بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Frequency of *Entamoeba histolytica* and *Giardia lamblia* among the patients attending Khartoum hospital

معدل الإصابة بالدسنتاريا الأميبية والقارضيا عند المرضي في مستشفي الخرطوم

Athesis submitted in partial fulfillment of the requirements for M.Sc degree in medical parasitology

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2014

الآية

بسم الله الرحمن الرحيم

قال تعالي:

{ إقرأ باسم ربك الذي خلق{1} خلق الإنسان من علق{ 2} اقرأ وربك الأكرم {3} الذي علم بالقلم {4} علم الإنسان مالم يعلم {5} }.

صدق الله العظيم

سورة العلق الآيات من { 1-5 }

Dedication

To

Whom the deep worm-ness is fully generated from, and smooth love is completely formed in ,to the one and only sweet durable ***Mother***.

To

Whom I could easily struggle find when ever am need wisdom is placed and to my source of supercilious to my wonderful ***Father***.

To

My brothers (***Mohammed and Abdalla***) and my sisters (***Amna and Amany***) who help me in my life from (A to Z).

To

My college of Medical Laboratory and Sciences in Sudan University of Science and Technology.

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First and heart fully thanks to Allah.

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Thanks for all patients who help me.

I appreate my teachers in parasitology and medical entomology department for their efforts.

Abstract

The study was conducted in Khartoum hospital during the period from October 2013 to September 2013. The aim of the study was to determine the frequency of infection by *E.histolytica* and *G.lamblia* in Khartoum hospital .Hundred patients were included in this study, 61 were males and 39 were females with different age ranging from 1-70 years. Stool samples were collected from all patients. The stool samples were examined to detect the cyst of *E.histolytica* and *G.lamblia* by using wet preparation and count the cyst by using concentration technique (formal ether concentration technique).

22 out of 100 were infected with *E.histolytica* and 14 out of 100 were infected with *G.lamblia.*

30 out of 100 have previous infection with *E.histolytica* and 14 out of 100 have previous infection with *G.lamblia*. The infection increase among the age group (11-20) years. The infection was high among patients with low education level (48%).

Statistically, there was no relationship between infection and age, sex, education level and type of latrine ($P > 0.05$).

ملخص الدراسة

أجريت هذه الدراسة في مدينة الخرطوم في الفترة بين شهر اكتوبر 2013 إلي شهر سبتمبر 2012 وقد هدفت الدراسة لتحديد مدي الإصابة بالدسنتاريا والقارضيا وتضمنت هذه الدراسة 100 شخص منهم 61 من الرجال و39 من النساء تتراوح أعمارهم بين 1-70 سنة. عينات الفسحة تم جمعها من جميع الأشخاص كما تم الحصول علي بيانات تتعلق بموضوع الدراسة وتم تسجيلها .

عينات الفسحة تم فحصها بإستخدام طريقة التحضير الرطب وطريقة التركيز بإستخدام محلول الفورمال إيثر.22 شخص من أصل كانوا100 مصابين بالدسنتاريا الأميبية و14 من أصل 100 كانوا مصابين بالقارضيا.

30 من اصل 100شخص كانت لديهم إصابة مسبقة بالدسنتاريا و14 من اصل 100 شخص كانت أيضا لديهم إصابة مسبقة بالقارضيا .

الإصابة كانت تتركز في الأعمار التي تتراوح من 11 الي 20 سنة ومعظم المصابين مستوي التعليم لديهم منخفض .

إحصائيا خلصت هذه الدراسة الي انه ليس هناك علاقة بين الإصابة والعمر ,الجنس, المستوي التعليمي ونوع دورات المياه المتواجدة في المنازل حيث كانت (القيمة المعنوية اكبر من 05 .0).

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Chapter One

Introduction and literature review

1.1 *Entamoeba histolytica*

1.1.1 Definition:

Entamoeba histolytica, the causative agent of amoebaisis, is believed to infect more than 10% of the world's population with majority of infection occurring in the developing world (Petri *et al*., 2003). The parasite has been reported as the third major cause of death attributable to parasitic infections globally, after malaria and schistosomiasis (Smith, 1995).

1.1.2 Etiology:

Amebiais, pronounced [ăˊmē-bīˊă-sis] is caused by an infection with the parasite *Entamoeba histolytica* (CDC, 2008).

Entamoeba histolytica is Protozoa which are unicellular organisms which can only divide in the host organism. Intestinal amebae with identical morphologic appearances can be one of three species, *E. histolytica* or *E. dispar* or *E. moshkovskii* (Petri *et al*., 2006). The latter two are non-pathogenic. Only the *E. histolytica* which is genetically distinct causes invasive disease. 90% of *Entamoeba* infections are due to *E. dispar* (www.cdc.gov, 2008). There are two distinct stages, the cyst stage and the trophozoite stage. The first stage is not motile but is very resistant to therapy while the latter is motile and is only active in the host and is sensitive to antibiotic therapy (Petri *et al*., 2006).

1.1.3 Scientific Classification:

Domain : Eukaryota

Phylum: Amoebozoa

Class: Archamoebae

Order: Amoebida

Genus: Entamoeba

Species: *E. histolytica*

Binomial Name: *Entamoeba histolytica*

(Loftus *et al.*, 2005).

1.1.4 Historical background:

In 1875, Fedor Losch first described amebiasis in St. Petersburg, Russia.11 years later, Kartulus, showed that the amoeba were a pathogen for the diarrhea and liver disease in patients with amebiasis. In 1891 doctor Johns Hopkins differentiated amoebic from bacterial dysentery. Over 20 years later, Walker and Sellards, working in the Phillipines were able to document the pathogenesis of the amebae. There may be up to 100,000 persons worldwide who die annually from amebic infections (Vinod *et al.*, 2008).

1.1.5 Epidemiology and distribution:

Occurs worldwide; the highest incidence and prevalence is found in areas with poor sanitation where as many as 80% of a population may be infected. Highest in children >5 years of age; more prevalent in males than in females; common in mental hospitals, prisons, orphanages .

Developing tropical areas such as Mexico, Central and South America, Asia, and Africa are at highest risk of infection with amebiasis.

Approximately 1% of the US population has an amebic infection.

Worldwide, estimates of infection are as high as 50,000,000 people annually. Outbreaks are common in endemic areas of the world,

especially associated with the occurrence of natural disasters which affect the ability to provide clean water and which affect hygene.

(Vinod *et al*., 2008)

1.1.6 Reservoirs:

Human beings are the reservoirs for the protozoa *Entamoeba histolytica* 90% of patients who become infected with *Entamoeba histolytica* will remain asymptomatic (Gathiram and Jackson, 1987). Immunosuppression with steroids or due to other reasons increase the risk of invasive disease. Incubation of the disease may be from 2 days to years, although it usually from within days to months (Harrison, 2010).

1.1.7 Transmission:

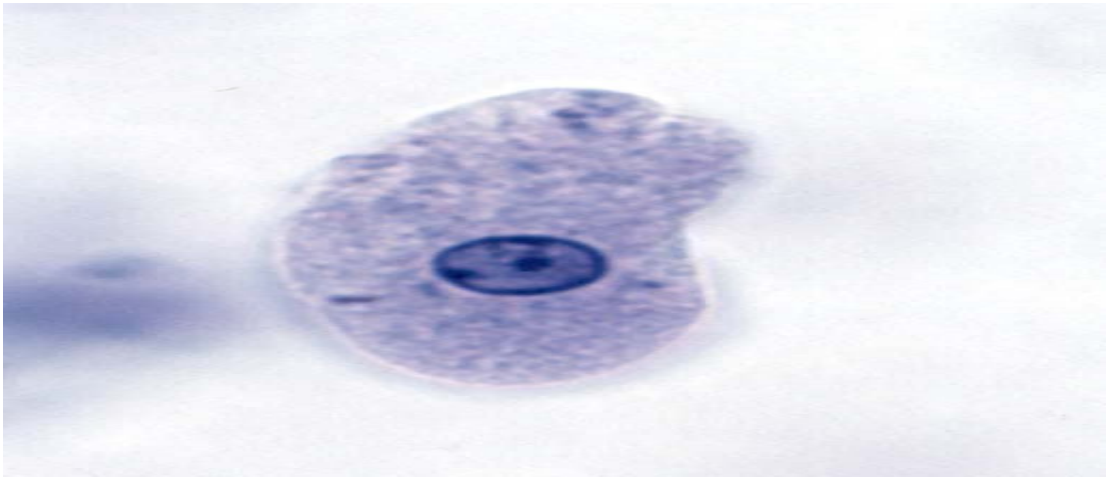
Amebiais is spread in humans ingestion of fecally contaminated food or water or through oral-anal sex, anilingus (Bobbi *et al.*, 2008). The food is usually uncooked food such as salads or vegetables, although contaminated water may be the source. Poor hand washing is also a factor in transmission of the disease. Inadequate sanitation and crowding increase the risk of infection. The cysts are ingested and undergo encystations in the small intestine and then divide into trophozoites which invade the colonic mucosa. Trophozoites are killed in the stomach if ingested, while the cysts are resistant and pass to the small intestine unaffected (James, 1982).

1.1.8 Morphology:

1.1.8.1 Trophozoite:

Measure 10–60 micrometers (cm). Trophozoite cytoplasm is finely granular, and may contain inclusions. The presence of red blood cells is diagnostic for *E.histolytica*. The single nucleus has peripheral nuclear chromatin, which is usually evenly distributed in abead like arrangement, with a small, central, compact karyosome. Movement is progressive and

purposeful, and is characterized by protrusion of a clear pseudopod (Garcia and Bruckner, 1997).



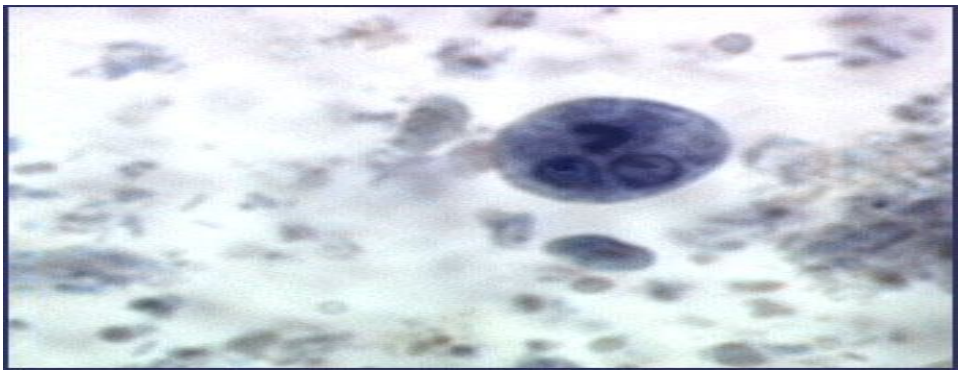
Figure(1.1):Trophozoite of E.histolytica Source: www.metapathogen.com

1.1.8.2 Cyst:

The spherical cyst of *E. histolytica* measures 10–20 cm .The mature cyst contains four nuclei, while the immature cyst may contain fewer than four. The presence of evenly distributed peripheral nuclear chromatin is important in the identification of *E. histolytica*. All species of the genus *Entamoeba*, but no other genus of amebae, possess peripheral nuclear chromatin in different arrangements. The karyosome is tiny and usually centrally located with evenly distributed peripheral nuclear chromatin.

The elongated chromatoidal bars have blunt, smooth, rounded ends.

Glycogen vacuoles may be present in immature cysts, but usually disappear as cysts mature (Garcia and Bruckner, 1997).



Figure(1.2):Cyst of E.histolytica Source:www.metapathogen.com

1.1.9 Life cycle of *E. histolytica:*

The organism exists in two forms, the trophozoite or the dividing form and the cyst which is the dormant form. Human infection usually begins with the ingestion of the cyst which is present in food and/or water contaminated with human fecal material. Cysts survive the acidic pH of the stomach and pass into the intestine. In the ileo-cecal region, cysts undergo excystment and each cyst gives rise to eight trophozoites. These migrate to and multiply in the colon. In most cases, trophozoites in the intestine live as commensals. Occasionally, however, trophozoites attack and invade the intestinal mucosa causing dysentery and/or progress through the blood vessels to extra-intestinal locations like liver, brain and lungs, where they may form life-threatening abscesses. In the intestine many of the trophozoites encyst and produce quadrinucleated cysts. Both trophozoites and cysts are excreted along with the feces. Cysts can survive for prolonged periods outside the host while the trophozoites survive only for a few hours. Trophozoites play no role in transmission of the disease but are responsible for producing tissue pathology. The reservoir of human infection is the "carrier" or asymptomatic human host who continuously passes cysts (Bhattacharya *et al.,* 1992).

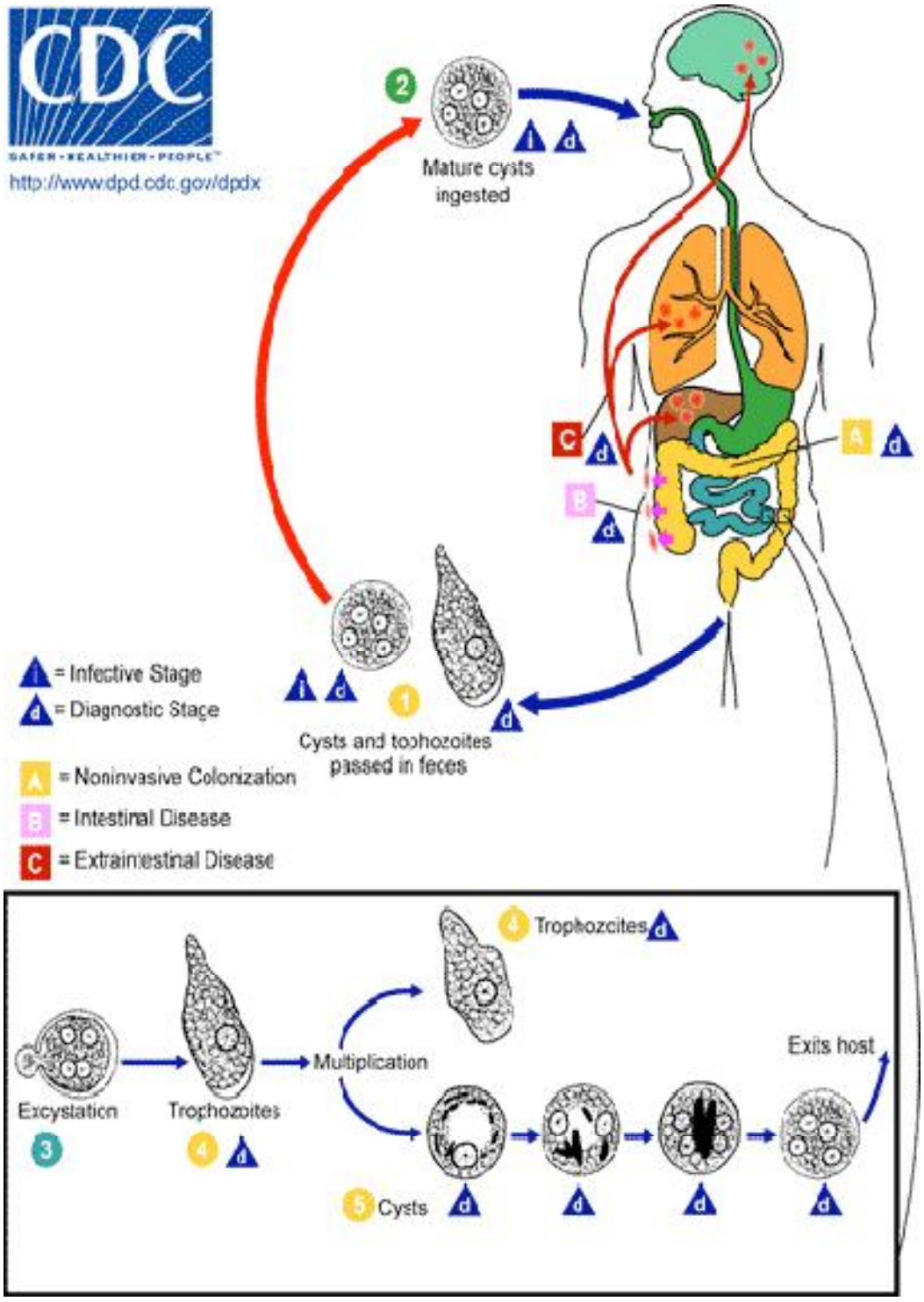


Figure (1.3):Life cycle of E.histolytica Source:www.dpd.cdc.gov,2007

1.1.10 Signs and Symptoms:

While in most, the disease is asymptomatic, symptoms often start with a mild diarrhea and lower abdominal pain. This may progress to malaise, weight loss and increasing abdominal pain. The pain may mimic acute appendicitis. The diarrhea will progress to frank dysentery with bloody mucus like watery diarrhea passing up to 20 times daily as infiltration of the colon wall increases. As opposed to bacterial enteritis, less than 40% of patients will become febrile unless fulminate intestinal infection occurs which may cause high fevers, severe abdominal pain with profuse diarrhea. The patient may develop a toxic megacolon (Harrison's 2010) Patients with chronic disease may present like those with inflammatory bowel disease or may present with an asymptomatic abdominal mass which is intraluminal and may be difficult to distinguish from a carcinoma (Radvin *et al*., 1995). They may have diarrhea every fewdays alternating with constipation as well as weakness and weight loss.

Extra-intestinal disease most often presents in the liver. Spread to the liver is through the portal circulation. Abcesses are solitary in around 2/3rds of cases and 80% of the time, these abscesses are found in the right lobe of the liver. Jaundice is present in up to 1/3rd of patients and is usually mild. The liver is usually enlarged and tender. Aspiration rarely shows the trophozoites or white blood cells. Spread to the liver is more common in males than females for an unknown reason (James, 1982).

1.1.11 Pathogenicity of amoebiasis:

E. histolytica causes intestinal and extraintestinal amoebiasis based on the site of infection. Though most infections do not harm the host (asymptomatic infections), establishment in the colonic mucosa via the Galactose/N-acetyl Galactosamine inhibitable lectin (Gal-lectin) is a pre-requisite for the disease (Chadee *et al*., 1987). Pathogenic forms of the parasite are known to secrete enzymes that facilitate their invasion into the mucosa and sub-mucosa causing deep-flask shaped ulcers and in some cases entering the circulation and reaching internal organs like the liver, lungs, skin, etc.

The disease in the colon is the most common with acute diarrhoea and dysentery accounting for 90% of the clinical amoebiasis cases (Espinosa and Martnez, 2000) and only 1% involve the liver (Haque *et al*., 2003).

1.1.11.1 Asymptomatic colonization:

Asymptomatic infections are characterized by the parasite living in perfect harmony within the host. *E. histolytica* trophozoites have developed elusive tactics to prevent them from being purged from the host By modulating signals by intestinal epithelial cells (IEC), trophozoites direct anti-inflammatory host responses leading to a tolerogenic/hyporesponsive immune state favourable to their survival (Kammanadiminti *et al*., 2006).

1.1.11.2 Intestinal amoebiasis:

After an incubation period of 1-4 weeks, the parasite invade the colonic mucosa, producing characteristic ulcerative lesions and a profuse bloody diarrhea (amoebic dysentery). Amoebic invasion through the mucosa and into the submucosa is the hallmark of amoebic colitis. Contact of the trophozoites via the Gal/GalNAc lectin triggers a signaling cascade initiating the death of the host cell through different mechanisms such as phagocytosis, cytotoxicity and caspase activation instigating the invasive

(intestinal and/or extraintestinal) stages of the disease. Other molecules involved in the disease process include: a serine-rich *E.histolytica* protein (SREHP), amoebapores, and cysteine proteases (Boettner *et al*., 2002). Activation of damaging inflammatory and non inflammatory responses following contact of the trophozoites to the gut wall induces a massive neutrophil infiltration across the epithelium into the underlying tissues resulting in weakening of epithelial cells and the mucous layer and allowing trophozoites to invade the intestinal epithelium and disseminating to other bodily sites (Ackers, 2000).

The ulcers formed may be generalized involving the whole length of the large intestine or they may be localized in the ileo-caecal or sigmoido-rectal regions. Ulcers are normally disconnected with sizes varying from pin-head size to more than 2.5 cm in diameter. They may be deep or superficial. Base of the deep ulcers is generally formed by the muscularis layer. Nonetheless, superficial ulcers do not extend beyond the muscularis layer.

Alarge number of fatalities results from perforated colons with concomitant peritonitis. *E. histolytica* also causes amoebomas. These are pseudotumoural lesions, whose formation is associated with necrosis, inflammation and oedema of the mucosa and submucosa of the colon. These granulomatous masses may obstruct the bowel.

While the serine rich *E. histolytica* protein (SREHP) have been shown to promote adhesion of the trophozoites to host cells, cysteine proteases (CP), are known for their virulence in other protozoa as well as in tumour metastasis. Five *E. histolytica* proteins (EhCP1, 2, 3, 5 and 112) have been identified. All are alleged to play a role in the destruction of host cells, phagocytosis, together with the recruitment of neutrophils and macrophages and the induction of intestinal inflammation (Mortimer and Chadee, 2010).

Although most intestinal invasions heal following an acute inflammatory response, *E.histolytica* evades destruction in a modest number of individuals and a chronic state is established. This chronic state is associated with the development of a non-protective adaptive immune response. Human data, in vitro and in vivo models support a paradigm that Th1 responses in the gut clear *E. histolytica*, while Th2 responses through the production of IL-4 are anti-protective, likely through suppressing IFN-・. It is not yet clear what signals drive an anti-protective Th2 immune response instead of an effective protective Th1 response towards the infection.

1.1.11.3 Extraintestinal amoebiasis:

About 5% individuals with intestinal amoebiasis, 1-3 months after the disappearance of the dysenteric attack, develop extraintestinal amoebiasis. Once in the blood, the parasite uses many different strategies to avoid elimination by the host and reaches other sites in the body (such as the liver, lungs, brain, etc). The most common extraintestinal site affected by the parasite is the liver and an Amoebic liver abscess (ALA) is its most common manifestation, predominantly seen in adult males. This chronic stage of ALA is characterized by defective cell-mediated immunity and the suppression of T cells and their defective proliferative responses (Campbell *et al.*, 1999). *E. histolytica* trophozoites reaching the liver create their unique abscesses, which are well circumscribed regions of cytolysed liver cells, liquefied cells, and cellular debris. The lesions are surrounded by connective tissue enclosing few inflammatory cells and trophozoites. Parenchymal cells adjacent to the lesion are often unaffected. However, lysis of neutrophils by *E. histolytica* trophozoites might release mediators that lead to the death of liver cells, and extend damage to hepatocytes not in direct contact with the parasite. In ALA from humans, the small numbers of amoebas relative to the size of the

abscess suggests that *E. histolytica* can kill hepatocytes without direct contact .From the liver, *E. histolytica* trophozoites may enter into the general circulation and reach other organs (Stanley 2003).

1.1.12Diagnosis:

Intestinal amoebiasis:

Laboratory confirmation of intestinal amoebiasis is by:

Examination of afresh dysenteric faecal specimen or rectal scraping for motile *E.histolytica* amoebae.

The finding of motile amoebae containing red cells is diagnostic of amoebic dysentery.

Examination of formed or semiformed faeces for *E.histolytica* cysts.

The finding of cysts indicates infection with either pathogenic or non pathogenic strain of *E.histolytica*.

1- Examination of faeces for *E.histolytica* cysts:

the cyst may be found in formed and semiformed specimen by using:

1-Direct method (wet preparation) of examining faeces for *E.histolytica* cyst.

2- Concentration technique:

E.histolytica cyst can be concentrated by the Formal Ether Technique, Formal Detergent Sedimentation Technique (overnight sedimentation) or by Zinc Sulphate Flotation Technique.

2- Examination of faeces or rectal scraping for *E.histolytica* amoebae:

Specimen : the specimen should be examined as soon as possible after collected (within 15 minutes).it should be kept in a warm environment because amoeboid movement is reduced in cold surroundings.

Amoebic liver abscess:

Laboratory tests which are helpful in diagnosing amoebic liver abscess include:

1- Cellulose Acetate Precipitin (CAP) test to detect significantly raised levels of anti-*E.histolytica* antibodies.

This a simple inexpensive technique which is of value in confirming a diagnosis of amoebic liver abscess . It has high specificity with positive results being obtained only if invasive amoebasis is presnt . A positive result does not persist more than a few months after cure.

The CAP test relies on the surface diffusion of specific globulins and soluble antigens to produce a line of precipitation where they meet which can be visualized by staining.

Result:

A positive test is indicated by seeing a precipitin are between the serum and the antigen.

Negative result there is no precipitin are between the serum and antigen.

2- White Blood Cell (WBC) total count and differential count. A leucocytosis with neutrophilia is found in about 80% of patients with amoebic liver abscess.

3- Erythrocyte sedimentation rate (ESR) which is always raised usually over 50 mm / h.

4- Haemoglobin which is frequently low (usually normocytic , normochromic anaemia).

5- Serum alkaline phosphatase which is often raised.

6- Serum albumin which is reduced in some patients.

Specific tests for identification

Microscopy does not distinguish between pathogenic and non pathogenic *Entamoeba histolytica*. Enzyme immunoassay or polymerase chain reaction can be used to detect the infection. The enzyme immunoassay is readily available as a commercial test and is as accurate as PCR examination.

Serologic tests do not distinguish new versus past infections and even in patients with amebic liver abcesses, these tests may be falsely negative initially for the first week when symptomatic.The indirect hemagglutination assay measures antibody titers for the galactose-inhibitable adherence lectin It is no more sensitive than the enzyme immunoassay test. Either one of these two tests takes about 2 hours to perform (Vinod *et al.*, 2008).

1.1.13 Treatment:

There are many kinds of effective drugs:

1.1.13.1 Intestinal Infection:

Usually nitroimidazole derivatives are used because they are highly effective against the trophozoite form of the amoeba. Since they have little effect on amoeba cysts, usually this treatment is followed by an agent (such as paromomycin or diloxanide furoate) that acts on the organism in the lumen (Caler and lorenzi, 2010).

1.1.13.2 Liver abscess:

In addition to targeting organisms in solid tissue, primarily with drugs like metronidazole and chloroquine, treatment of liver abscess must include agents that act in the lumen of the intestine (as in the preceding paragraph) to avoid re-invasion. Surgical drainage is usually not necessary except when rupture is imminent (Caler and lorenzi, 2010).

1.1.13.3 Asymptomatic Patients:

For asymptomatic patients (otherwise known as carriers, with no symptoms), non endemic areas should be treated by paromomycin, and other treatments include diloxanide furoate and iodoquinol. There have been problems with the use of iodoquinol and iodochlorhydroxyquin, so

their use is not recommended. Diloxanide furoate can also be used by mildly symptomatic persons who are just passing cysts (Caler and lorenzi, 2010).

1.1.14 Control:

- Treatment of patients.
- Examination and treatment of food handlers.
- Environmental sanitation.
- Personal prophylaxis.
- Human faeces should not be used as fertilizers (Catherine *et al*., 2007).

1.1.15 Vaccination:

Currently there is no vaccine available for amebiasis in humans. However, in 2007 a vaccine was developed which was tested in gerbils and confirmed immunity. The vaccine was designed to induce an immune response to the surface lecithin responsible for adherence of the ameboe to the intestinal wall (Catherine *et al*., 2007).

1.2 Giardia lamblia

1.2.1 Definition:

Giardiasis, a gastrointestinal disease characterized by acute or chronic diarrhea, is caused by protozoan parasites in the genus *Giardia*. *Giardia duodenalis* is the major species found in mammals, and the only species known to cause illness in humans (Dawson, 2005).

1.2.2 Etology:

The protozoan genus *Giardia* (Family Giardiidae, order Giardiida) contains at least six species that infect animals and/or humans. In most mammals, giardiasis is caused by *Giardia duodenalis*, which is also called *G. intestinalis* (Abe *et al.*, 2010).

1.2.3 Scientific Classification:

Domain: Eukaryota

Phylum: Metamonada

Order: Diplomonadida

Family: Hexamitidae

Genus: Giardia

Species: *G.* lamblia

Binomial name:

Giardia lamblia

Synonyms:

Giardia intestinalis

Lamblia intestinalis

Giardia duodenalis

(Loftus *et al.*, 2005).

1.2.4 History:

The trophozoite form of *Giardia* was first observed in 1681 by Antonie van Leeuwenhoek in his own diarrhea stools. The genus was chosen to honour Professor Alfred Mathieu Giard of Pari.

The names for the human parasite *Giardia duodenalis, Giardia lamblia and Giardia intestinalis* are all in common current use despite the potential for confusion this has created.

Van Leeuwenhoek's observations were recreated, using a single-lens microscope of the kind he used, by British microbiologist Brian J. Ford, who showed how clearly one could view *Giardia* through a primitive microscope(Betancourt and Rose, 2004).

In 1998, a highly publicised *Giardia* and *Cryptosporidium* outbreak was reported in Sydney, Australia, but it was found to be due to mismeasurement of the concentrations of microbes in the water supply. A 2004 outbreak in Bergen (Norway) hastened work on adding UV treatment to the water facilities (Betancourt and Rose, 2004).

In October 2007, *Giardia* was found in the water supply for parts of Oslo, prompting authorities to advise the public to boil drinking water;[32] but subsequent test showed levels of contamination too low to pose a threat, so this advice has since been cancelled.

In 2008, *Giardia* was identified as one of the causes of the dysentery afflicting Crusaders in Palestine in the 12th and 13th centuries (Betancourt and Rose, 2004).

1.2.5 Epidemiology:

Giardia is a common cause of gastrointestinal disturbance in both high- and low-income countries. The incidence of *Giardia* is generally higher in low-income countries (e.g. many countries of Africa, Asia, and South and Central America) where access to clean water and basic sanitation is lacking. Nearly all children in this setting will acquire *Giardia* at some point in their childhood, and the prevalence of the parasite in young children can be as high as 10%-30%. In areas such as Western Europe and the United States of America, *Giardia* infection is associated with ingestion of contaminated water, person-to-person spread, recent foreign travel, and recreational swimming. *Giardia* may be a cause of 2-5% of cases of diarrhoea in high-income countries (Hill and Nash, 2006).

1.2.6 Transmission:

Giardia can be found in humans and many non human mammalian reservoirs such as sheep and cattle. The role of non-human mammals in transmission of *Giardia* to humans remains unclear.

Infection is acquired via the faecal-oral route often through the ingestion of *Giardia* cysts from faecally-contaminated water. Person-to-person transmission occurs in conditions of poor faecal-oral hygiene, particularly in low-income settings amongst children, between young children in day care facilities, and amongst men who have sex with men. Transmission of *Giardia* via food is uncommon (CDC, 2012).

1.2.7 Morphology:

1.2.7.1 Trophozoite:

- Four pairs of flagella - one pair located anterior, two pair located ventral, and one pair located posterior. An axostyle and parabasal bodies

are present. Motility resembles a “falling leaf” uses “sucking discs” to adhere to intestinal wall; interferes with absorption of nutrients (Garcia and Bruckner, 1997).

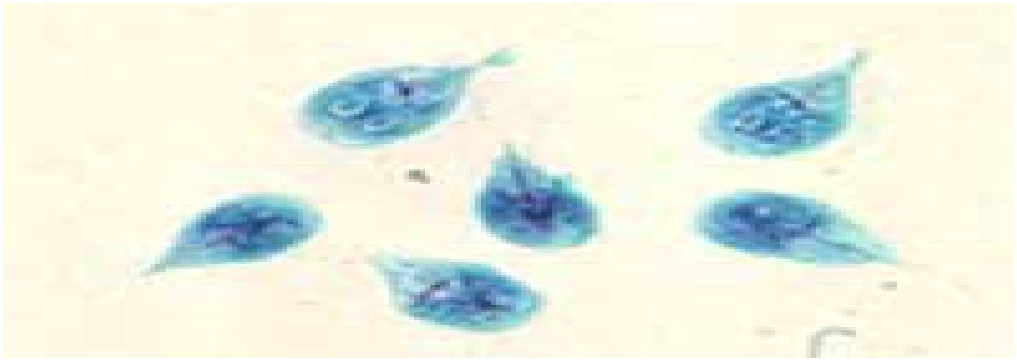


Figure (1.4):Trophozoite of Giardia lamblia Source: msd.com.mx

1.2.7.2 Cyst

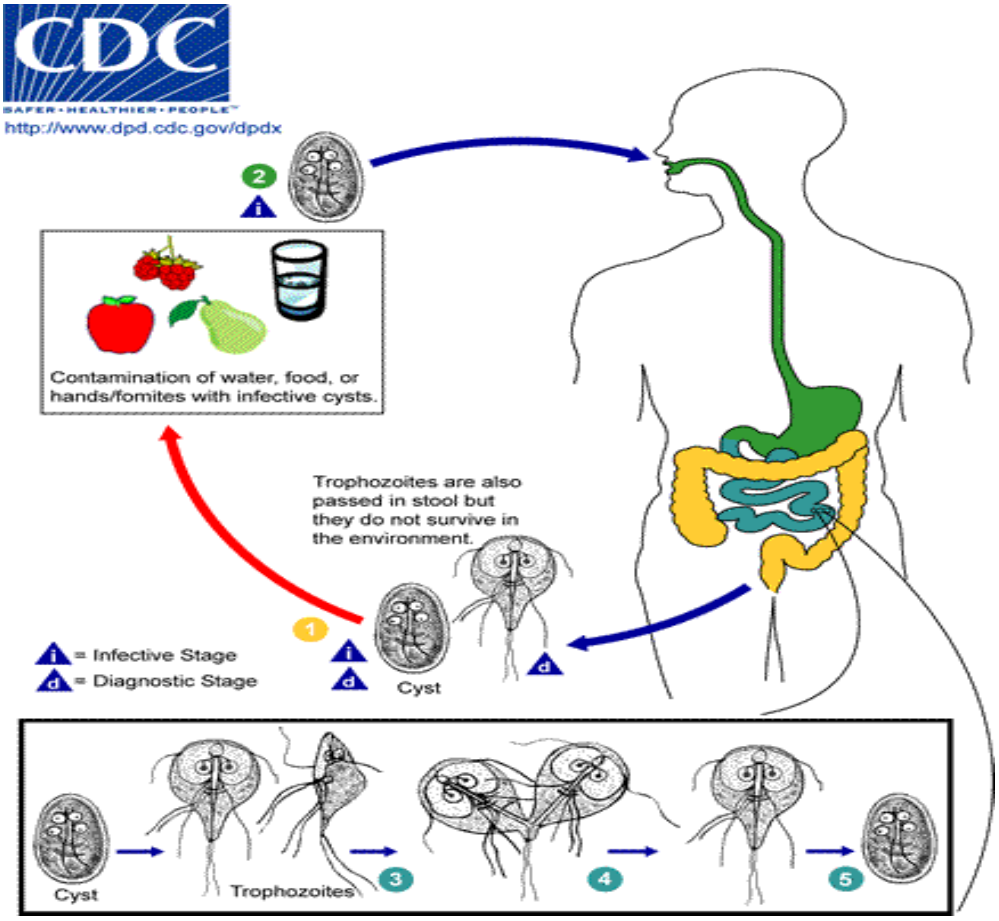
- Measures 9 x 12 micrometers and contain 2 to 4 nuclei; the karyosome is centrally located, with little or no peripheral chromatin; parabasal bodies are present (Garcia and Bruckner, 1997).

Figure (1.5):Cyst of Giardia lamblia Source: msd.com.mx

1.2.8 Life cycle of *Giardia lamblia:*

The life cycle begins with a non infective cyst being excreted with the faeces of an infected individual. The cyst is hardy, providing protection from various degrees of heat and cold, desiccation, and infection from other organisms. Once ingested by a host, the trophozoite emerges to an

active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission. The resulting trophozoites and cysts then pass through the digestive system in the faeces. While the trophozoites may be found in the faeces, only the cysts are capable of surviving outside of the host (Tovar *et al*., 2003).



Figure(1.6):Life cycle of Giardia lamblia Source:www.dpd.cdc.gov,2007

1.2.9 Signs and Symptoms:

Most cases of giardiasis are asymptomatic. In those that do experience clinical illness, the incubation period is usually between 1 and 2 weeks. Therefore symptoms may begin after a traveller has returned home. The most common symptoms are a gradual onset of nausea, anorexia and

diarrhoea, accompanied by abdominal cramps, bloating and flatulence. Diarrhoea can persist for several days or weeks and be accompanied by weight loss and lactose intolerance. Severe cases can be associated with malabsorption. Less common are vomiting and fever. Urticaria is seen rarely.

Symptoms often last for more than 10 days and sometimes longer than a month and may come and go (Hill and Nash, 2006).

1.2.10 Pathogenesis:

Infection with *G.lamblia* is initiated by ingestion of cysts. Gastric acid stimulates excystation, with the release of trophozoites in duodenum and jejunum. The trophozoites can attach to the intestinal villi by the ventral sucking discs without penetration of the mucosa lining, but they only feed on the mucous secretions. In symptomatic patients, however, mucosa-lining irritation may cause increased mucous secretion and dehydration. Metastatic spread of disease beyond the GIT is very rare *(*Pavl *et al.*, 1984).

1.2.11 Clinical features:

Clinical disease**:** Giardiasis

Symptomatic giardiasis ranges from mild diarrhea to severe malabsorption syndrome. Usually, the onset of the disease is sudden and consists of foul smelling, watery diarrhea, abdominal cramps, flatulence, and streatorrhoea. Blood & pus are rarely present in stool specimens, a feature consistent with the absence of tissue destruction (Pavl *et al.*, 1984).

1.2.12 Laboratory diagnosis:

Giardiasis can be diagnosed by direct observation of the trophozoites or cysts in the feces. Either stained preparations (e.g., preserved with polyvinyl alcohol or 10% formalin) or unstained wet mounts can be used.

Because they are small and can resemble other fecal components, *Giardia* cysts and trophozoites can sometimes be difficult to identify by morphology alone.

Direct smears or fecal wet mounts can be used to look for trophozoites. This stage usually observed only in fresh, watery stools.Cysts can be found in formed as well as unformed stools. Various flotation or sedimentation processes can be used to concentrate the cysts. Repeated sampling may be necessary when there are few organisms. Because shedding occurs intermittently; cysts are more likely to be found if specimens from 3 different days are examined.

If chronic giardiasis is suspected, but repeated stool examinations are negative, the intestinal contents can be examined directly for trophozoites. One technique is the “string test” (Entero-test), in which the patient swallows a gelatin capsule on a string, and the string is later retrieved and examined for trophozoites. Aspiration of duodenal contents has also been used.

Infections can also be diagnosed by enzyme-linked immunosorbent assays (ELISAs) and immunochromatographic tests to detect *G. duodenalis* antigens in the feces, as well as by direct-immunofluorescence. Antigen shedding may persist for weeks after elimination of the parasite. Rapid tests such as ELISAs should supplement but not replace routine ova and parasite examination by microscopy, as the latter test can also diagnose other diseases. PCR assays can detect *Giardia* in clinical samples. Genetic characterization of isolates at the assemblage level is usually employed only in epidemiological studies and research.

Serology has been used in epidemiologic investigations and other research. *Giardia* can be cultured *in vitro*, but this technique is used only in research (Dryden *et al.*, 2006).

1.2.13 Treatment:

For asymptomatic carriers and diseased patients the drug of choice is quinacrine hydrochloride or metronidazole (Caler and Lorenzi, 2010).

1.2.14 Prevention and control:

- Asymptomatic reservoirs of infection should be identified & treated.
- Avoidance of contaminated food and water.
- Drinking water from lakes and streams should be boiled, filtered and/or iodine treated.
- Proper waste disposal and use of latrine (Catherine *et al*., 2007).

Rationale

E.histolytica and *G.lamblia* infection continues to be a major health problem in developing countries. The most cases of infection are asymptomatic. The infection associated with low socioeconomic status and poor hygiene.

The seriousness of the complication of infection with *E.histolytica* is liver abscess.

Diagnosis is usually performed using wet preparation which may lead to false negative result. The use of more sensitive and advanced techniques must be used.

Objectives

General objective:

To determine the frequency of *E.histolytica* and *G.lamblia* among the patients attending Khartoum hospital.

Specific objectives

-To detect cyst of *E.histolytica* and *G.lamblia* by using wet - preparation .

-To count number of cysts of *E.histolytica* and *G.lamblia* by using concentration technique (formal ether concentration technique).

Chapter Tow

Materials and methods

2.1 Study design

It is a cross sectional study.

2.2 Study population

Males and females with different ages and occupations residing in different locations in Khartoum city.

2.3 Study area

The study was conducted in Khartoum state.

2.4 Sample size

Seventy patients have been included in this study. Stool samples from all patients were collected.

2.5 Methods

Practical aspect of the study include performance of the following test:

Stool examination:

E.histolytica and *G.lamblia* were detected in stool samples using wet preparation and concentration technique .

2.5.1 Wet preparation

2.5.1.1 Reagents and materials

Stool containers.

Wood stick.

Slides .

Cover glass.

Normal saline.

Iodine.

Olympus microscope.

2.5.1.2 Procedure

Stool samples were collected in clean dry containers. Adrop of fresh physiological saline was placed on a slide. A small amount of stool was placed on the slide and mixed by wood stick to make smooth thin preparation which was then covered with cover glass. Slides were examined under microscope (x10, x40) (Cheesbrogh, 1987).

2.5.2 Concentration technique:

Formal ether concentration technique:

2.5.2.1 Reagents and materials:

Stool containers.

Slides.

Cover glass.

Formal saline (10% v/v).

Ether (diethyl).

Sieve with small holes (400-450µm).

Conical tubes.

Centrifuge.

Olympus microscope.

2.5.2.2 Procedure

1 gram of stool was estimated and emulsified in 4 ml of 10% formal water. Then further 4 ml of formal saline was added, mixed by shaking for 20 second. The emulsified faeces was sieved.The sieved suspension was collected in a beaker then transferred to conical tube. Equal volume of ether was added, mixed for 1 minute. Centrifuge was used at 3000 rpm for 1 minute. The tube was inverted to discard the ether, faecal debris and formal saline. A Pasteur pipette was used to mix the sediment, then sediment was transferred to slide and covered with cover glass, examined microscopically using x10, x40 and the number of parasites was counted (Cheesbrogh, 1987).

2.5.2.3 Result:

Number of parasite /one gram of faeces

2.6 Ethical consideration:

Informed concepts will be taken from study subjects after explaining the study purpose.

2.7 Data analysis:

All information and data was been interred in computer for analysis using Statistical Package Social Science (SPSS) version 14.

Chapter three

Results

3.1 General characteristics of the studied population

The age of the study subjects included in the present study ranged between 5-64 years, mean of age was 22 years. The age was divided into 7 groups: 1-10, 11-20, 21-30, 31-40, 41-50, 51-60 and 61-70 years (table 3.1). Male: female ratio was 61:39 (61% and 39% respectively) (table 3.2).

Table 3.1:Age groups of studied population

	Age	Frequency	Percentage
	1-10	10	10.0
	11-20	51	51.0
	21-30	13	13.0
	31-40	13	13.0
	41-50	9	9.0
	51-60	3	3.0
	61-70	1	1.0
	Total	100	100.0

Table 3.2: Frequency of gender

Gender	Frequency
Male	**61**
Female	**39**
Total	**100**

3.2 Detection of *E.hostolytica* and *G.lamblia* in stool sample

Atotal of 100 stool samples were examined for *E.hitolytica* and *G.lamblia*. All samples were examined by using wet preparation. 22 samples were positive for *E.histolytica* and 14 samples were positive for *G.lamblia* (tables 3.3 and 3.4).

Table 3.3: Frequency of infection with E.histolytica

Result	Frequency
+ve	**22**
-ve	**78**
Total	**100**

Table 3.4: Frequency of infection with G.lamblia

Result	Frequency
+ve	**14**
-ve	**86**
Total	**100**

3.3 Previous infection with *E.histolytica* and *G.lamblia*

Thirty out of 100 (30%) have previous infection with *E.histolytica* and 14 (14%) have previous infection with *G.lamblia* 56 (56%) while 56 (56%) have no previous infection with *E.histolytica* or *G.lamblia* (table 3.5).

Table 3.5: Frequency of previous infection

Previous infection	Frequency
Yes *(E.histolytica)*	**30**
Yes (*G.lamblia*)	**14**
No	**56**
Total	**100**

3.4 Duration of infection:

Sixteen out of 30 (16%) which have previous infection with *E.histolytica* the duration of infection was less than 5 months ,3 (3%) between 5 to 11 months and 11(11%) more than 12 months (table 3.6)

Table3.6: Frequency of Duration of infection with E.histolytica

Duration of infection	Frequency
Less than 5 month	**16**
Between 5 to 11 month	**3**
More than 12 month	**11**
Total	**30**

Seven out of 14(7%) who have previous infection with *G.lamblia* the duration of infection was less than 5 months , 4 (4%) between 5 to 11 months while 3 (3%) more than 12 months (table 3.7).

Table 3.7: Frequency of Duration of infection with G.lamblia

Duration of infection	Frequency
Less than 5 month	**7**
Between 5 to 11 month	**4**
More than 12 month	**3**
Total	**14**

3.5 Education level

Thirty one out of 100 (31%) have received high level of education, 21 (21%) have received medium level of education while 48 (48%) have received low level of education (table 3.8).

Table 3.8: Frequency of education level

Level	Frequency
High	**31**
Medium	**21**
Low	**48**
Total	**100**

3.6 The relationship between wet preparation of *E.histolytica* and concentration technique

The relationship between wet preparation of *E.histolytica* and concentration technique was detected by using Chi- squire test when there was less than 30 cyst was 7, between 31 to 50 cyst was 9 while more than 51 cyst was 6 (table 3.9).There was six samples which were negative by wet preparation but positive by concentration technique, 2 samples the count of cyst was more than 51 cyst and 4 samples the count of cyst was less than 30 cyst.

Table 3.9: The relationship between wet preparation of E.histolytica and concentration technique

Wet preparation	**Concentration technique**			**Total**	**P.value**
	more than 51 cyst	between 31 to 50 cyst	less than 30 cyst		
+ve	6	9	7	22	**.15**
-ve	2	0	4	6	
Total	8	9	12	28	

3.7 The relationship between wet preparation of *G.lamblia* and concentration technique

The relationship between wet preparation of *G.lamblia* and concentration technique was detected by using Chi- squire test when there was less than 30 cyst was 9, between 31 to 50 cyst was 3 while more than 51 cyst was 1(table 3.10). There was one sample which was negative by wet

preparation but positive by concentration technique and the count of cyst was 31 to 50 cyst.

Table 3.10: The relationship between wet preparation of G.lamblia and concentration technique

Wet preparation	**Concentration technique**			**Total**	**P.value**
	more than 51 cyst	between 31 to 50 cyst	less than 30 cyst		
+ve	9	3	1	13	
-ve	0	1	0	1	**.26**
Total	9	4	0	14	

3.8 The relationship between infection with *E.histolytica* and age group

The relationship between infection with *E.histolytica* and age group of studied population was detected by using Chi- squire test (table 3.11).

Table 3.11: The relationship between infection with E.histolytica and age group

Infection	**Age group**							**Total**	**P.value**
	1-10	11-20	21-30	31-40	41-50	51-60	61-70		
+ve	2	10	4	3	1	2	0	22	**.52**
-ve	8	41	9	10	8	1	1	78	
Total	**10**	**51**	**13**	**13**	**9**	**3**	**1**	**100**	

3.9 The relationship between infection with *G.lamblia* and age group:

The relationship between infection with *G.lamblia* and age group of studied population was detected by using Chi- squire test (table 3.12).

Table 3.12: The relationship between infection with G.lamblia and age group

Infection	Age group							Total	P.value
	1-10	11-20	21-30	31-40	41-50	51-60	61-70		
+ve	2	10	1	1	0	0	0	14	**.59**
-ve	8	41	12	12	9	3	1	86	
Total	**10**	**51**	**13**	**13**	**9**	**3**	**1**	**100**	

3.10 The relationship between infection with *E.histolytica* and gender:

The relationship between infection with *E.histolytica* and gender of studied population was detected by using Chi- squire test (table 3.13). Infected males were 9 out of 22 while infected females were 13 out of 22.

Table 3.13: The relationship between infection with E.histolytica and gender

Gender	Infection by *E.histolytica*		Total	P.value
	+ve	-ve		
Male	9	52	61	**.09**
Female	13	26	39	
Total	22	78	100	

3.11The relationship between infection with *G.lamblia* and gender:

The relationship between infection with *G.lamblia* and gender of studied population was detected by using Chi- squire test (table 3.14). Infected males were 10 out of 14 while infected females were 4 out of 14.

Table 3.14: The relationship between infection with G.lamblia and gender

Gender	**Infection by *G.lamblia***		**Total**	**P.value**
	+ve	-ve		
Male	10	51	61	**.38**
Female	4	35	39	
Total	14	86	100	

3.12 The relationship between infection with *E.histolytica* and educational level:

The relationship between infection with *E.histolytica* and educational level was detected by using Chi- squire test (table 3.15). 7 out of 22 have high level of education, 5 out of 22 have medium level of education and 10 out of 22 have low level of education.

Table 3.15: The relationship between infection with E.histolytica and educational level

Education level	**Infection by *E.histolytica***		**Total**	**P.value**
	+ve	-ve		**.95**
High	7	24	31	
Medium	5	16	21	
Low	10	38	48	
Total	22	78	100	

3.13 The relationship between infection with *G.lmblia* and educational level:

The relationship between infection with *G.lmblia* and educational level was detected by using Chi- squire test (table 3.16).

4 out of 14have high level of education, 0 out of 14 have medium level of education and 10 out of 14 have low level of education.

Table 3.16: The relationship between infection with G.lamblia and educational level

Education level	**Infection by *G.lamblia***		**Total**	**P.value**
	+ve	-ve		
High	4	27	31	**.07**
Medium	0	21	21	
Low	10	38	48	
Total	14	86	100	

3.14 The relationship between infection with *E.histolytica* and type of latrine:

The relationship between infection with *E.histolytica* and latrine was detected by using Chi- squire test (table 3.17). 13 out of 22 have primary latrines while 9 out of 22 have modified latrines.

Table 3.17: The relationship between infection with E.histolytica and type of latrine

Type of latrine	**Infection by *E.histolytica***		**Total**	**P.value**
	+ve	-ve		
Primary	13	37	48	**.83**
Modified	9	41	52	
Total	22	78	100	

3.15 The relationship between infection with *G.lamblia* and type of latrine:

The relationship between infection with *G.lamblia* and latrine was detected by using Chi- squire test (table 3.18). 7 out of 14 have primary latrines while 7 out of 14 have modified latrines.

Table 3.18: The relationship between infection with G.lamblia and type of latrine

Type of latrine	**Infection by *G.lamblia***		**total**	**P.value**
	+ve	-ve		**.87**
Primary	7	41	48	
Modified	7	45	52	
Total	14	86	100	

Chapter four

Disscussion

Amoebiasis caused by the intestinal parasite *Entamoeba histolytica*, has an estimated worldwide prevalence of 500 million infected people and is responsible for 40,000 - 100,000 deaths each year. It is an important health problems, especially in developing countries (Sebastiaan *et al.*, 2007).

Giardiasis, caused by *Giardia lamblia*, is a frequent cause of diarrhea that can have a negative impact on growth and development of children and affects approximately 200 million people worldwide (WHO, 1997).

This study was conducted in Khartoum city .For this purpose, 100 stool samples were collected .The cysts of *E.hisotlytica* and *G.lamblia* were detected by using wet preparation and concentration technique (formal ether concentration technique).

The results of this study showed that male : female ratio was 61:39 as the samples were collected randomly.

The infection of *E.histolytica* and *G.lamblia* was high among the age group 11-20 years.

22 out of 100 were positive with *E.histolytica* and 14 out of 100 were positive with *G.lamblia*.

30 out of 100 have previous infection with *E.histolytica*. 16 out of 30 the infection was before less than 5 month, 3 out of 30 the infection was before 5 to 11 month and 11 out of 30 the infection was before more than 12 month and 22 still have infection that mean 8 patient were cleared from infection and the treatment was effective .

14 out of 100 have previous infection with *G.lamblia* and still have infection which means that they are not cleared from infection which migh indicate either that the treatment was not effective or it is a new infection. 7 out of 14, the infection was before less than 5 month, 4 out of 14 the infection was before 5 to 11 month and 3 out of 14 the infection was before more than 12 month.

There is no relationship between the infection with *E.histolytica* and age groups (P.value 0.52) which means that the infection was not affected by age groups. These result were not in agreement with previous study of Jamila (2014) in Jeddah city which showed that there is a relationship between the infection with *E.histolytica* and age groups and the prevalence of infection was high in age group 1 month-6 years and more than 6 years.

There is no relationship between the infection with *G.lamblia* and age groups (P.value 0.59) which means that the infection not affected by age groups.

There is no relationship between the infection by *E.histolytica* and gender (P.value 0.09) which means that the infection was not affected by gender. Thes results were not in agreement with previous study of Munazza *et al*.,(2011) in Pakistan who showed that there is relationship between infection with *E.histolytica* and gender and the infection was more in females than males (31.5% and 19.6% respectively).

There is no relationship between the infection with *G.lamblia* and gender (P.value 0.38) which means that the infection was not affected by gender. These results were not in agreement with previous study of Brelet , (2000) in Agboville area who showed a significant association between the prevalence of *G. lamblia* and sex .

There is no relationship between the infection with *E.histolytica* , *G.lamblia* and level of education (P.value .95 and.07 respectively). These result were in agreement with previous study of Albonico *et al* ,(1999) in Brazil who showed that there is no relationship between the infection of parasites and educational level.

There is no relationship between the infection with *E.histolytica* and *G.lamblia* and type of laterine (P.value 0.83 and 0.87 respectively). These results were in agreement with previous study of Albonico *et al.*, (1999) in Brazil who showed that there is no relationship between the infection of parasites and latrine .

All results in this study showed there is no relationship between infection and age, sex, education level and type of latrine. This in our opinion may probably be attributed to the small size of sample used in this study.

Chapter five

1.5 Conclusions

- The infection with *E.histolytica* and *G.lamblia* is not affected by age.

-The infection with *E.histolytica* and *G.lamblia* is not affected by sex.

-The infection with *E.histolytica* and *G.lamblia* is not affected by education level.

-The infection with *E.histolytica* and *G.lamblia* is not affected by type of latrine.

2.5 Recommendations

-For further study are has to increase the sample size.

-For detection of the cyst of *E.histolytica* and *G.lamblia* in stool sample by using wet preparation, more than one slide must be prepared and examined.

-Using advance techniques such as ELISA (Enzyme Linked Immune Sorbent Assye) and PCR (Polymearase Chain Reaction) for such studies.

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Appendices

Table of result

No	Sex	Age	Education level	Previous infection	Duration of infection	Type of latrine	*E.histolytica*	*G.lamblia*	Concentration technique
1	Female	11	Low	No	-	Primary	+ve	-ve	24
2	Female	14	Low	Yes	4month	Modified	+ve	-ve	43
3	Male	43	High	No	-	Primary	-ve	-ve	-
4	Male	12	Low	No	1 year	Primary	+ve	-ve	12
5	Male	22	High	Yes	-	Modified	-ve	-ve	-
6	Female	37	High	No	3month	Modified	-ve	+ve	34
7	Male	11	Low	No	-	Modified	-ve	-ve	-
8	Female	13	Low	No	-	Primary	-ve	-ve	-
9	Male	12	Low	Yes	2 year	Primary	+ve	-ve	-
10	Female	13	Low	No	2 year	Primary	-ve	-ve	-
11	Male	48	Medium	No	-	Modified	-ve	-ve	-
12	Male	16	Low	No	-	Primary	-ve	-ve	-
13	Female	20	Medium	Yes	2months	Modified	-ve	-ve	-
14	Female	29	High	Yes	2months	Primary	-ve	-ve	-
15	Female	20	High	Yes	1 year	Primary	-ve	+ve	58
16	Male	16	Low	Yes	4months	Primary	-ve	+ve	20
17	Male	14	Low	Yes	6months	Modified	+ve	+ve	31
18	Female	13	Low	No	-	Modified	-ve	-ve	-
19	Male	29	High	Yes	2 weeks	Primary	-ve	-ve	58
20	Female	43	Medium	No	-	Modified	-ve	-ve	-
21	Female	45	Medium	Yes	3 weeks	Primary	-ve	-ve	53
22	Male	16	Low	No	-	Modified	-ve	-ve	-
23	Female	18	Low	Yes	3 years	Modified	-ve	-ve	-
24	Male	23	High	Yes	2 years	Modified	-ve	-ve	-
25	Male	45	Low	No	-	Primary	-ve	-ve	-
26	Female	20	High	No	-	Modified	+ve	-ve	26
27	Female	8	Low	Yes	5months	Primary	+ve	-ve	42
28	Male	19	High	No	-	Modified	-ve	-ve	-
29	Female	18	High	Yes	3years	Modified	+ve	-ve	51
30	Male	12	Low	No	-	Primary	-ve	-ve	-
31	Male	14	Low	No	-	Modified	-ve	-ve	-
32	Female	17	Medium	Yes	1years	Modified	-ve	-ve	-
33	Male	13	Low	No	-	Modified	-ve	-ve	-
34	Male	20	High	No	-	Primary	-ve	-ve	-
35	Female	14	Low	No	-	Modified	-ve	-ve	-
36	Female	9	Low	Yes	3years	Modified	+ve	-ve	82
37	Male	16	Low	No	-	Modified	+ve	-ve	15
38	Female	5	Low	No	-	Primary	-ve	-ve	-
39	Male	14	Low	No	4months	Modified	-ve	+ve	30
40	Male	16	Medium	No	-	Modified	-ve		-
41	Male	14	Low	Yes	2months	Primary	-ve	+ve	11
42	Female	22	High	No	-	Primary	+ve	-ve	60
43	Female	10	Low	Yes	2years	Primary	-ve	-ve	-
44	Male	40	Low	No	-	Modified	-ve	-ve	-
45	Male	60	Low	Yes	5years	Primary	+ve	-ve	33

46	Male	44	Medium	No	-	Modified	-ve	-ve	-
47	Male	20	High	No	-	Modified	-ve	+ve	58
48	Female	13	Low	No	3months	Primary	-ve	-ve	-
49	Female	14	Low	No	6months	Primary	-ve	+ve	14
50	Male	14	Low	No	-	Primary	-ve	-ve	-
51	Female	32	High	No	-	Modified	+ve	-ve	-
52	Male	12	Low	Yes	7months	Primary	-ve	-ve	-
53	Male	10	Low	No	-	Modified	-ve	-ve	-
54	Male	13	Low	Yes	4months	Modified	+ve	-ve	45
55	Male	8	Low	Yes	2years	Primary	-ve	-ve	52
56	Female	6	Low	No	2months	Modified	-ve	-ve	-
57	Female	31	High	Yes	1months	Primary	-ve	-ve	-
58	Male	5	Low	No	-	Modified	-ve	-ve	-
59	Male	44	High	No	-	Primary	-ve	-ve	-
60	Male	20	High	No	-	Modified	-ve	-ve	-
61	Female	32	High	No	-	Primary	-ve	-ve	21
62	Male	24	High	No	-	Primary	-ve	-ve	-
63	Male	14	Low	No	-	Modified	-ve	-ve	-
64	Male	33	Low	No	-	Primary	-ve	-ve	-
65	Male	13	Low	No	2months	Primary	-ve	-ve	-
66	Male	16	Medium	No	-	Modified	-ve	-ve	-
67	Male	7	Low	Yes	1month	Primary	-ve	+ve	22
68	Female	30	High	No	-	Primary	+ve	-ve	32
69	Female	45	Medium	No	-	Modified	+ve	-ve	5
70	Male	34	Medium	No	-	Modified	-ve	-ve	70
71	Female	33	Medium	Yes	5months	Modified	+ve	-ve	63
72	Female	23	High	No	-	Primary	-ve	-ve	-
73	Male	21	High	No	-	Modified	+ve	-ve	55
74	Male	45	Medium	No	-	Modified	-ve	-ve	-
75	Male	22	High	No	-	Primary	+ve	-ve	25
76	Female	35	Medium	Yes	4months	Primary	+ve	-ve	42
77	Male	39	Medium	Yes	5months	Modified	-ve	-ve	-
78	Male	12	Low	No	-	Modified	-ve	-ve	-
79	Male	14	Low	No	-	Primary	-ve	+ve	14
80	Male	16	Medium	Yes	3months	Modified	-ve	-ve	-
81	Female	12	Low	No	-	Primary	-ve	-ve	-
82	Male	10	Low	Yes	2month	Primary	-ve	+ve	30
83	Female	19	Medium	Yes	1month	Modified	-ve	-ve	-
84	Male	35	High	Yes	3weeks	Modified	-ve	-ve	-
85	Male	31	High	No	-	Primary	-ve	-ve	-
86	Female	12	Low	Yes	9months	Primary	-ve	+ve	28
87	Male	28	High	No	-	Modified	-ve	+ve	40
88	Female	25	High	No	-	Primary	-ve	-ve	-
89	Male	17	Medium	Yes	3months	Primary	-ve	-ve	-
90	Male	14	Low	No	-	Modified	-ve	+ve	4
91	Male	12	Low	No	-	Modified	-ve	-ve	-
92	Male	45	High	No	-	Primary	-ve	-ve	20
93	Female	58	Medium	Yes	4years	Modified	+ve	-ve	35
94	Female	56	Medium	No	-	Primary	-ve	-ve	-
95	Male	64	Low	No	-	Modified	-ve	-ve	-
96	Female	30	High	No	-	Modified	-ve	-ve	-
97	Male	11	Low	Yes	3months	Primary	-ve	-ve	39

98	Male	40	High	Yes	2years	Modified	-ve	-ve	-
99	Male	12	Low	No	-	Primary	+ve	-ve	51
100	Male	11	Low	No	-	Modified	-ve	-ve	-

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Questionnaire Form

Name:.....

Age:.....

Gender: Male ..... Female.....

Eeducation level:

low..... medium.....high.....

Laterine: Primary.....Modified.....

Previous infection: Yes ..... No.....

Duration of infection: .....