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RESEARCH PAPER

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Comparison between Direct Stool Examination and Culture Technique in Diagnosis of *Giardia lamblia* in Patient's attending to Kosti Teaching Hospital, White Nile State, Sudan

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ABSTRACT

Giardia lamblia is the most common flagellate of the intestinal tract, causing Giardiasis. It is more common in warm climates and the humans are the only important reservoir of the infection in parts of the world where sanitation is poor. The aimed of the current comparative study design is to compare between direct stool examination and culture technique for Giardia lamblia among patients suffering from Giardiasis attending Kosti Teaching Hospital, Kosti Locality, White Nile State, Sudan during the year 2015. The data were collected using questionnaire and by the Stool examination. Two techniques Direct Stool Examination and Culture Technique were used. The data was analyzed using Statistical Package for Social Sciences (SPSS) version 21.

Sixty samples of stool of those who suffered from Giardia disease have been checked randomly. 33.3% of the samples have been tested directly, while the other samples 66.7% have been tested through culture technique. Our results stated that testing the parasite of Giardia lamblia through culture technique is better than testing of stool directly. Finally the study recommended that it is necessary more useful true and more accurate for fitness of peoples using culture technique for testing the parasite of Giardia lamblia than direct examination technique.
Keywords: Giardiasis, Direct stool examination, Kosti, Sudan.

INTRODUCTION

Giardia lamblia is a flagellate of world-wide distribution. It is more common in warm climates than temporal climates. It is the most common flagellate of the intestinal tract, causing Giardiasis. Humans are the only important reservoir of the infection. The infection is most common in parts of the world where sanitation is at its lowest standarad. Giardiasis is an infection of the upper small bowel, which may cause diarrhoea (CDC, 2005). Giardiasis is transmitted by ingesting the parasite *Giardia lamblia* which is a very simple eukaryote that is a unicellular protozoan. The parasite has two stages in its lifecycle, the cyst and the trophozoite. Van Leeuwenhoek first saw Giardia through a microscope in 1681. He described it being rather slow moving, but making quick motions with their "paws", moving in a helical motion, which we now know are the flagella (Gillin, 1996). The name of the parasite has been through a lot of controversy and at differenttimes, has been called *G. duodenalis* and *G. intestinalis* , but *G. lamblia* has been the name most accepted. Although, the scientific community was aware of Giardia, they did not realize it was the cause of diarrhea out breaks until the 1970's.Until that time, they had thought Giardia was harm less inhabitant of the intestines (CDC, 2005). According to Levine et al. (1980) and Meyer. (1994). Giardia is a flagellated protozoan and both taxonomy and host specificity of which remain the subject of considerable debate. The organism has been found in more than 40 animal species (Meyer, 1994). The distribution of *G. lamblia* is worldwide (WHO, 2015). The parasite infects nearly 2% of adults and 6% to 8% of children in developed countries worldwide. Nearly 33% of people in developing countries have had giardiasis. In the United States, *Giardia* infection is the most common intestinal parasitic disease affecting humans. *Giardia* infection rates have been known to go up in late summer between 2006-2008 in the United States, known cases of giardiasis were twice as high between June-October as they were between January-March (CDC, 2005). The causative agent of Giardiasis is *G. lamblia.* It causes a severe infection in the small intestines of humans, called gastroenteritis; it is a water-borne microscopic parasite, known as *Giardia intestinalis*. *Giardia intestinalis* belong to the Genus *Giardia*, a species that is pathogenic to humans and to the mammals. The cyst *Giardia* can survive outside the host for months, as long as its environment is cool and moist, where it waits for a new host (CDC, 2005). *G. lamblia* is transmitted through ingestion of cysts in contaminated water or food. Cysts can survive outside the body for several weeks under favorable conditions. The main symptoms are abdominal pain flatulence, and episodic diarrhoea with steatorrhea and periodical soreness in severe cases. No blood or mucus is normally seen. However 50% of *G. lamblia* infections are symptomless, although severe infections may develop in immunocom promised hosts. Cysts can be found by examination of the deposit of a formol-ether concentrate of a stool preparation. The oval cysts with thick walls serve as characteristic features for these organisms. The flagella disintegrate and form a central ‘streak’ which becomes visible when stained with iodine or MIF (merthiolate-

iodine-formaldehyde). Cysts may be excreted intermittently; therefore it is important to examine more than one stool. (Jones *et al*, 1974). The standard treatment for Giardiasis can involve one of the following medications that a physician can prescribe: Metronidazole, tinidazole, quinacrine hydrochloride or furazolidone. Children can be treated with the drug, Nitazoxanide. The best measure for preventing the infection of Giardiasis, is to follow proper hygiene rules regularly, taking special care when in a potentially infectious area like a day care center (Boreham PFL*, et al* 1984).

MATERIAL AND METHOD

The present comparative study was conducted in Kosti Teaching Hospital, White Nile State, Sudan. This work was carried out in the emergency laboratory in patients with different age admitted with diarrhea. Sixty stool samples were collected randomly from patients in accident ward. Information was collected regarding individual number, age and sex. Also information includes toilet, education and diarrhea duration. Questionnaire was constructed and explained to the patients in simple Arabic. Before samples were collected, patients were advised on health hygiene and were given instruction on how to take the samples and the amount of stool needed. Patients also instructed not to contaminate stool with urine and water. After informed concient about 50g of stool was collected from each patient in clean dry wide mouth plastic with screw cap stool container. Each container was labeled clearly with patient number and name, then immediately transferred to the laboratory for examined using direct stool examination technique and culture techniques. The detection of *Giardia lamblia* cyst and trophozoite in stool sample was conducted according to the (Cheesbrough, 1987) method as flows:

Firstly a drop of fresh physiological saline was placed on slide using a piece of stick, a small amount of specimen (about 2mg), was mixed to make smooth thin preparation, then covered with cover glass and examined microscopically using 10x & 40x objectives. The stool sample is inoculated immediately into tubes of growth media (LES medium) and incubated at 35 for 24hr, 48hr and 72hrs. After that a drop of culture media is removed by sterile Pasteur pipette in to glass microscope slide and cover with cover glass, then examined microscopically using 40x objective. The results are presented as appositive trophozoite or negative trophozoite (Diamond, 1983). To prepare 1Litter of physiological saline solution, 8.5g NaCl and 1000ml Distilled water are measured and mixed until the salt is fully dissolved label the bottle and store it at room temperature (Cheesbrough, 1987).

To prepare a 1litter of Locke's solution 9gNaCl, 0.2gCaCl2, 0.4gKCl, 0.2gNaHCO3 and 2.5g Glucose are measured and dissolved in 1000ml Distilled water, autoclaved and storage (Diamond, 1983). The Blood sample was collected from normal human to plain container and stand for 15minet then centrifugation done and the serum transferred in to small test tube then put in the water bath with 56̊C temperature for 30minte and leave to cool, labeled with date and transfer to freezer (-20̊C) for storage according to (Mackie,2006). Four eggs were washed and shells were wiped with 70% alcohol and broken in to a sterile flask containing glass beads. Then some of Locke's solution is added and shaken until homogenous. The medium is dispensed so that the slant of 1 to 1.5 (1in=2.54Cm) is produced in the bottom of the tube, the tube is plugged and placed in aslant position in an inspissator at 70 C˚ until the slant solidifies. Inspissator condition in achieved in the autoclave by leaving the door jar (non pressurized system).The tubes were then autoclaved at121C˚ for 20 minute and any damaged slants were discarded. A mixture of 8 parts sterile Locke's solution to 1 part sterile inactivated human serum was prepared, sterilized by

filtration and incubated at 37C˚ for 24to 48hrs before used. The slants were covered to a depth of 1Cm with the sterile solution and loop full sterile rice powder was added (Diamond, 1983).

RESULTS

Sixty stool samples were collected and screened for *G. Lamblia* using direct stool examination and culture techniques. The number of infected cases for *G. Lamblia* in stool samples were 20(33.3%) using direct examination technique and 40(66.7%) using culture technique; The results obtained is presented in the following table:-

Table 1. The number and percentage of infected and non infected cases with *G. Lamblia* using the two different techniques.

Techniques / Cases	Direct saline	Culture (LES medium)
Infected cases	20 (33.3%)	40 (66.7%)
Non infected cases	40 (66.7%)	20 (33.3%)
Total	60	60

Table 2. The number and percentage of infected cases with *G. Lamblia* using the two different techniques correlated with age group.

Techniques / Age groups	Direct saline	Culture(LES medium)
10-16 years	12 (60%)	28 (70%)
17-23 years	6 (30%)	10 (25%)
Over 24 years	2 (10%)	2 (5%)
Total	20	40

Table 3. The number and percentage of infected cases with *G. Lamblia* in relation to sex using the two different techniques.

Techniques / Sex	Direct saline	Culture(LES medium)
Male	15 (25%)	26 (43.3%)
Female	5 (8.3%)	14 (23.3%)

Table 4. The number and percentage of infected cases with *G. Lamblia* according to the history of diarrhoea using the two different techniques.

Techniques / Cases	Direct saline	Culture(LES medium)
With a history	17 (28.3%)	20 (33.3%)
Without a history	3 (5%)	20(33.3%)

Table 5. The number and percentage of infected cases with *G. Lamblia* in according to toilet using the two different techniques.

Techniques / Cases	Direct saline	Culture(LES medium)
Have toilet	3 (5%)	9 (15%)
Without a toilet	17 (28.3%)	31 (51.6%)

DISCUSSION

Out of 60 stool samples collected and examined for *G. lamblia* using Direct saline examination and culture technique (LES medium); The greater number of parasite was detected by culture technique (Table 1). The study is in agreement with the finding of Rezaian and Ghalhari (1995), who used culture technique as a diagnostic aid in the detection and identification of *G. lamblia* from stool sample beside the direct saline examination in their study conducted in Iran. The greater numbers of parasite were detected in the age group 10-16 years; Table 2. This may be due to the bad habits like playing in river, drinking from contaminated water and who have not toilet or poor latrine. Rose, *et al* (1991) was conducted in Korea the infection of *G. lamblia* in agreement to age group one for bad habits. As a sex is concerned, infection with *G. lamblia* is higher among males than female; table 3. This may be due to the habits such as playing in water drinking contaminated water and who have not washing hand before eating food. The result was in agreement with the finding of Rose, *et al* (1991). According to the history of diarrhea, the greatest of number of parasites were detected in individual who have such history; Table 4. This may be due to the discard of trophozoites and cyst which produce intermittently in low amount and the result is stated by Buret A (1994). The highest number infected cases were found to be among those have no toilet in their houses; Table 5. This because the parasite is associated with poor hygiene as stated by Rose, *et al* (1991).

CONCLUSION

The overall , prevalence of *G. lamblia* among patients was 33.3%-66.7% using direct examination and culture technique (LES medium)respectively The prevalence was higher among age group 10-16 years (60%-70% and male showed higher prevalence rate than female (8.3%-25%) to (23.3%-43.3%) using the two different techniques. The prevalence rate was higher among patients have no toilet in their houses and was ranged between (28.3%-51.6%) using the two different techniques. From the finding, the study showed that

the culture technique by LES medium is the best than the direct saline stool examination which give a false negative result of 33.4%.

RECOMMENDATIONS

Mass treatment should be continued to eradicate the infection of *G. lamblia*. Improving the hygiene through good sanitation and provision of latrines which reduce water and food contamination. Health education programs should be held to teach the people how to avoid giardiasis. Improve the routine laboratory techniques for helpful diagnosis and treatment.

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