoan parasitic infection was 20% in the study area. Females were more affected than males. The prevalence of intestinal protozoan parasitic infection was higher in age group 1-14 years than other age groups. The most common

causative agents of intestinal protozoan parasitic infection in study area were *G.lamblia*.

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