Chapter one

Introduction and literature review

1.1 Introduction:

Gastrointestinal infections are major causes of morbidity and mortality throughout the world, and particularly in developing countries. Causes of gastrointestinal disease include a wide variety of bacteria, viruses and parasites (Samie *et al.*, 2009).

In low-income countries co-infections involving several different pathogens commonly occur. Several recent cross-sectional studies from different locations, have reported a potential association between *Giardia lamblia* and *Helicobacter pylori*. Both organisms colonize the gastrointestinal tract in their human hosts and both organisms are known to infect children at a high rate (Ankarklev *et al.*, 2012).

Giardiasis (disease caused by *Giardia lamblia*) occurs worldwide and may infect up to a third of the population in developing countries. The disease was reported from other mammals also, which serves to make it difficult to eradicate (Ridely, 2010). Approximately about 200 million of people in the world are with clinically manifested giardiasis, with 500,000 new cases per year (Ivanov, 2010).

H. pylori is one of the most common bacterial infectious agents; it inhabits the stomachs of more than half of the world's population. Globally, the prevalence of *H. pylori* infection in developing countries is markedly higher than that in developed countries. Moreover, the acquisition of *H. pylori* seems to occur at higher rates in developing countries (Khalifa *et al.*, 2010).

1.2 Literature review:

1.2.1 *Giardia Lamblia*:

1.2.1.1 Definition:

Giardia lamblia (Giardia duodenalis, Giardia intestinalis) is a flagellated protozoan parasite that cause very common intestinal disease called giardiasis. It

was firstly discovered by Van Leeuwenhoek in 1681 when he examined his own stool microscopically (Prescott *et al.*, 2002). It was initially named as *Cercomonas intestinalis* by Lambl in 1859. Then it was renamed *Giardia lamblia* by Stiles in 1915 in honor of Professor A. Giard of Paris and Dr. F. Lambl of Prague (CDC, 2015).

1.2.1.2 Classification:

Kingdom: Protista

Sub kingdom: Protozoa

Phylum: Sarcomastigophora

Sub phylum: Mastigophora

Class: Zoomastigophora

Genus: *Giardia*

Species: *Giardia Lamblia*

(Prescott *et al*., 2002).

More than 50 *Giardia* species were reported, distinguished by the host species in which they parasitize and protozoan morphology. At present, five *Giardia* species are recognized: *G. duodenalis*, *G. agilis*, *G. muris*, *G. ardeae* and *G. psittaci*. The species *G. duodenalis* is the only one encountered in men and more domestic and wild mammals (Ivanove, 2010).

Recent molecular studies have identified eight different genetic groups for *G. lamblia* (referred as assemblages A-H). Only assemblage A and B have been isolated from human hosts, as well as recovered from numerous species of animals, including mice, beavers, and live stock, suggesting that both human to human and animal to animal modes of transmission exist (Lamb, 2012).

1.2.1.3 Epidemiology:

G. lamblia has a worldwide distribution. Epidemiological surveys have shown that parasitic diarrhoea in children is primarily due to *G. lamblia* infection, particularly in areas with poor sanitation. It has been estimated that about 200 million people are infected each year in Africa, Asia and Latin America. In the

industrialized countries, overall prevalence rate of giardiasis is 2-5%. However, in developing countries, *G. lamblia* infects children early in life with a prevalence rate of 15-20% in children younger than 10 years is common (Nkrumah and Nguah, 2011).

Large community outbreaks have been attributed to contaminated drinking water, whereas in smaller outbreaks contaminated food and contact with contaminated recreational water have been implicated (Espelage *et al.*, 2010).

1.2.1.4 Morphology:

Giardia exists in two forms: the trophozoite and the cyst forms. The trophozoite is bilaterally symmetrical; each structure being paired, roughly pear shaped. Body length ranges from 9 to 21µm and width from 5 to 15 µm. The anterior portion of the ventral surface is modified to form a sucking disk, which serves for attachment of organism, two nuclei lie within sucking disk. Two curved rods known as median bodies, axonemes which divide the body into halves throughout most of its length. Trophozoite has characteristic falling leaf motility observed in wet preparation (John and Petri, 2006). The cyst is oval and a range from 8 to 17µm by 7to 10 µm. Mature cyst contains four nuclei, median bodies and longitudinal fibers. The cyst characterized by clear zone between the cytoplasm and the cell wall (Ridely, 2012) (figure 1.1).

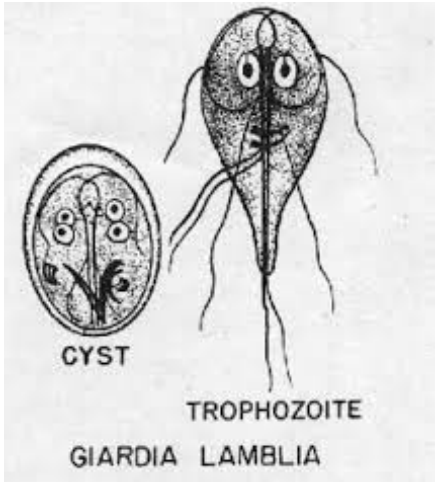

Figure (1.1): Appearance of *Giardia lamblia* cyst and trophozoite

(www.Giardiahubpages.com., 2014).

1.2.1.5 Transmission:

G. lamblia is transmitted by swallowing cysts in contaminated food and water or fecal-oral contact. While the parasite can be spread in different ways, water (drinking water and recreational water) is the most common mode of transmission (CDC, 2015).

1.2.1.6 Life cycle:

Following ingestion, each cyst is triggered by acid in the stomach to release trophozoite in to the small intestine that begins multiplying via binary fission.

Trophozoites either remain free or attach to the intestinal mucosa through ventral adhesive disk. As trophozoites migrate down the digestive tract, change in the environment, particularly reductions in the amount of cholesterol and other lipids enhance encystment occurs (Lamb, 2012).

Cysts are immediately infective upon release, and remain viable for several months in the environment (Bauman, 2011). Although infected person might shed 1-10 billion cysts daily in their feces, swallowing as few as 10 cysts might cause someone to become ill (CDC, 2015) (figure 2.2).

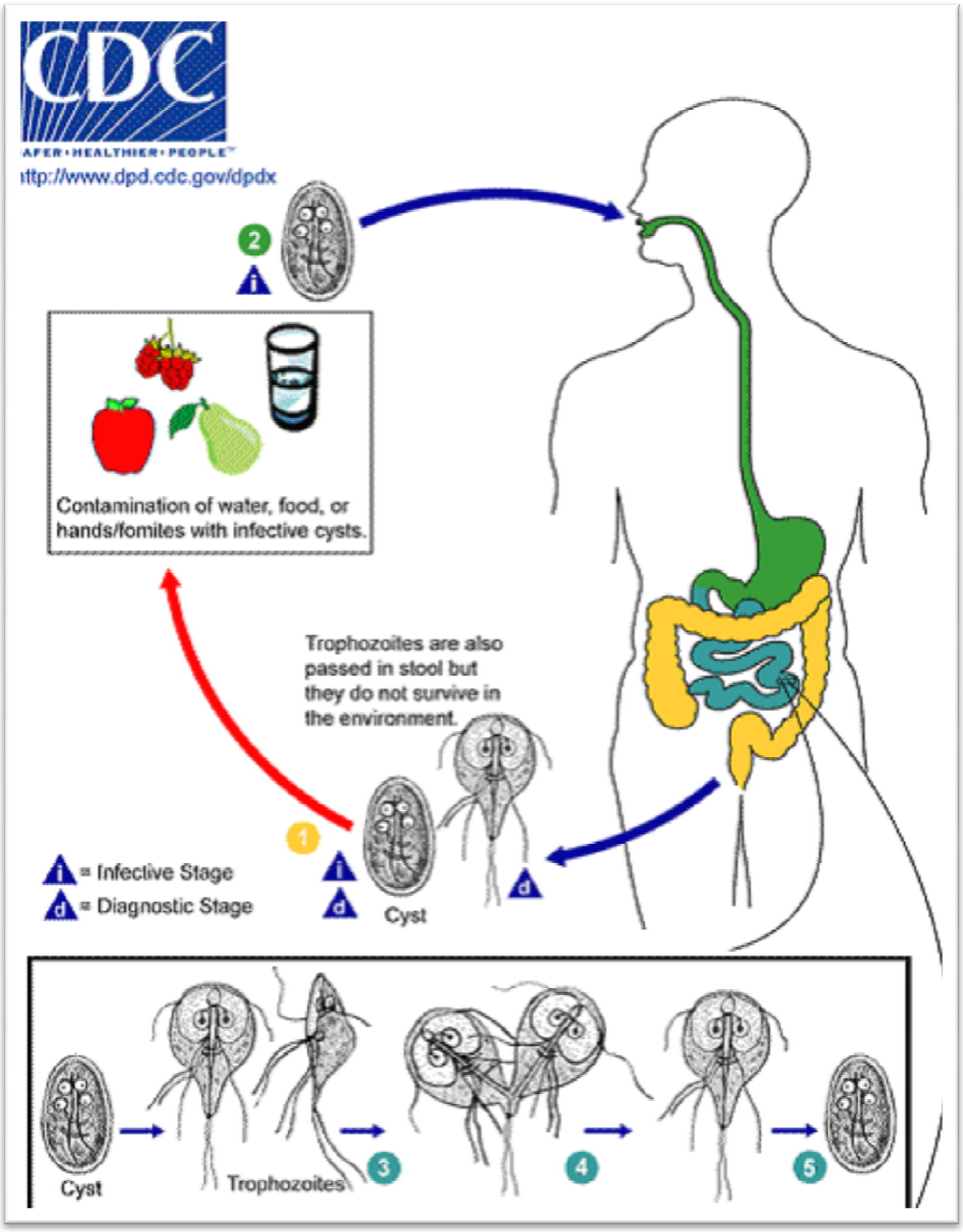

Figure (1.2): Life cycle of *Giardia lamblia*

(Centers for Disease Control and Prevention, 2013).

1.2.1.7 Pathology:

Giardiasis varies in severity, the disease can be acute or chronic and a symptomatic carriers are common (Prescott *et al.*, 2002). Children are more frequently affected than adults, although all ages may acquire the infection. Achlorhydria may predispose to symptomatic giardial infection, as well as hypogammaglobulinemia, or deficiency in secretory IgA in the small bowel (John and Petri, 2006).

Following an incubation period of 2 to 3 weeks after exposure to the organism, water and smelly diarrhea, abdominal cramps, flatulence, and anorexia, sometimes accompanied by nausea, may occur. Vitamin deficiencies, particularly the fat- soluble vitamins A, D, E and K, along with folic acid, may create a number of health problems. Weight loss and malabsorption syndrome as well as steatorrhea (fatty stools) may occur (Ridley, 2012).

In some persons, however, large numbers of parasites attached to the bowel wall may cause irritation and low-grade inflammation of the duodenal or jejunal mucosa, with consequent acute or chronic diarrhea associated with crypt hypertrophy, villous atrophy or flattening, and epithelial cell damage. Stools may be watery, semisolid, greasy, bulky, and foul smelling at various times during the course of the infection (Brooks *et al.*, 2013). On the other hand, chronic giardiasis is characterized by intermittent diarrhea, with periodic appearance and remission of symptoms (Prescott *et al.*, 2002).

The trophozoite may penetrate down into the secretary tubules of the mucosa and are found at times in the gall bladder and biliary drainage, also giardiasis of the pancreas with reversible pancreatic exocrine dysfunction has been reported in an elderly diabetic woman (John and Petri, 2006).

1.2.1.8 Immunology:

1.2.1.8.1 Innate immunity:

The initial recognition of *Giardia* by host occurs through innate immune system. The epithelial cells lining the wall of the intestine respond to *Girdia* trophozoite contact by production of cytokines and chemokines. Dendritic cells are also able to recognize and respond to *Giardia* trophozoite. On the other hand resistance to *Giardia* infection can be due to the presence of beneficial microbes in the intestinal tract. Recent studies showed that Nitric oxide could inhibit trophozoite replication as well as differentiation in to cyst (Lamb, 2012). Various reports describe the capability of normal human milk to kill trohpozoites of *Giardia*, the killing activity was depends on lipase that present in human milk (John and Petri, 2006).

1.2.1.8.2 Acquired immunity:

Infection with *G. lamblia* typically results in strong antibody response against the parasite. Incubation of trophozoites with specific antibody can lead to inhibition of parasite replication as well as parasite death, depending on the particular antibodies and the presence of complement. While Immunoglobulin G (IgG) is made in significant amounts, Immunoglobulin A (IgA) is believed to be more important in parasite control, which represent the most abundant isotype in intestinal secretions, as well as in mother's milk. Passive transfer of anti-*Giardia* antibodies in milk can help protect human new born (Lamb, 2012). T.cells are essential for proper control of *Giardia* infections; CD4+ helper T cells are primarily responsible for this protective effect. Mast cells activation affects intestinal motility through increase smooth muscle contractions. Conversely, variant specific surface proteins (VSPs) coat the surface of *Giardia* trophozoite may contribute to pathogenecity, and allow *Giardia* to evade immunological memory responses (Lamb, 2012).

1.2.1.9 Laboratory diagnosis:

The diagnosis of *Giardia* is initially based on clinical signs and symptoms and confirmed by the presence of cysts and trophozoites in stool samples (Julio *et al.*, 2012). Collecting multiple samples is recommended to increase chance of detecting cysts in stool (Brooks *et al.*, 2013).

The direct wet preparation is more useful for detection of characteristic motility of trophozoite (John and Petri, 2006). Diagnosis of *Giardia* by conventional microscopic methods following the application of fecal concentration techniques, especially Zinc sulphate flotation and centrifugation remains a relatively reliable indicator of infection (Salman, 2014). Other methods of diagnosis include examination of duodenal contents by aspiration or biopsy with endoscopy with the aid of permanent stains is essential for more identification (Ridley, 2012).

Enzyme immunoassay (ELISA) is highly sensitive and specific. For these reasons, in the last years, ELISA coproantigen test developed as alternative methods for the diagnosis of giardiasis (Chakarova, 2010). Molecular techniques particularly polymerase chain reaction (PCR) based procedures have greater sensitivity and specificity than the conventional diagnostic methods for diagnosis of *Giardia* (Salman, 2014).

Histological test such as jejunal biopsy reveals villous atrophy by using special stains, as well as mucosal invasion by *Giardia* has been noted (Lamb, 2012).

1.2.1.10 Treatment:

Metronidazole is the primary treatment for giardiasis, a five day doses is recommended. Recently Tinidazole has been approved by Food and Drug Administration (FDA) for treatment of *Giardia* and numerous studies have shown Albendazole to be equally effective (Lamb, 2012). Oral rehydration therapy may be required for severe cases of giardiasis (Bauman, 2011).

1.2.1.11 Prevention and control:

A sanitary life style as well as safe water source would prevent most of the *giardia* infection worldwide (Ridley, 2012).

Giardia cyst has ability to withstand filtration and chlorination, so purification of drinking water by saturated solution of iodine is more effective in killing the cyst with a 20-minute exposure at 20^0 C(68^0F) (John and Petri, 2006).

The filtration, flocculation, flowcytometry, immunomagnetic separation and monoclonal antibody immunofluorescence are the methods usually used for detection of *Giardia* in tap water (Ivanov, 2010).

Improve personal hygiene as well as treatment of infected patients to avoid transmission to family member (Bauman 2011).

1.2.2 *Helicobacter pylori:*

1.2.2.1 Definition:

Helicobacter pylori (*H. pylori*) is spiral shaped-gram negative rods which represent the most common causes of bacterial infection in human beings (Hestvik *et al.*, 2010). It was successfully cultured in Perth, Australia, in1982 and named as *Campylobacter*, then in 1993 it is name was changed to *Helicobacter pylori* (Prescott *et al.*, 2002).

H.pylori is the main causative agent of gastrointestinal diseases including chronic gastritis, peptic ulcer associated disorders, gastric and duodenal carcinomas leading to morbidity and mortality in humans (Hamid and Eldaif, 2014).

1.2.2.2 Classification:

Kingdom: *Protista*

Phylum: *Proteobacteria*

Class: *Epsilonproteobacteria*

Genus: *Helicobacter*

Species: *Helicobacter pylori*

(Prescott *et al.*, 2002).

1.2.2.3 Epidemiology:

Infection with *H.pylori* occurs worldwide, with about 50% of the world's population is estimated to be infected. In developing countries 70-90% of the populations are infected, while developed countries ranged from 25 to 50% (Prescott *et al.*, 2002).

H. pylori is present on the gastric mucosa of fewer than 20% of persons younger than 30 years but increases in prevalence to 40-60% of persons age 60 years, including persons who are asymptomatic. Acute epidemics of gastritis suggest a common source for *H. pylori* (Brooks *et al.*, 2013).

In Sudan, information about the prevalence of *H. pylori* infection is very patchy, and there is only one study which showed high prevalence (80%) of *H. pylori* infection among patients with symptoms of gastritis, 56% with duodenal ulcer, while 60% with duodenitis and 16% apparently look normal (Hamid and Eldaif, 2014).

1.2.2.4 Morphology:

H. pylori is a spiral shaped gram negative rod, It has multiple flagella at one pole. It is actively motile and about 3 micrometers long with a diameter of about 0.5 micrometers (Mehmood *et al.*, 2010) (figure 1.3).

Figure (1.3): Morphology of *H. pylori*

(www.dreamstime.com., 2015).

1.2.2.5Transmission:

H. pylori is usually transmitted from person to person, as well as contaminated food or water. Support for the person-to-person transmission comes from evidence of clustering within families and from reports of higher prevalence in residents of custodial institutions and nursing homes (Prescott *et al.*, 2002).

H. pylori that is acquired in the developing countries at very early ages is comparatively common up to 80-90% (Zeyrek *et al.*, 2008).

1.2.2.6 Pathology:

Individual host factors and virulence factors of the organisms are major determinants of the pattern of gastritis and subsequent disease outcome (Sabet *et al.*, 2009).

H. pylori posses many virulence factors that enable it to colonize the human stomach such as; a protein that inhibit acid production by stomach, flagella for movement, adhesins that facilitate binding to gastric cells, enzymes that inhibit phagositic killing, and urease enzyme that degrade urea in gastric juice to highly alkaline ammonia (Bauman, 2011).

H. pylori penetrate the gastric mucus layer to reach the underlying epithelial cells where it multiplies. With the aid of previously mentioned virulence factors the bacteria can stimulate inflammatory response and destruction of mucus secreting cells allowing the mucus layer to become thin, allowing acidic gastric juice to digest the stomach lining and become ulcerated (Bauman, 2011) (figure 1.4). Acute infection can yield an upper gastrointestinal illness with nausea and pain; vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the *H. pylori* infection persists for years and may develop about 90% of duodenal ulcers and 50–80% of those with gastric ulcers. Recent studies confirm that *H. pylori* also are a risk factor for gastric carcinoma and lymphoma (Brooks *et al.*, 2013).

H. pylori have been isolated from the gastric mucosa of 95% of patients with gastric ulcer, and virtually 100% of those with chronic gastritis (Prescott *et al.*,

2002). Higher risk of gastric malignancy leading the world health organization international agency for research in cancer to categorize *H. pylori* as a class I carcinogen (Suarez *et al.*, 2006).

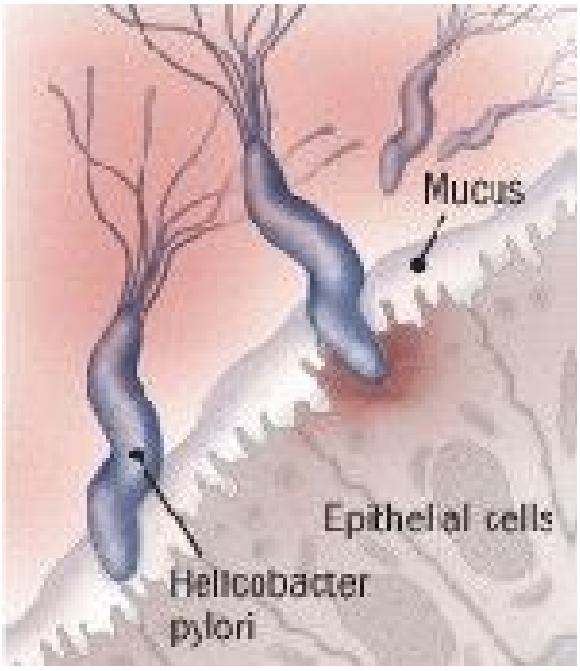

Figure (1.4): Pathogenesis of *H. pylori*

(Mehmood *et al.*, 2010)

1.2.2.7 Immunology:

Infection with *H. pylori* can induce both innate and acquired immune responses by the host.

1.2.2.7.1 Innate immunity:

The gastric epithelium has typically been regarded as a physical barrier; however, multiple studies have provided evidence to suggest that the gastric epithelium plays a key role in the inflammatory and immune responses induced by *H. pylori* through release of an array of chemokines and cytokines. In addition to the epithelium is the only cell phenotype in the gastric mucosa that is in direct contact with the pathogen. The infected gastric mucosa is infiltrated by neutrophils, mono-nuclear cells as well as lymphocytes. Mast cells represent

another innate cell phenotype that is found within the *H.pylori*-infected gastric mucosa of humans and mice. These cells represent an innate defense component that may kill bacteria through the release of proteases and other mediators (Suarez *et al.*, 2006).

1.2.2.7.2 Acquired immunity:

Patients infected with *H. pylori* develop an Immnuoglobulin M (IgM) antibody response to the infection. Subsequently, IgG and IgA are produced, and these persist, both systemically and at the mucosa, in high titer in chronically infected persons (Brooks *et al.*, 2013).

There is a demonstrated infiltration of T. cells in the gastric mucosa and most of those are CD4+ T.cells with markers of activation, various studies have tried to address the inefficiency of the host response in clearing the infection. Different studies have demonstrated that *H. pylori* infection can decrease T. cell responses as well as induce T.cell anergy (Suarez *et al.*, 2006).

1.2.2.8 Laboratory diagnosis:

Diagnostic methods are divided into invasive and non invasive categories. Invasive methods that require endoscopy include; culture, Campylobacter like organism test (CLO), direct gram stain, histology, PCR, and fluorescence insitue hyperdization (FISH). While the non invasive methods that do not require endoscopy include; serology, urea breath test (UBT) and *H. pylori* stool antigen test (Hp sAg) (Abdalsadeg *et al.*, 2012).

Laboratory identification of *H. pylori* by culture of gastric biopsy specimens, examination of stained biopsies for the presence of bacteria, or detection of urease activity in the biopsies. Urinary excretion of ammonia also can be used for diagnosis (Prescott *et al.*, 2002).

Urea breath test which in vivo test, based on detection for urease activity in patient breath which indicate *H. pylori* infection (Brooks *et al.*, 2013). Serological tests include presence of human IgG antibodies against *H. pylori.*

Antibody levels decline after treatment for infection and hence the positive antibody levels may indicate current or past infection (Pahwa *et al.*, 2010).

The faecal monoclonal antigen test has a high sensitivity, specificity and accuracy. The faecal test can be performed on humans in all age groups and gives a rapid result without the need for sophisticated laboratory equipment (Hestvik *et al.*, 2010). PCR can be performed rapidly and cost- effectively, and it can be used to identify different strains of bacteria for pathogenic and epidemiologic studies (Mehmood *et al.*, 2010).

1.2.2.9 Treatment:

Triple therapy with metronidazole and either bismuth sub salicylate or bismuth sub citrate plus either amoxicillin or tetracycline for 14 days. An acid suppressing agent given for 4-6 weeks to enhance ulcer healing. Inhibition of urease production through Proton pump inhibitors (PPI) can eradicate *H. pylori* infection. The preferred initial therapy is 7-10 days of a PPI plus amoxicillin and clarithromycin or a quadruple regimen of a PPI metronidazole, tetracycline, and bismuth for 10 days (Brooks *et al.*, 2013).

1.2.2.10 Prevention and control:

The prevention of *H. pylori* infection involves good personal hygiene, adequate sewage treatment, proper food handling and water purification (Bauman, 2011). Eradication of the infection in individuals, who had symptoms including dyspepsia, gastritis and peptic ulcers, may prevent gastric cancer. Rising antimicrobial resistance increases the need for a prevention strategy for the bacteria. There have been extensive vaccine studies in mouse models, for different adjuvants, antigens and routes of immunization, with most of the research only recently moving from animal to human trials. Studies have recently been published suggesting that *H. pylori* activity could be suppressed via dietary methods (Mehmood *et al.*, 2010).

1.2.3 Co-infections of *G. lamblia* and *H. pylori*:

1.2.3.1 Definition of co-infection:

Co-infection means; the simultaneous presence of two or more infections, which may increase the severity and duration of one or both (Segen, 2012). Concomitant *H. pylori* and *Giardia* infection is common for their similar mode of transmission and strong correlation to socioeconomic levels (Sabet *et al.*, 2009).

1.2.3.2 Epidemiology:

Improved understanding of co-infection prevalence is greatly needed, partly because co-infecting pathogens can interact either directly with one another or indirectly via the host's resources or immune system. Compared to infections of single pathogen species, these interactions within co-infected hosts can alter the transmission, clinical progression and control of multiple infectious diseases. Establishing the nature and consequences of co-infection requires integrated monitoring and research of different infectious diseases, but such data are rare (Griffiths *et al.*, 2011).

Several recent, cross-sectional studies from different locations, have reported a potential association between *G. lamblia* and *H. pylori* (Ankarklev *et al.*, 2012). Shafie *et al.* study the prevalence of *G. lamblia* and *H. pylori* co-infections in Iran; co-infection was investigated in a group of 130, patients with dyspepsia. Using three methods of duodenal aspiration sample, duodenal biopsy samples and evaluation of stool samples. Co-infections of *G. lamblia* and *H. pylori* were found in 4 patients (3.8%) (Shafie *et al.*, 2009).

1.2.3.3 Predisposing factors:

There is increasing evidence that polymicrobial infections in which microorganisms present specific pathologies may act in a synergistic or inhibitory fashion, impacting on either tissue, host cell destruction or the maintenance of health (Nimri *et al.*, 2004).

G. lamblia and *H. pylori* co-infection can be caused by common risk factors, transmission routes, probability of synergism in metronidazole resistance and common laboratory findings leading to gastrointestinal (GI) metaplasia in both organisms. Gastritis resulting from each organism predisposes the person to the other one (Shafie *et al.*, 2009). Further research is needed to identify the role of predisposed risks to co-infection (Griffiths *et al.*, 2011).

1.2.3.4 Impact of co-infections:

Overall recently published reports of co-infection in humans show co-infection to be detrimental to human health. Understanding the nature and consequences of co-infection is vital for accurate estimates of infectious disease burden. In particular, more holistic data on infectious diseases would help to quantify the size of the effects of co-infection on human health. Improved knowledge of the factors controlling an individual's risk of co-infection, circumstances when co-infecting pathogens interact, and the mechanisms behind these pathogen-pathogen interactions, especially from experimental studies, will also aid the design and evaluation of infectious disease management programmes. To date, most disease control programs typically adopt a vertical approach to intervention, dealing with each pathogen infection in isolation. If co-infecting pathogens generally interact to worsen human health, as suggested here, control measures may need to be more integrated and specialist treatments developed for clinical cases of co-infection (Griffiths *et al.*, 2011).

Acute infection with *H. pylori* is accompanied by hypochlorhydria, which facilitates the acquisition of other enteropathogens because of removal of the gastric acid barrier, which then results in diarrheal disease and iron-deficiency anemia (IDA). This is likely to occur most frequently in developing regions where the prevalence of *H. pylori* infection is disproportionately high and multiple enteric co-infections are common. The consequent synergistic impact of diarrheal disease and micronutrient deficiency on growth and cognitive

function in children has significant public health implications for socioeconomic development in these countries (Windle *et al.*, 2007).

Hypochlorhydria induced by *H. pylori* increases susceptibility to enteric infections such as typhoid and non typhoid salmonellosis, cholera, giardiasis, and other infections. Evidence from case-controlled studies that examined the association between proton pump inhibitor-induced hypochlorhydria and increased relative risk of acquiring enteric infections including *Clostridium difficile, Giardia*, and *Salmonella* supports the view that a reduction in gastric acid secretion may result in the acquisition of infections. Thus, *H. pylori*-induced hypochlorhydria may predispose to enteric infections, particularly in regions of the developing world where enteric infections are endemic. *H. pylori* infection and hypochlorhydria result in development of IDA and acquisition of other enteric infections, which promotes a vicious cycle of malnutrition and growth impairment (Windle *et al.*, 2007).

Rationale

G. lamblia and *H. pylori* are the most common pathogens which infect human worldwide. Many studies focus on establishing the prevalence of infection by these organisms as a common causative agent for diarrheal disease and gastrointestinal disorder. But there is fewer studies concerning the association between *H. pylori* and *G. lamblia*, particularly in those who had persistent epigastric pain after *H. pylori* eradication.

It is now known that *Giardia* can also infect biliary tree and stomach, this confirms that symptoms of *Giardia* infection are similar to that for *H. pylori.* So, the differentiation between two pathogens is of critical value. This study was carried out to determine the prevalence of *G. lamblia*/ *H. pylori* co-infections, in order to provide health planners with fundamental data, which may be necessary for diagnosis, treatments and eradication.

Objectives

General objectives:

To study the prevalence rate of *Giardia lamblia/ Helicobacter pylori* co-infections in Khartoum state, Sudan.

Specific objectives:

-To determine the prevalence of *G.lamblia* and *H. pylori* in Khartoum State.

-To compare between wet mount and formal ether concentration techniques for detection of *G. lamblia*.

-To estimate the existence of co-infection between *G. lamblia* and *H. pylori.*

Chapter two

Materials and methods

2.1 Study design:

A cross-sectional study.

2.2 Study area:

The study was conducted in Khartoum State, the capital of the Sudan. It lies between longitudes 31.5-34east and latitude 15-16 north in an area, about 28.165 square kilometers. It is bordered on the north and the east sides by the River Nile State, on the northwestern side by the Northern State, and on the eastern and southern sides by Kassala, Gedaref and Gezira States **(**Ministry of Human Development and labour, 2015).

2.3 Study duration:

The study was conducted in the period from May to December 2015.

2.4 Study population:

The study was carried out on patients that were clinically suspected to have gastrointestinal disorder.

2.5 Sample size:

Sample size was obtained according to the following equation:

$N=t^{2*}p\ (1-p)/M^2)$

N= sample size

t= the normal standard deviation (t=1.96)

p= the frequency of occurrence of *G. lamblia* (13%)

M=the degree of precision (0.05%)

N= 1.96*1.96(1-0.13)/0.05*0.05=133

The study was conducted on hundred clinically suspected patients.

2.6 Sampling:

Hundred stool samples were collected from participants. 100 questionnaires were filled by the same participants.

2.7 Sampling methods:

2.7.1 Collection of faecal samples:

Patients were advised to pass the stool samples directly into a plastic cup with a tight fitting lid. About 20- 40 grams of formed stools or 5- 6 spoonfuls for watery stools were collected. All specimens were labeled with patient's name, age, sex, and date of collection (Ichhpujani and Bhatia, 2002).

2.7.2 Parasitological methods:

For detection of *G. lamblia*, stool samples were screened using direct wet mount and formal ether concentration technique as described by Chakarova (2010) and Cheesbrough (1998) respectively.

2.7.2.1 Direct wet mount:

A small sample of faeces was placed on a glass slide and mixed with a drop of 0. 9% solutions of NaCl and the slide was covered with a glass cover slip and examined for the presence of cysts of parasites at 10× and 40× magnification.

2.7.2.2 Formal ether concentration technique (FECT):

About 1 g of faeces (pea-size) was emulsified in 4 ml of 10% formal saline. 3-4 ml of 10% formal saline were added and mixed well by shaking. Then sieved in a beaker and transferred to centrifuge tube. A 3- 4 ml of diethyl ether were added, stoppered and mixed for 1 minute. Then centrifuged at 750- 1000 rpm for 1 minute, layers of faecal debris, ether and formal saline were discarded by using plastic bulb pipette. The sediment was resuspended, mixed and transferred to slide, covered with cover glass, and then examined microscopically using (10x, 40x). FECT used to determine the intensity of *G. lamblia* infection as described by Cheesbrough (1998) was expressed as:

Scanty: 1-3 stage/ preparation

Few: 4-10 stage /preparation

Moderate: 11-20 stage /preparation

Many: 21-40 stage /preparation

Very many: over 40 stage /preparation

2.7.2.3 Sensitivity and specificity of direct wet mount:

The sensitivity and specificity were calculated regarding FECT as gold standard, as described by Lalkhen and McCluskey (2008):

Sensitivity = TP⁄(TP+FN)

TP= True positive

FN= False negative

Specificity= TN⁄(TN+FP)

TN= True negative

FP= False positive

2.7.3 Bacteriological methods:

2.7.3.1 *H. pylori* antigen rapid test (*H. pylori* Ag Rapid Test):

The use of rapid immune chromatographic test (ICT) for the qualitative detection of *H. pylori* antigen in fresh fecal samples as described by manufacturer.

Instructions given by the manufacturer were followed. Stool collection device was opened and using collection stick to pierce the stool sample, then the collection stick was replaced to stool device and was shaked vigorously. On the test device, 2 drops of the solution was dispended into the sample well. Results were read after 15 minutes of adding the specimen.

2.8 Data collection:

The primary data were collected by using questionnaire to obtain information that of help to the study. Variables included in the questionnaire were: age, gender, education levels, occupation, symptoms and previous infections (appendix).

2.9 Data analysis:

The results obtained were analyzed by computerized program of statistical package for social science (SPSS version 16) by using frequency, mean, and chi-squire test. Then data were presented in figures and tables.

2.10 Ethical consideration:

The approval was taken from 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 for this study.

Chapter three

Results

3.1 General characteristics of study population:

A total of 100 study subjects were included in this study. Age ranging from 1-80 years, the mean age was 29± 19 years old. Out of 100 participants, 37(37%) were males and 63(63%) were females (table 3.1). Study subjects were divided into six age groups as follow: (1-15), (16- 25), (26-35), (36-45), (46-65), and (>66 years old), frequencies of age groups were 27 (27%), 20 (20%), 17 (17%), 15 (15%), 19 (19%) and 2 (2%) respectively (table 3.2).

Table (3.1): Frequency of study subjects according to gender:

Gender	Frequency	Percentage (%)
Male	37	37%
Female	63	63%
Total	100	100%

Table (3.2): Frequency of study subjects according to age groups:

Age groups (years)	Frequency	Percentage (%)
1- 15	27	27%
16- 25	20	20%
26- 35	17	17%
36- 45	15	15%
46- 65	19	19%
>66	2	2%
Total	100	100%

3.2 Parasitological results:

3.2.1 Prevalence of *G. lamblia* by using direct wet mount and FECT:

Out of 100 subjects, 14 (14%) and 22 (22%) were positive for *G. lamblia* when using direct wet mount and FECT respectively (table 3.3).

Table (3.3): Prevalence of *G. lamblia* by using direct wet mount and FECT:

Technique	*G. lamblia*		Prevalence
	No. examined	No. positive	
Direct wet mount	100	14	14%
FECT	100	22	22%

3.2.2 Prevalence of *G. lamblia* by using direct wet mount and FECT according to gender:

Out of 100 subjects, the prevalence rate of *G. lamblia* was higher in females 11 (17.5%) than in males 3 (8.1%) by using direct wet mount (p=0.193) (table 3.4), and when using FECT was increased to 18 (28.6%) in females and 4 (10.8%) in males (p=0.038) (table 3.5).

Table (3.4): Prevalence of *G. lamblia* by using direct wet mount according to gender:

Gender	No. examined	No. positive	Prevalence
Male	37	3	8.1%
Female	63	11	17.5%

p=0.193

Table (3.5): Prevalence of *G. lamblia* by using FECT according to gender:

Gender	No. examined	No. positive	Prevalence
Male	37	4	10.8%
Female	63	18	28.6%

p=0.038

3.2.3 Prevalence of *G. lamblia* by using direct wet mount according to age groups:

The prevalence rate of *G. lamblia* by using direct wet mount according to age groups (1- 15), (16- 25), (26- 35), (36- 45), (46- 65), and >66 years old were 7.4%, 25%, 5.9%, 0%, 26.3%, and 50% respectively (p=0.053) (table 3.6).

Table (3.6): Prevalence of *G. lamblia* by using direct wet mount according to age groups:

Age groups (years)	No. examined	No. positive	Prevalence
1- 15	27	2	7.4%
16- 25	20	5	25%
26- 35	17	1	5.9%
36- 45	15	0	0%
46- 65	19	5	26.3%
>66	2	1	50%

p=0.053

3.2.4 Prevalence of *G. lamblia* by using FECT according to age groups:

The prevalence rate of *G. lamblia* using FECT according to age groups (1- 15), (16- 25), (26- 35), (36- 45), (46- 65), and >66 years old were 14.8%, 30%, 17.6%, 6.7%, 36.8%, and 50% respectively (p=0.209) (table 3.7).

Table (3.7): Prevalence of *G. lamblia* by using FECT according to age groups:

Age groups (years)	No. examined	No. positive	Prevalence
1- 15	27	4	14.8%
16- 25	20	6	30%
26- 35	17	3	17.6%
36- 45	15	1	6.7%
46- 65	19	7	36.8%
>66	2	1	50%

p=0.209

3.2.5 Prevalence of *G. lamblia* by using direct wet mount according to education levels:

Out of 14 positive cases for *G. lamblia*, 7 (50%) with low level of education, while 7 (50%) with high level of education (p=0.019) (table 3.8).

Table (3.8): Prevalence of *G. lamblia* by using direct wet mount according to education levels:

Education levels	*G. lamblia*		Prevalence
	No. examined	No. positive	
Low	48	7	50%
Medium	25	0	0%
High	27	7	50%
Total	100	14	100%

p=0.019

3.2.6 Prevalence of *G. lamblia* by using direct wet mount according to occupation:

Out of 14 positive cases for *G. lamblia*, 7 (50%) with student, 5 (35.7%) with house wives, 2 (14.3%) with officer and there was no infection with preschool and free laborers (p=0.160) (table 3.9).

Table (3.9): Prevalence of *G. lamblia* by using direct wet mount according to occupation:

Occupation	*G. lamblia*		Prevalence
	No. examined	No. positive	
Pre-school	10	0	0%
Students	33	7	50%
House wives	25	5	35.7%
Officer	15	2	14.3%
Free laborers	17	0	0%
Total	100	14	100%

p=0.160

Table (3.10): Relationship between *G. lamblia* and clinical symptoms:

Symptoms	*G. lamblia*		Total	p. value
	Positive	Percentage (%)		
Diarrhea	6	13.6%	44	0.926
Nausea	8	13.8%	58	0.944
Fatigue	10	16.4%	61	0.388
Abdominal pain	14	15.5%	90	0.179
Bloating	9	24.3%	37	0.023
Loss of weight	9	21.5%	42	0.068
Loss of appetite	5	8.5%	59	0.056
Vomiting	1	8.3%	12	0.546
Headache	1	5.5%	18	0.254
Fever	0	0%	7	0.268

3.2.7 Prevalence of *G. lamblia* according to previous infection:

Out of 100 subjects, 15 (15%) had previous infection with *G. lamblia*, among those 5 (33.4%) were positive and 10 (66.6%) were negative for *G. lamblia*. While 85 (85%) had no previous infection with *G. lamblia*, among those 9 (10.5%) were positive and 76 (89.4%) were negative for *G. lamblia* (p=0.019) (figure 3.1).

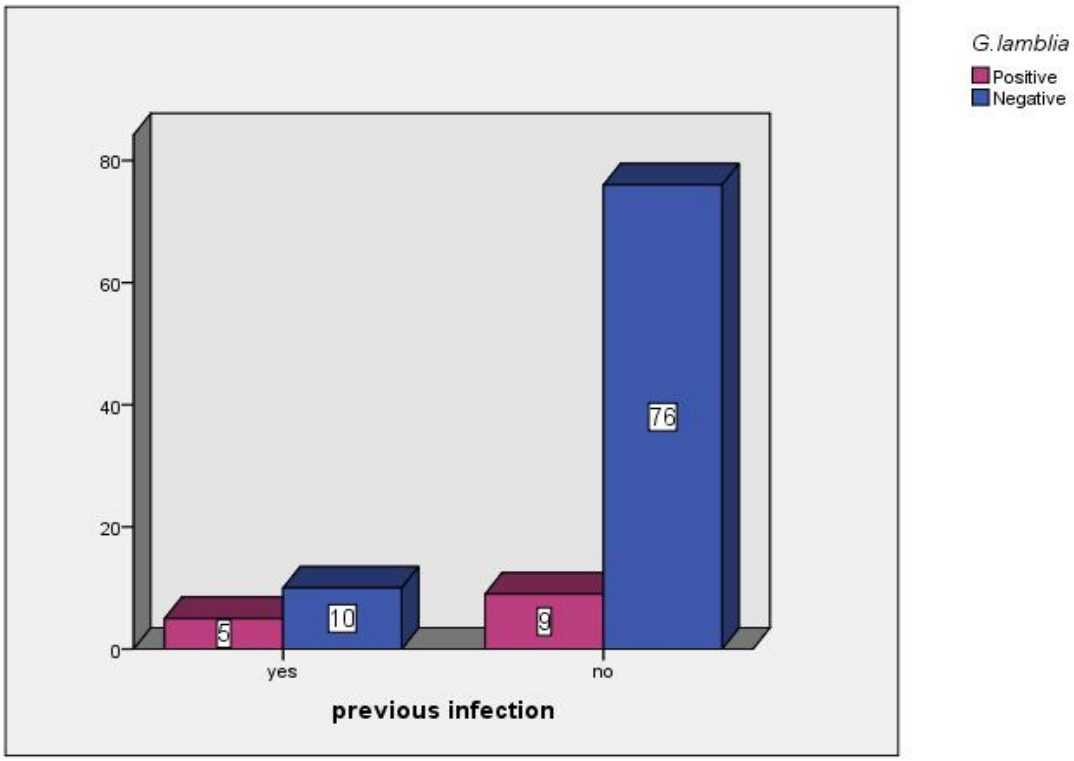

Figure (3.1): Prevalence of *G. lamblia* according to previous infection

3.3 Bacteriological results:

3.3.1 Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test:

Out of 100 subjects, 30 (30%) were found to be positive for *H. pylori* by using stool antigen test (table 3.11).

Table (3.11): Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test:

Technique	*H. pylori*		
	No. examined	No. positive	prevalence
H.pylori Ag Rapid Test	100	30	30%

3.3.2 Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to gender:

Out of 100 subjects, the prevalence rate of *H. pylori* was higher in males 13 (35.1%) than in females 17 (27%) (p=0.390) (table 3.12).

Table (3.12): Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to gender:

Gender	No. examined	No. positive	Prevalence
Male	37	13	35.1%
Female	63	17	27%

p=0.390

3.3.3 Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to age groups:

The prevalence rate of *H. pylori* according to age groups, (1- 15), (16- 25), (26- 35), (36- 45), (46- 65), and >66 years old were 33.3%, 15%, 35.3%, 20%, 42.1% and 50% respectively (p=0.424) (table 3.13).

Table (3.13): Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to age groups:

Age groups (years)	No. examined	No. positive	Prevalence
1- 15	27	9	33.3%
16- 25	20	3	15%
26- 35	17	6	35.3%
36- 45	15	3	20%
46- 65	19	8	42.1%
>66	2	1	50%

p=0.424

3.3.4 Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to education levels:

Out of 30 positive cases for *H. pylori*, 20 (66.6%) with low level of education, while 5 (16.6%) with medium and 5 (16.6%) with high levels of education (p=0.023) (table 3.14).

Table (3.14): Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to education levels:

Education levels	*H. pylori*		Prevalence
	No. examined	No. positive	
Low	48	20	66.6%
Medium	25	5	16.6%
High	27	5	16.6%
Total	100	30	100%

p=0.023

3.3.5 Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to occupation:

Out of 30 positive cases for *H.pylori*, 7 (23.4%) with pre-school, 4 (13.4%) with students, 11(36.6%) with house wives, 3 (10%) with officer and 5 (16.6%) with free laborers (p=0.008) (table 3.15).

Table (3.15): Prevalence of *H. pylori* by using *H. pylori* Ag Rapid Test according to occupation:

Occupation	*H. pylori*		Prevalence
	No. examined	No. positive	
Pre-school	10	7	23.4%
Students	33	4	13.4%
House wives	25	11	36.6%
Officer	15	3	10%
Free laborers	17	5	16.6%
Total	100	30	100%

p=0.008

Table (3.16): Relationship between *H. pylori* and clinical symptoms:

Symptoms	*H. pylori*		Total	p. value
	Positive	Percentage (%)		
Diarrhea	21	47.7%	44	0.001
Nausea	17	29.3%	58	0.860
Fatigue	19	31.1%	61	0.754
Abdominal pain	30	33.3%	90	0.029
Bloating	15	40.5%	37	0.078
Loss of weight	17	40.5%	42	0.052
Loss of appetite	18	30.5%	59	0.894
Vomiting	3	25%	12	0.687
Headache	2	11.1%	18	0.053
Fever	1	14.2%	7	0.347

3.3.6 Prevalence of *H. pylori* according previous infection:

Out of 100 subjects, 22 (22%) had previous infection with *H. pylori*, among those 6 (27.3%) were positive and 16 (72.7%) were negative for *H. pylori*. While 78 (78%) had no previous infection with *H. pylori*, among those 24 (30.7%) were positive and 54 (69.3%) were negative for *H. pylori* (p=0.752) (figure 3.2).

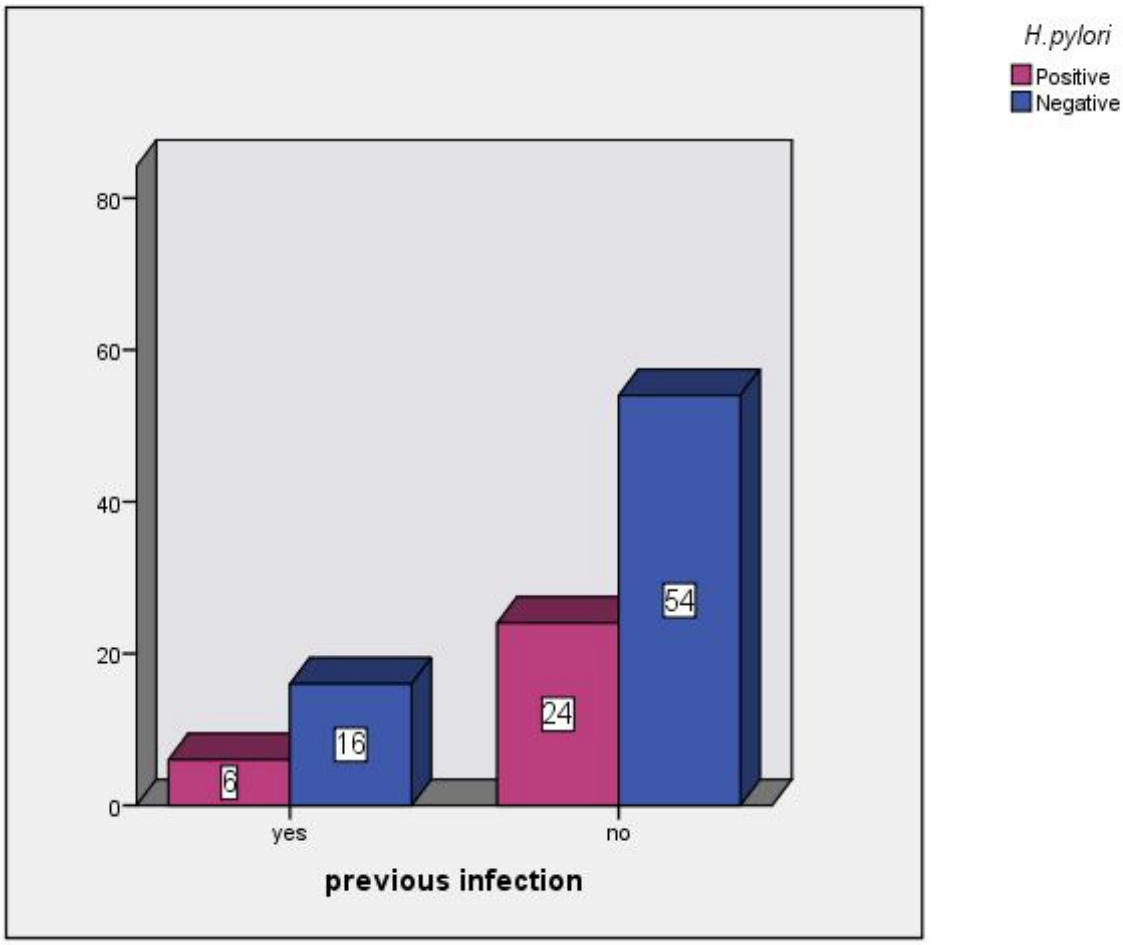

Figure (3.2): Prevalence of *H. pylori* according to previous infection

3.4 Co-infections of *G. lamblia* and *H. pylori*:

3.4.1 Co-infections of *G. lamblia* and *H. pylori* by using direct wet mount and *H. pylori* Ag Rapid Test:

Out of 100 subjects, co-infection was present in 5 (5%) of study subjects when using direct wet mount (p=0.615) (table 3.17).

Table (3.17): Co-infections of *G. lamblia* and *H. pylori* by using direct wet mount and *H. pylori* Ag Rapid Test:

		H. pylori		**Total**
		Positive	**Negative**	
G.lamblia	Positive	5	9	14
	Negative	25	61	86
	Total	30	70	100

p=0.615

3.4.2 Co-infections of *G. lamblia* and *H. pylori* by using FECT and *H. pylori* Ag Rapid Test:

Out 100 subjects, co-infection was present in 9 (9%) of study subjects when using FECT (p=0.291) (table 3.18).

Table (3.18): Co-infections of *G. lamblia* and *H. pylori* by using FECT and *H. pylori* Ag Rapid Test:

		H. pylori		**Total**
		Positive	**Negative**	
G. lamblia	Positive	9	13	22
	Negative	21	57	78
	Total	30	70	100

p=0.291

3.5 Comparison between direct wet mount and FECT:

Out of 100 stool samples, 14 (14%) were positive for *G. lamblia* when using direct wet mount, 8 samples were negative by direct wet mount and positive by FECT, while 22 (22%) were found to be positive by FECT (p=0.000) (table 3.19).

Table (3.19): Comparison between direct wet mount and FECT:

		FECT		**Total**
		Positive	**Negative**	
Direct Wet mount	Positive	14	0	14
	Negative	8	78	86
	Total	22	78	100

p=0.000

3.6 Sensitivity and specificity of direct wet mount:

According to the formula mentioned in materials and methods the sensitivity of direct wet mount was (63.6%) (table 3.20). While the specificity was (100%) (table 3.21).

Table (3.20): Sensitivity of direct wet mount:

		FECT		Total	Sensitivity %
		Positive	Negative		
Direct Wet mount	Positive	14	0	14	63.6%
	Negative	8	78	86	
	Total	22	78	100	

Table (3.21): Specificity of direct wet mount:

		FECT		Total	Specificity %
		Positive	Negative		
Direct Wet mount	Positive	14	0	14	100%
	Negative	8	78	86	
	Total	22	78	100	

3.7 Detection of intensity of *G. lamblia* by using FECT:

Out of 22 (22%) positive samples, the intensity of *G. lamblia* was expressed as; 9 (40.9%) were few infection, 7 (31.8%) were moderate and 6 (27.3%) were many infection (p=0.000) (figure 3.3).

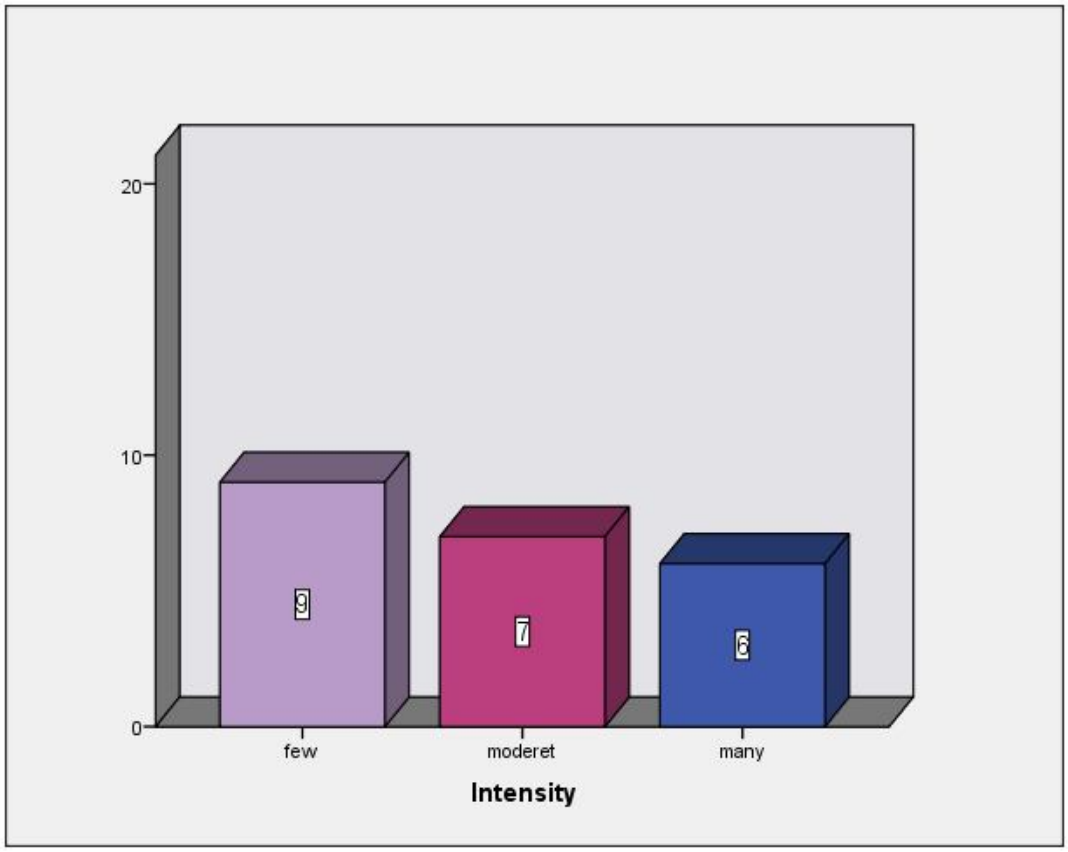

Figure (3.3): Detection of intensity of *G. lamblia* by using FECT

Chapter four

Discussion

Co-infection with several different pathogens occurs commonly in developing countries. *G. lamblia* parasite is considered the most common protozoan infection in human. *H. pylori* was accepted as a main cause of gastritis and gastritis associated diseases, Co-infection of *H. pylori* and *G. lamblia* is common for their similar mode of transmission and strong correlation to socio-economic levels (Sabet, 2009).

The present study showed that the prevalence rates of *G. lamblia* as detected by using direct wet mount was (14%), while it was increased to (22%) when using formal ether concentration technique (FECT) (p=0.000). These results were similar to results obtained by Eltayeb *et al.* (2012) and disagreed with the result which was reported by Gabbad and Elawad (2014) in Khartoum State (33.4%).

The current study revealed that the prevalence of *G. lamblia* was higher in females (17.5%) than in males (8.1%), by using direct wet mount (p=0.193) and when using FECT was increased to (28.6%) in females and (10.8%) in males (p=0.038), these results were not in agreement with Yakoob *et al.* (2005) who found that the prevalence of *G. lamblia* was higher in males (72%) than in females (28%) in Pakistan.

The present study showed that the prevalence rate of *G. lamblia* was higher (50%) in the age group >66 years old by using direct wet mount (p=0.053) and when using FECT the highest infection rate (50%) was found in the same age group >66 years old (p=0.209), these results were not in line with an Iraqian study which was done by Raza and Sami (2009) who showed that the highest rate of infection (17%) was among the age group (6-10) years old. The reason for the differences in the gender and age distribution was probably due to varying sample size, age groups, geographical locations, and time periods of the studies (Mohammed, 2014).

With regard to educational levels, the high infection rate of *G. lamblia* was reported among those with low and high education levels (50%) (p=0.019) which indicated that the prevalence of *G. lamblia* was affected with level of education.

In the present study, the highest infection rate of *G. lamblia* according to occupation was found among the students (50%) (p=0.160). This result was similar to that obtained by Raza and Sami (2009).

The present study showed that there was no relationship between *G. lamblia* and clinical symptoms except bloating (p=0.023) this finding disagreed with the finding obtained by Yakoob *et al.* (2005) who revealed that the most common symptoms were abdominal pain and diarrhea.

The current study showed that, 15 out of 100 subjects (15%) had previous infection with *G. lamblia*, among those, 5 (33.4%) were positive and 10 (66.6%) were negative for *G. lamblia*. While 85 (85%) had no previous infection with *G. lamblia*, among those, 9 (10.5%) were positive and 76 (89.4%) were negative for *G. lamblia* (p=0.019). It means that an infection was affected by previous one.

In the present study, the prevalence of *H. pylori* was (30%) when using stool antigen test, this result was not consistent with another Sudanese study which based on antibody detection (74.7%) (Hamid and Eldaif, 2014), the higher prevalence rate may be due to detection of antibodies which are relatively remainng positive for years after successful eradication of *H. pylori*.

The result of this study, showed that the prevalence rate of *H. pylori* infection was higher in males (35.1%) than in females (27%) (p=0.390). This result was disagreed with a study conducted in Yemen by Bin Mohanna *et al* (2014) who found that the prevalence in females was (67%) and in males was (33%).

According to age groups, the highest infection rate (50%) in this study was detected in >66 years old (p=0.424). This result disagreed with a study done by

Hamid and Eldaif (2014) in Sudan which showed the high prevalence rate of infection among age group (30-50) years old.

According to educational levels the results showed that the highest infection rate of *H. pylori* was (66.6%) among those with low education, followed by (16.6%) in both medium and high levels (p=0.023). This result indicated that the infection was affected by education level which was reflected in the degree of personal hygiene.

In the current study, the prevalence of *H. pylori* according to occupation was found to be higher in house wives 11 (36.6%), followed by pre-school 7 (23.3%), students 4 (13.3%), officer 3 (10%), and free laborers 5 (5%) (p=0.008).

The present study showed that the most common symptoms in *H. pylori* infection were abdominal pains (30%) (p=0.029) and diarrhea (21%) (p=0.001); so that abdominal pain and diarrhea symptoms occurred significantly more frequent in *H. pylori* infected patients.

The present study revealed that, out of 100 subjects, 22 (22%) had previous infection with *H. pylori*, among those, 6 (27.3%) were positive and 16 (72.7%) were negative for *H. pylori*, while 78 (78%) had no previous infection with *H. pylori*, among those, 24 (30.7%) were positive and 54 (69.3%) were negative for *H. pylori* (p=0.752).

The results showed that FECT was more accurate than direct wet mount for detection of *G. lamblia* (p=0.000). The sensitivity of direct wet mount was (63.6%), while the specificity was (100%).

In the present study, co-infections of *G. lamblia* and *H. pylori* were (5%), (9%) by using direct wet mount and FECT respectively (p=0.291). These results disagreed with the results of Shafie *et al.* (2009), who revealed that co-infections of *G. lamblia* and *H. pylori* were found in 4 patients out of 130 (3.8%) in Iran.

The present study showed that *G. lamblia* and *H. pylori* infections were more distributed in the study area; this may increase the chance for development of co-infections between them.

Chapter five

5.1 Conclusion:

The study concluded that *G. lamblia* infection was less prevalent compared with *H. pylori* infection in the study area, prevalence rates of co-infections with *H. pylori* and *G. lamblia* were (5%), (9%) in subjects under study by using direct wet mount and FECT respectively. Females were found to be more affected than males in infection by both pathogens. The prevalence of *G. lamblia* and *H. pylori* was higher in the age group >66 years old than other age groups. The study concluded that co-infections of *G. lamblia* and *H.pylori* are possibly present in the study area.

5.2 Recommendations:

1. Accurate diagnosis is essential for the effective treatment and management of infections caused by *G. lamblia* and *H. pylori*.
2. Improvement of health measures such as personal hygiene, and water purification are useful ways for elimination of the infection.
3. Further studies with the aid of more advanced techniques such as PCR are recommended to assess the presence of *G. lamblia* and *H. pylori* co-infection.
4. FECT should be used as the best method for detection of *G. lamblia* than wet preparation.

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Appendix

Questionnaire form:

Sudan University of Science and Technology

College of Graduate Studies
Questionnaire

Patient ID No……..... Date…….

Age……....

Gender: Male ☐ Female ☐

Education Levels:

Low ☐ Medium ☐ High ☐

Occupation…….

Symptoms:	Yes ☐	No ☐
1-Diarrhoea	Yes ☐	No ☐
2-Nausia	Yes ☐	No ☐
3-Fatigue	Yes ☐	No ☐
4-Abdominal pain	Yes ☐	No ☐
5-Bloating	Yes ☐	No ☐
6-Loss of weight	Yes ☐	No ☐
7-Loss of appetite	Yes ☐	No ☐
8-Vomiting	Yes ☐	No ☐

9- Others……

Previous infection with *G. lamblia*:

Yes ☐ No ☐

Previous infection with *H. pylori*:

Yes ☐ No ☐