

Sudan University of Science and Technology

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**Correlation between *Helicobacter pylori* Infection and Presence of
Intestinal Parasites in Khartoum State**

العلاقة بين البكتريا الحلزونية والطفيليات المعوية في ولاية الخرطوم

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(اِقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ۝ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ۝ اِقْرَأْ
وَرَبُّكَ الْأَكْرَمُ ۝ الَّذِي عَلَّمَ بِالْقَلَمِ ۝ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ)

سورة العلق الايات من 1 - 5

Dedication

I dedicate this work to....

My parents who support me to be the best

My brothers and sisters whose gave my live a meaning

My teachers whose make my dream reality

*My friends and colleagues who encourage and stand with
me step by step.*

Acknowledgment

Thanks firstly and finally to Allah almighty for the blessing of success and for giving me the power to complete this project.

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Abstract

This study was conducted in different hospitals in Khartoum state. The study involved two hundred stool samples, 100 patients with *H.pylori* and 100 persons as controls, the test for *H.pylori* was already done by hospitals using ICT for detection *H.pylori* Ag in stool sample , during the period between June and October 2018. The results showed that 23 (23%) of *H.pylori* patients as well as 10 (10%) of the control were harboring gastrointestinal parasites.

The study showed that *Entamoeba histolytica* was seen in 12% of the *H.pylori* cases followed by *Entamoeba coli* in 9% of cases and *Giardia lamblia* in 4%. Lower rates were reported among the control group where *Entamoeba histolytica* was seen in 5% followed by *Giardia lamblia* in 3% of ontrols and *Entamoeba coli* in 2%.

The result demonstrated that the prevalence rate of gastrointestinal parasites among males and females was almost close (24% and 22% respectively) . On the other hand, the prevalence of gastrointestinal parasites among males and females in the control group was found to be 9% and 11% respectively . The highest prevalence rates (40% and 38%) were found among the 1-15 and 46-60 years old groups respectively, while the lowest rate (10.7%) was found among the 31-45 years old group for *H.pylori* patients. For the control group , the highest prevalence rate (15%) was found among the 31-45 years old group , while the lowest prevalence rate (8%) was found among the 16-30 years old group .

At the end we found that , gastrointestinal parasites are more occurrence among *H.pylori* patients.

Also we found that the infection rate was not affected by gender , while its affected by age group.

مستخلص الدراسة

تم اجراء هذا البحث في المستشفيات المختلفة من ولاية الخرطوم , حيث شملت الدراسة 200 عينة براز جمعت من 100 مريض مصاب بـ *H.pylori* ومن 100 اخرين غير مصابين بـ *H.pylori* , تم إجراء اختبار *H.pylori* بالفعل من قبل المستشفيات باستخدام ICT للكشف عن *H.pylori* Ag في عينة البراز , في خلال الفترة من يونيو الي اكتوبر 2018 .

اظهرت النتيجة ان 23 (23%) من مرضى الـ *H.pylori* مصابين بالطفيليات المعوية وكذلك في 10 (10%) من الاشخاص السليمين.

اظهرت الدراسة ان *Entamoeba histolytica* ظهرت في 12% من حالات الـ *H.pylori* يليها *Entamoeba coli* في 9% من الحالات و *Giardia lamblia* في 4% من الحالات .

سجلت معدلات اقل في الاشخاص السليمين *Entamoeba histolytica* ظهرت في 5% من الحالات تليها *Giardia lamblia* في 3% من الحالات و *Entamoeba coli* 2% .

بينت الدراسة ان معدل الانتشار للطفيليات المعوية كان متقاربا في الاناث و الذكور (22% و 24% علي التوالي) .

من جهة اخرى , كان معدل الانتشار في الاشخاص السليمين في الاناث و الذكور 11% و 9% علي التوالي .

اعلى معدل انتشار (40% و 38%) سجل في الفئات العمرية 1-15 و 46-60 سنة علي التوالي بينما اقل معدل 10.7% قد تم تسجيله في الفئة العمرية 31-45 سنة في حالة المصابين بـ *H.pylori* .

في الاشخاص السليمين تم تسجيل اعلى معدل انتشار 15% في الفئة العمرية 31-45 سنة , بينما اقل معدل انتشار 8% تم تسجيله في الفئة العمرية 16-30 سنة .

في النهاية وجدنا أن ، الطفيليات المعدية المعوية هي أكثر حدوثا بين مرضى *H.pylori*.

كما وجدنا أن معدل الإصابة لم يتأثر بنوع الجنس ، بينما تأثر بالفئة العمرية.

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Chapter one

Introduction and literature review

Chapter one

Introduction and literature review

1.1 Introduction:

Intestinal parasitic infections are amongst the most common infections worldwide. It is estimated that some 3.5 billion people are affected, and that 450 million are ill as a result of these infections. These infections are regarded as serious public health problem, as they cause iron deficiency anemia, growth retardation in children and other physical and mental health problems (Okyay *et al.*, 2004).

Pathogenic intestinal protozoa produce disease by infecting the small or large intestine, or both. Intestinal protozoa are found in highest prevalence in developing countries, where they are responsible for a substantial burden of disease. (Biggs and Brown, 2001)

Health impacts vary with age: the small intestinal protozoa *Giardia lamblia* and *Cryptosporidium* spp. have their major impact in children, while the large bowel pathogen *Entamoeba histolytica* infects all age groups with its most profound effects in adults. Some of the protozoa, particularly *Cryptosporidium* and *Isospora belli*, have a greatly increased morbidity in immunodeficiency states, whereas the severity of disease due to giardiasis and amoebiasis is little affected. (Biggs and Brown, 2001)

The majority of the more serious infections occur in tropical regions, particularly in less-developed countries, so most dwellers within temperate, industrialized regions are unaware of the magnitude of the problem. The

global prevalence (proportion of a population infected) of *Ascaris lumbricoides* was estimated in 2003 at 26%, that of *Trichuris trichiura* at 17%, and of hookworms at 15% (Smyth, 1990).

1.2 Intestinal parasites

Parasitic infection caused by intestinal helminthes and protozoan parasites, are among the most prevalent infections in humans in developing countries (Savioli and Albonico, 2004).

1.2.1 Intestinal Protozoa

The term protozoa refers to single-celled parasites that inhabit various parts of the body, some of which are capable of causing serious damage, whereas others may be scarcely noticed by carriers of the organisms. These forms of parasitic organisms include amoebae, cryptosporidiosis, giardiasis (Ridley, 2012).

1.2.1.1 Amoeba

Amoebae are irregularly shaped microorganisms that infect chiefly the end of the smaller intestine and colon. Amebiasis is the most common protozoal infection by a pathogenic species and is caused by the species *Entamoeba histolytica* (Ridley, 2012).

1.2.1.1.1 Morphological features

(a) Trophozoites:

Viable trophozoites vary in size from about 10-60 μm in diameter. Motility is rapid, progressive, and unidirectional, through pseudopods. The nucleus is characterized by evenly arranged chromatin on the nuclear membrane and

the presence of a small, compact, centrally located karyosome. The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In the case of dysentery, however, RBCs may be visible in the cytoplasm (figure1-1), and this feature is diagnostic for *E.histolytica* (Assafa *et al.*, 2006).

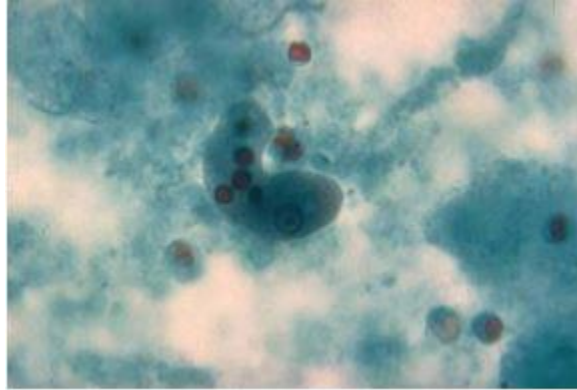


Figure 1-1. *Entamoeba histolytica* trophozoite (Cuomo *et al.* 2012)

(b) Cyst:

Cysts of *E. histolytica* are 10-15 μ m in diameter and contain one to four nuclei (figure1-2) (Cuomo *et al.* 2012). The immature cyst has inclusions namely; glycogen mass and chromatoidal bars. As the cyst matures, the glycogen completely disappears; the chromatoid bars may also be absent in the mature cyst (Assafa *et al.*, 2006).



Figure 1-2. *Entamoeba histolytica* cyst . (Cuomo *et al.* 2012)

1.2.1.1.2 Biology:

Trophozoites may live and multiply indefinitely within the crypts of the large intestine mucosa, apparently feeding on starches and mucous secretions and interacting metabolically with enteric bacteria. However, such trophozoites commonly initiate tissue invasion when they hydrolyze mucosal cells and absorb the predigested product. Invasive amebas erode ulcers into the intestinal wall, eventually reaching the submucosa and underlying blood vessels. From there they may travel with the blood to other sites such as liver, lungs, or skin. Although these endogenous forms are active, healthy amebas that multiply rapidly, they are on a dead-end course. They cannot leave the host and infect others and so perish with their luckless benefactor. Mature cysts in the large intestine, on the other hand, leave the host in great numbers. An individual that produces such cysts is usually asymptomatic or only mildly afflicted. Cysts of *E. histolytica* can remain viable and infective in a moist, cool environment for at least 12 days, and in

water they can live up to 30 days; however, they are rapidly killed by putrefaction, desiccation, and temperatures below 5°C and above 40°C. They can withstand passage through the intestines of flies and cockroaches. The cysts are resistant to levels of chlorine normally used for water purification. When swallowed, cysts pass through the stomach unharmed and show no activity while in an acidic environment. When they reach the alkaline medium of the small intestine, metacysts begin to move within their cyst walls, which rapidly weaken and tear. Quadrinucleate amebas emerge and divide into amebulas that are swept downward into the cecum. This is the organisms' first opportunity to colonize, and their success depends on one or more metacystic trophozoites making contact with the mucosa. The amebas possess several hydrolytic enzymes, including phosphatases, glycosidases, proteinases, and an RNase. Major metabolic end products are CO₂, ethanol, and acetate, whose proportions vary with the extent to which the parasites are deprived of oxygen (Smyth, 1990).

1.2.1.1.3 Pathogenesis:

The complete life cycle of *E. histolytica* consists of four consecutive stages: the trophozoite, precyst, cyst and metacyst. The cyst is resistant to gastric acid, and on ingestion it passes into the small intestine. The amoeba within the cyst becomes active in the neutral or alkaline environment of the small intestine. The cyst wall is digested, probably by the digestive enzymes within the lumen of the gut. The encysted amoeba becomes very active and each of the four nuclei in the emerging *E. histolytica* undergoes one round of division, thus forming eight amoebae, smaller than the trophozoites seen in the colon, from a single cyst. They are carried into the caecum where they complete their maturation. They multiply by binary fission, the nucleus

dividing by modified mitosis. As the amoebae pass down the colon they become dehydrated and assume a spherical shape known as a precyst. A thin cyst wall is secreted, forming an unripe cyst. Two mitotic divisions occur, resulting in a cyst that contains four nuclei. They are evacuated in the stool and discharged into the environment. Cysts remain viable and infective for several days in faeces and water, but are easily killed by desiccation.

E. histolytica has the capacity to destroy almost all tissues of the human body. The intestinal mucosa, the liver and, to a lesser extent, the brain and skin are affected most commonly. Even cartilage and bone can be eroded by *E. histolytica* trophozoites. Several virulence factors have been identified, such as adhesion molecules, contact-dependent cytolysis, proteases, haemolysins and phagocytic activity. To produce damage, trophozoites must first colonize the colon. The presence of bacteria is essential for colonization as they provide an environment with low oxygen tension and probably supply other metabolic needs. Trophozoites then penetrate through the mucus layer and adhere to the host cells. *E. histolytica* enhances mucus secretion, alters its composition and depletes goblet cells of mucin, thereby making epithelial surfaces more vulnerable to invasion. The parasite also induces the expression of cathelicidin, an antimicrobial peptide to which *E. histolytica* is resistant through its cysteine protease,¹⁸ which reduces competition. Once the mucus barrier has been broken down, *E. histolytica* reaches the luminal surface of enterocytes and initially produces a contact-dependent focal and superficial epithelial erosion. Trophozoites adhere to colonic mucins and host cells through the N-acetyl-d-galactosamine-inhibitable lectin, a 260-kDa protein also known as the Gal/GalNAc adherence lectin. The first contact of the trophozoite with the immune

system is through the epithelial intestinal cells. *E. histolytica* stimulates human intestinal epithelial cells to secrete interleukin (IL)-8 and tumour necrosis factor (TNF) α . Neutrophils are rapidly recruited and activated in response to the proinflammatory cytokine IL-8. Cell infiltration around invading amoebae leads to rapid lysis of inflammatory cells followed by tissue necrosis. The primary pathogenetic event is host cell death: *E. histolytica* is well named. There are four steps in this pathway; • Lectin-mediated contact • Calcium influx • Tyrosine dephosphorylation • Caspase 3 activation leading to the final steps of apoptosis. Amoebae probably spread from the intestine to the liver through the portal circulation. The presence and extent of liver involvement bears no relationship to the degree of intestinal amoebiasis, and these conditions do not necessarily coincide. It is thought that liver damage is not caused directly by the amoebae but rather by the lysosomal enzymes of lysed polymorphonuclear neutrophils (PMNs) and monocytes that accumulate around the parasite. During experimental infection, hypocomplementaemic and leukopaenic animals demonstrate reduced amoebic-induced liver damage when compared with normal animals. In severe cases, especially in patients treated with corticosteroids, amoebic trophozoites can be found in virtually every organ of the body, including the brain, lungs and eyes (Farrar *et al*, 2014).

1.2.1.1.4 Laboratory diagnosis

Laboratory diagnosis is made by finding the characteristic cysts in an iodine stained, formolether concentration method or by detecting the characteristic trophozoites in a wet preparation or a permanent stained preparation (Cuomo *et al*, 2012).

1.2.1.2 *Giardia lamblia*:

Giardia lamblia is a flagellate of world-wide distribution. It is more common in warm climates than temperate climates. It is the most common flagellate of the intestinal tract, causing Giardiasis. Humans are the only important reservoir of the infection. The infection is most common in parts of the world where sanitation is at its lowest. Giardiasis is an infection of the upper small bowel, which may cause diarrhea. Only *Giardia* spreads disease (Cuomo *et al*, 2012)

1.2.1.2.1 Morphology:

Trophozoites are 12 μm to 15 μm long, rounded at their anterior ends and pointed at the posterior. The organisms are dorsoventrally flattened and convex on the dorsal surface. The flattened ventral surface bears a concave, bilobed adhesive disc, which actually is a rigid structure, reinforced by microtubules and fibrous ribbons, surrounded by a flexible, apparently contractile, striated rim of cytoplasm. Application of this flexible rim to a host intestinal cell, working in conjunction with ventral flagella, found in a ventral groove, is responsible for the organism's remarkable ability to adhere to host cells. The pair of ventral flagella as well as three more pairs of flagella arise from kinetosomes located between the anterior portions of the two nuclei. Axonemes of all flagella course through cytoplasm for some distance before emerging from the cell body; those of the anterior flagella actually cross and emerge laterally from the adhesive disc area on the side opposite their respective kinetosomes (Smyth, 1990). A pair of large, curved, transverse, dark-staining median bodies lies behind the adhesive disc. These bodies are unique to *Giardia*. Various authors have regarded

them as parabasal bodies, kinetoplasts, or chromatoid bodies, but ultrastructural studies have shown they are none of these. Their function is obscure, although it has been suggested that they may help support the posterior end of the organism, or they may be involved in its energy metabolism (Smyth, 1990). Cysts are 8µm to 12 µm by 7 µm to 10 µm in size. Newly formed cysts have two nuclei, but older ones have four (Smyth, 1990).

1.2.1.2.2 Pathogenesis:

The pathogenesis of giardiasis is complex and multifactorial. Major contributors to pathogenesis include epithelial remodeling (increased turnover, increased apoptosis), reduced barrier function, brush border (microvillous) effacement, increased transit, bile salt metabolism, and inhibition of pancreatic enzymes. Giardia attaches to the intestinal epithelium, probably by a variety of mechanisms, although it seems likely that the ventral disc plays a major part, either by flagella-generated hydrodynamic forces beneath the disc or by direct disc movement mediated by its contractile proteins, particularly those in the peripheral regions of the disc. Giardia also possesses a mannose binding surface lectin that appears to exist as a prolactin in the cytoplasm and is activated by trypsin. Experiments in attachment models using mammalian intestinal epithelial cells or culture cell lines suggest that both disc and lectin-mediated mechanisms are important, at least in vitro. In human giardiasis the full spectrum of abnormalities of villous architecture has been described, ranging from normal to subtotal villous atrophy. A majority of infected individuals have relatively mild abnormalities of villous architecture, with associated crypt hyperplasia. Infections in experimental models produce similar changes but,

as in human infection, the abnormalities are generally mild. Ultrastructural abnormalities such as shortening and disruption of microvilli are apparent, especially at the point of ventral disc attachment, where microvillus effacement is obvious. This brush border damage is associated with reduction in disaccharidase activity. In animal models, diarrhoea is at its peak when disaccharide activities are most profoundly reduced. However, other mechanisms of epithelial cell damage operate. Work in tissue culture cell lines has shown that *Giardia* trophozoites induce localized condensation of F-actin and loss of peri-junctional α -actinin, and tight junction changes have been associated with alterations in claudin. These cytoskeletal rearrangements could account for early changes in epithelial cell function. There is also evidence to suggest that *Giardia* trophozoites produce cytopathic substances that might be responsible for this disruption of epithelial structure and function. There is increasing evidence to suggest that T-cell activation within the intestinal mucosa can produce villous atrophy. This mechanism also operates in graft-versus-host disease and in coeliac disease. Giardiasis has been associated with increased numbers of aerobic and/or anaerobic bacteria in the upper small intestine of the indigenous Indian population or in travellers to the Indian subcontinent. Bacterial overgrowth can produce architectural abnormalities in the small intestine similar to those seen in giardiasis and thus may have a role in the pathogenesis of mucosal damage. Bile salts have an important role in the life cycle of *Giardia*. There is evidence to suggest that the organism takes up bile salts during growth by an energy-requiring active transport process, possibly involving a membrane carrier. Although the precise metabolic advantages to the parasite have not been defined, a secondary effect of this process could be the depletion of intraluminal bile salt, thus impairing micellar

solubilization of dietary fats and at the same time inhibition of pancreatic lipase, which is dependent on bile salts for full expression of hydrolytic activity. *Giardia* trophozoites are also able to inhibit trypsin and lipase activity in vitro. The precise mechanisms by which the organism achieves this have not been established, although it seems likely that this may relate to a direct effect of parasite-derived proteinases on the secreted pancreatic proteins. Until specific virulence factors have been identified it is unlikely that the relative importance of these mucosal and luminal mechanisms in pathogenesis will be established. At present it seems reasonable to assume that the process is multifactorial, involving a combination of varying degrees of mucosal injury combined with disruption of the luminal phases of digestion and absorption (Farrar *et al.*, 2014).

1.2.1.2.3 Laboratory diagnosis:

Examination of diarrhoeal stool- trophozoite or cyst, or both may be recovered in wet preparation. In examinations of formed stool (e.g. in asymptomatic carriers) only cysts are seen. *Giardia* species may occur in “showers”, i.e. many organisms may be present in the stool on a given day and few or none may be detected the next day. Therefore one stool specimen per day for 3 days is important (Assafa *et al.*, 2006)

1.2.1.3 The coccidian

The coccidia are small protozoa within the subphylum sporozoa. The important intestinal coccidian include *Cryptosporidium parvum* and *Isospora belli*. They are obligate tissue parasites with sexual and asexual stages in their life cycle (Washington *et al.*, 2006).

1.2.1.3.1 *Cryptosporidium parvum*:

Causes cryptosporidiosis, the main symptom of which is diarrhea. It is most severe in immunocompromized patients, e.g., those with AIDS. The organism is acquired by faecal-oral transmission of oocysts from either human or animal sources. The oocysts excyst in the small intestine, where the trophozoite (and other forms) attach to the gut wall. Invasion does not occur. The jejunum is the site most heavily infested. The pathogenesis of the diarrhea is unknown; no toxin has been identified. Diagnosis is made by finding oocysts in fecal smears when using a modified Kinyoum acid-fast stain. Serological tests are not available (Assafa *et al.*, 2006).

1.2.2 Intestinal helminthes:

Intestinal worm infections are widely prevalent in tropical and subtropical countries and occur where there is poverty and poor sanitation. Soil transmitted helminthes (STH) infections form the most important group of intestinal worms affecting two billion people world wide and the main species are *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whip worms) and *Necator americanus/Ancylostoma duodenale* (hookworms) (Sodeman and William, 1990).

Recent estimates suggest that *A. lumbricoides* can infect over a billion, *T. trichiura* 795 million, and *hookworms* 740 million people (de Silva et al, 2003). Other species of intestinal helminthes are not widely prevalent. Intestinal helminthes rarely cause death. Instead, the burden of disease is related to less mortality than to the chronic and insidious effects on health and nutritional status of the host (Stephenson et al, 2000)

In addition to their health effects, intestinal helminthes infections also impair physical and mental growth of children, thwart educational achievement, and hinder economic development (Drake et al, 2000).

1.2.2.1 Taenia species:

Taenia spp are long, segmented, parasitic tapeworms (family: Taeniidae, subclass: Cestoda). These parasites have an indirect life cycle, cycling between a definitive and an intermediate host (Beaver et al, 1984).

Taenia infections are estimated to affect 100 million people worldwide, with major endemic areas located primarily in the developing countries of South America, Africa, India, China and Southeast Asia. *Taenia* infections are less common in North America; however, neurocysticercosis has been recognized as an important health problem in California. Although this disease is mainly seen in migrant workers from Latin American, it has also been reported in US residents who have not traveled to endemic countries (Abhay et al, 2009).

Taenia species are hermaphrodite and are very long enough to measure in metres (*T. solium* measure 3-5 meters while that of *T. saginata* measures 5 - 10 meters). The body is divided into head, neck and a long segmented body (strobilla). The head size is about 1 mm in diameter. Head of both species bears four suckers. The head of *T solium* has a rostellum armed with hooklets. In contrast, the head of *T. saginata* does not have the armed rostellum instead a depression. Each segment contains independent male and female sex organs. The terminal mature segments measuring 15-25 x 5-7 mm keep on detaching from the body and are passed in the feces. There are more than 15 lateral uterine branches in each segment of *T. saginata* whereas the *T. solium* segment contains less than 15 lateral uterine branches. The eggs

are golden brown in color, measure 30-40 um in diameter and are indistinguishable morphologically. The onchosphere bears three pairs of hooklets (Rai et al, 1996).

Humans become infected after eating uncooked or under cooked beef or pork containing cysticerci. After ingestion, the cysticerci attach to the intestinal mucosa and develop into adult worms (Tadesse et al, 2008).

Taeniasis is an infection with the adult tapeworm which usually remains confined to the small intestine. Most often, such infection results in minor gastrointestinal irritation and is frequently accompanied by nausea, diarrhea, constipation, hunger pains, chronic indigestion and passage of proglottids in the feces. Although these symptoms are usually milder when the infection is caused by *T. solium*, the risk of developing cysticercosis remains high (Abhay et al, 2009).

1.2.2.2 *Hymenolepis nana* :

Hymenolepis nana (dwarf tapeworm) is a common human parasite and the smallest tapeworm known to infect humans. The lifecycle of *H. nana* does not require an intermediate host, complete development occurring within a single host (Tadesse et al, 2008).

Hymenolepis nana is widely distributed in countries with warm climates including those of Africa, South America, Mediterranean region, and South East Asia, the infection is more frequently seen in children although adults are also infected (Tadesse et al, 2008).

Adult worm measures 1-3 cm in length. It is made up of head (scolex), neck and segmented body. The head carries four suckers and a rostellum armed with one row of hooks. The segments of the body are divided into

mature and gravid segments. In the mature segment, there are three testes in the middle. The egg, which is immediately infective when passed by the patient, is rounded, about 40 microns in diameter. It contains a six hooked oncosphere within a rigid membrane (the embryophore). This embryophore has two polar thickening or knobs from which project 4 to 8 long, thin filaments called polar filaments (Assafa et al, 2004).

Infection in man takes place by ingestion of eggs through contaminated foods or drinks. Autoinfection (the oncosphere hatched while the eggs being inside the intestine penetrate the villi and develop into cysticercoid larva that later develop into adult worm) also occurs (Rai et al, 1996).

1.2.2.3 *Ascaris lumbricoides*:

Round worm (*Ascaris lumbricoides*) is the largest of the human intestinal parasites. It lives and matures in the ileum and sometimes jejunum of the small intestine. Roundworm is often regarded as a parasite of children, but people of all ages may be infected (Obeng, 1997).

Ascariasis, a soil transmitted infection, is the most common human helminthes infection. Current estimates indicate that more than 1.4 billion people are infected world wide. In the United States, there are an estimated 4 million people infected, primarily in the southeastern states and among immigrants (Abhay et al, 2009).

Important factors associated with an increased prevalence of disease include socio-economic status, defecation practices and cultural differences relating to personal and food hygiene as well as housing and sewage systems. Most infections are sub clinical; more severe complications occur

in children who tend to suffer from the highest worm burdens (Abhay et al, 2009).

Round worm is long, cylindrical and tapers toward both ends. Female worms are longer than the males. Females measure from 200 to 400 mm long and the males are 150 to 300 mm (Obeng, 1997). At the anterior end, there are three prominent lips with dentigerous ridge. Posterior end of male is curved ventral. The tail is bluntly pointed. The spicules in male genital organ are simple and measure 2-3 mm in length. In female, vulva is present at about one third of the body length from the anterior end (Assafa et al, 2004). Since the sexes are separate, it requires infection with both male and female worms to produce fertile eggs in the host. It has been reported that, generally, infected persons harbor more females than male worms with an estimated ratio of 10 female worms to 3 male worms. There is always the possibility that a host may be infected only by female or by male worms. In such cases, the female worms produce the unfertilized eggs, which are incapable of developing further. Each female worm lays about 200,000 eggs per day, for as long as she is fertilized and in the intestine. Adult worms in the human host live for less than 10 months, with maximum life spans of up to 1.5 years. The fertile eggs are ovoid and measure 45 to 70 micrometers by 35 to 50 micrometers in size. Each has a protective durable shell. The eggs are discharged into the lumen of the intestine and leave the host with the feces into the environment (Obeng, 1997). Infection in man takes place by ingestion of embryonated eggs through contaminated food or drinks (unfertilized eggs are non-infectious) (Assafa et al, 2004).

The adult worm normally feeds on partly digested food from the intestine in humans. It has been reported that the host (having about 26 worms) may lose 10 per cent of his/her total daily intake of protein (Obeng, 1997).

Although most individuals infected with *Ascaris lumbricoides* are essentially asymptomatic, the burden of symptomatic infection is relatively high as a result of the high prevalence of infection on a worldwide basis. Symptomatic disease is usually related to either the larval migration stage or manifests as pulmonary disease, or to the intestinal stage of the adult worm (Abhay et al, 2009). The early symptoms of round worm infection are a pneumonitis with cough and sometimes blood stained sputum (which may contain larvae), dyspnea, wheezing, persistent nonproductive cough, substernal pain, fever and diarrhea; These symptoms begin 5 to 6 days after infection, usually last 10 to 12 days and are caused by the round worm larvae migrating and developing inside the human body. A heavy presence of adult worms in the small intestine may cause digestive disorders, nausea, abdominal pain, vomiting, restlessness and disturbed sleep (Obeng, 1997). The large size of the adult worms also presents problems, especially if the worms physically block the gastrointestinal tract. *Ascaris* seems to be especially sensitive to anesthetics, and numerous cases have been documented where patients in surgical recovery rooms have had worms migrate from the small intestine, through the stomach, and out the patient's nose or mouth (Assafa et al, 2004).

1.2.2.4 *Enterobius vermicularis*:

Enterobius vermicularis commonly referred to as pin worm, has the largest geographical distribution of any helminth. Discovered by Linnaeus in 1758, it was originally named *Oxyuris vermicularis* and the disease was referred to

as oxyuriasis for many years. It is believed to be the oldest parasite described and was recently discovered in ancient Egyptian mummified human remains as well as in DNA samples from ancient human coprolite remains from North and South America (Abhay et al, 2009). *Enterobius* is one of the most prevalent nematodes in the United States and in Western Europe. At one time, in the United States there are an estimated 42 million infected individuals. It is found worldwide in both temperate and tropical areas. Prevalence is highest among the 5-10 year-old age group and infection is uncommon in children less than two years old. Enterobiasis has been reported in every socioeconomic level; however, spread is much more likely within families of infected individuals, or in institutions such as child care centers, orphanages, hospitals and mental institutions. Humans are the only natural host for the parasite (Abhay et al, 2009).

Enterobius vermicularis is a spindle shaped parasite of man and attaches to the mucosa of the lower ileum, ceacum and ascending colon. Pinworm eggs are infective shortly after being excreted. After ingestion, the eggs hatch in the upper intestine and liberate larvae which migrate to the region of the ileum. Copulation (mating) of the worms takes place in the lower small intestine, and then the females migrate to the ceacum or lower bowel and pass through the anus where upon contact with the air they shower their sticky eggs on the perianal skin (Tadesse et al, 2008). The eggs of *E. vermicularis* naturally transparent and colorless, measure 50-60 x 20-30 mm in size, and are ovoid and asymmetrical, one side being more convex than the other. They embryonate in 6 h and can remain viable for 20 days in the environment (Burkhart and Burkhart , 2005).

Infection is facilitated by factors including overcrowding, wearing soiled clothing, lack of adequate bathing and poor hand hygiene, especially among young school aged children. Infestation follows ingestion of eggs which usually reach the mouth on soiled hands or contaminated food. Transmission occurs via direct anus to mouth spread from an infected person or via air borne eggs that are in the environment such as contaminated clothing or bed linen (Abhay et al, 2009).

The majority of enterobiasis cases are asymptomatic; however the most common symptom is perianal or perineal pruritus. This varies from mild itching to acute pain. Symptoms tend to be most troublesome at night and as a result, infected individuals often report sleep disturbances, restlessness and insomnia. The most common complication of infection is secondary bacterial infection of excoriated skin. Folliculitis has been seen in adults with enterobiasis. Gravid female worms can migrate from the anus into the female genital tract. Vaginal infections can lead to vulvitis, serous discharge and pelvic pain (Abhay et al, 2009).

1.2.2.5 Hookworms:

Hookworms are nematodes in the superfamily Ancylostomatoidea, Human hookworm infection is a soil transmitted helminthes infection caused primarily by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. It is one of the most important parasitic infections worldwide, ranking second only to malaria in terms of its impact on child and maternal health. An estimated 576 million people are chronically infected with hook worm and another 3.2 billion are at risk, with the largest number of afflicted individuals living in impoverished rural areas of sub-Saharan Africa, South east Asia and tropical regions of the Americas. *N. americanus* is the

most widespread hookworm globally, whereas *A. duodenale* is more geographically restricted in distribution (Abhay et al, 2009). The adult worms are brown at the time of passing. The buccal capsule is big and is armed with cutting plate or teeth. Lips are not present. Males have a conspicuous copulatory bursa, consisting of two broad lateral lobes and a smaller dorsal lobe supported by a fleshy ray. Spicules are simple and needle-like in appearance (Rai et al, 1996). Females' worms are bigger than males, Females have simple conical tail. The vulva is located at about three fifth of the body length from the anterior end (Rai et al, 1996). The eggs are colorless, oval in shape, measure 70 x 40 mm in size and contain four blastomeres in it. A rhabditiform larva (250-300 mm) is hatched from the egg which further grows into a non-feeding filariform larva (500-700 mm) (Rai et al, 1996). The route through which both *A. duodenale* and *N. americanus* enter the human host occurs by the infective larvae penetrating the skin of bare feet or hands that have contacted with contaminated soil (Beaver et al, 1984).

Adult worms bite into the tissues and suck blood. When a hookworm feeds on blood, it releases the secretion of anticoagulant, causing the lesions it created to continue bleeding even when the worm has stopped sucking blood. It has been estimated that a single *N. americanus* is responsible for a mean blood loss of 0.031ml (plus or minus 0.015 ml) per day. Similarly, an *A. duodenale* will cause a mean blood loss of about 0.08-ml (plus or minus 0.02 ml) per day. In case of heavy infections, there may be 500 to 1,000 worms in the host. At that level, the infected person would lose about 50 ml of blood per day (Beaver et al, 1984).

The clinical features of hookworm infection can be separated into the acute manifestations associated with larval migration through the skin and other tissues and the acute and chronic manifestations resulting from parasitism of the gastrointestinal tract by adult worms (Abhay et al, 2009).

Repeated exposure to *N. americanus* and *A. duodenale* filariform larvae can result in a hypersensitivity reaction known as "ground itch", a pruritic local erythematous and papular rash that appears most commonly on the hands and feet (Abhay et al, 2009).

Symptoms or signs of hookworm infection are abdominal pain, nausea, headache, rash, itching, weakness, fever, vomiting, diarrhea, dysentery and gastrointestinal bleeding. Hookworm infection causes iron deficiency anemia. Anemia is believed to be associated with high risk of maternal mortality and morbidity. In areas of very intense transmission heavy worm burdens can be built up in early childhood. In such cases, there may be retardation of mental and physical development (Beaver et al, 1984).

1.2.2.6 Diagnosis of intestinal helminthes:

Although clinical signs may evoke the suspicion of helminthiasis, diagnosis is still dependent upon the isolation and identification of helminthes from the feces. Adult worms or their segments can also be demonstrated macroscopically when the adult worm is spontaneously passed in stool or vomitus; administration of an antihelminthic drug may result in expulsion of the worm. The definitive methods usually involve microscopic detection of helminth eggs from fecal preparations via smears or after concentration. Microscopy, however, requires trained experts, has low sensitivity for detection of light and moderate infections, and may result in misdiagnosis

leading to delayed or inadequate treatment (Verweij et al, 2007). Numerous flotation and concentration methods are available, such as the Kato-katz, formol ethyl acetate sedimentation and zinc sulphate flotation techniques (Martin and Beaver, 1968).

Harada-Mori filter paper strip technique or charcoal culture method is the method of choice to distinguish the larvae of *A. duodenale* and *N. americanus* on epidemiological ground (Cooper, 1999). Commercial antibody detection tests are available for some STH infections (Verweij et al, 2007).

Colonoscopy is useful for the detection of whipworms in the rectum (Cooper, 1999).

The most successful diagnostic method for *Enterobius* is the "Scotch tape" or "cellophane tape" method; this is best done immediately after arising in the morning before the individual defecates or bathes (Abhay et al, 2009).

1.3 *H. pylori*:

Helicobacter pylori is the most common persistent bacterial infection of humans, infecting between 70 and 90% of the population of developing countries and 25–50% of the population of developed countries. (Gillespie and Hawkey, 2006). *Helicobacter pylori* colonizes the mucus layer of the human stomach and causes inflammation termed active chronic gastritis. *H. pylori* can easily be identified using simple techniques available in all microbiology laboratories (Guerrant *et al*, 2011) . It is a major cause of peptic ulcer and a risk factor for gastric malignancies (Lesbros *et al*, 2007).

Helicobacter pylori is a spiral, Gram-negative bacterium that is motile by means of four to eight sheathed flagella. The bacterium is an obligate

microaerobe, requiring 5–7% O₂ and 5–10% CO₂, and grows optimally in a humid atmosphere at 37°C on a variety of basal media supplemented with 5–10% horse or sheep blood. *Helicobacter pylori* will also grow in liquid media such as brain–heart infusion (BHI). (Gillespie and Hawkey, 2006). *Helicobacter pylori* is probably transmitted from person to person by the oral-oral or oral-fecal route. It can also be spread by houseflies acting as mechanical vectors (Talaro and Chess, 2018). Once inoculated, the incubation period is estimated to be 3–7 days and infection can last for the lifetime of the host. *H. pylori* exhibits tissue specificity exclusively for gastric mucosal epithelial cells and does not invade beyond these tissues. Detection of the organisms is best accomplished by biopsy of stomach tissue and subsequent testing for urease activity, by serologic testing, or by culture (Schaechter and Lederberg, 2004).

1.3.1 Pathogenesis and immunity

H. pylori colonizes gastric mucosal (epithelial) cells in the stomach, and metaplastic gastric epithelium in the duodenum or esophagus, but does not colonize the rest of the intestinal epithelium. The organism survives in the mucous layer that coats the epithelium, and causes chronic inflammation of the mucosa (Harvey *et al*, 2007).

1.3.1.1 Virulence factors

H.pylori produces several virulence factors that contribute to the pathogenesis of disease .

1. Urease: The enzyme urease is a very important factor for colonization of *H. pylori* in the gastric mucosa. The enzyme produces ammonia from urea, which in turn increases the pH of the gastric mucosa in the immediate

vicinity of the bacterial cell. It helps, therefore, to neutralize gastric acidity and also in colonization of the organism in gastric mucosa. The enzyme also stimulates monocytes and neutrophil chemotaxis and stimulates production of cytokines (Parija, 2012).

2. Flagella: Flagella are also very important for colonization of *H. pylori*. These help the organism to penetrate into gastric mucous layer; hence, it is protected from acidic environment of the stomach.

3. Adhesins: These include hemagglutinin, sialic acid-binding adhesins, and Lewis blood group adhesins. All these factors facilitate binding of *H. pylori* to gastric mucosa.

4. Enzymes: *H.pylori* produces many enzymes, such as mucinase, phospholipases, superoxide dismutase, and catalase. Both mucinase and phospholipase break down gastric mucus, while superoxide dismutase and catalase prevent phagocytic killing of the bacteria.

5. Heat shock protein (Hsp-B): The heat shock protein facilitates expression of the enzyme urease.

6. Acid inhibitory protein: This protein causes hypochlorhydria by blocking secretions of acid from parietal cells.

7. Cytotoxin: This causes vacuolation in epithelial cells of the host (Parija, 2012).

1.3.1.2 Pathogenesis of *H. pylori* infection:

H. pylori attaches to the mucus-secreting cells of the gastric mucosa. The production of large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa. Loss of the protective mucus coating predisposes to gastritis and peptic ulcer (Levinson, 2014). *H. pylori* also produces a protease that modifies the gastric mucus and further reduces the ability of acid to diffuse through the mucus (Brooks *et al.*, 2013). They produce pathogenic proteins, particularly vacuolating cytotoxin A (Vac A) and the product of the cytotoxin-associated gene A (Cag A), which detrimentally alter the epithelial cells' function. The bacteria evade the host's non-specific immune response, through catalase and superoxide dismutase, to neutralise reactive oxygen and nitrogen species. A pro-inflammatory T-cell response occurs, involving TH cells, IL-2, IL-17, IL-22 and IFN- γ but there is Treg activity which moderates the immune-mediated damage. In addition, the bacterial lipopolysaccharide and flagellin are not very immunogenic which limits the extent and effectiveness of the B-cell function (Pitt, 2018).

1.3.2 Clinical findings:

The clinical expression of chronic *Helicobacter pylori* (Hp) infection in humans is highly variable. Most infected individuals are asymptomatic. A minority suffers from dyspeptic symptoms and/or duodenal and gastric ulcer disease. Finally, gastric adenocarcinoma and primary gastric lymphoma are increasingly considered as potential end-stage consequences of chronic Hp associated inflammation (Brooks *et al.*, 2013).

1.3.3 Diagnosis:

1.3.3.1 Serological diagnosis:

There are numerous serological diagnoses, such as bacterial agglutination, complement fixation, indirect immunofluorescence test (IIF), enzyme immunoassay (EIA), and enzymelinked immunosorbent assay (ELISA), and ELISA is the most prevalent diagnosis (Kim, 2016).

Advantages and disadvantages of serological diagnosis

Serological diagnosis can be a useful way to detect *H. pylori* preliminary infection because it is easy, fast, noninvasive, and relatively economic and tends to show less false-negative results under certain situations, such as taking antibiotics or hemorrhagic ulcer condition, compared with other diagnoses. However, the serological diagnosis could show false-positive results for several months or years on samples because it hardly differentiates current active infection from previous infection, which has been treated and no more infection is shown (Kim, 2016). Thus, the serological diagnosis is not recommended after antimicrobial treatment because an average of 1 year was necessary until either antibody is undetectable or antibody titer is reduced to 50 % after antimicrobial treatment (Kim, 2016). Rather, it is utilized as a preliminary, selective diagnosis for *H. pylori* infection, not after the antimicrobial treatment (Kim, 2016). For instance, the serological diagnosis is used for epidemiological survey, not as a diagnosis before or after antimicrobial treatment, in China. In contrary, the diagnosis is recommended to be performed before or after antimicrobial treatment in Japan; *H. pylori* eradication is considered to be successful, if serological antibody titer that is

performed from 6 to 12 months after antimicrobial treatment is 50 % less than the titer that is performed before the treatment (Kim, 2016).

1.3.3.2 Urea breath test (UBT) :

The UBT is a preferred test for the diagnosis and confirmation of cure because it is a noninvasive, simple, and accurate method for diagnosis of active infections . Testing requires the patient to ingest a small quantity of urea in which the carbon is labeled with either the stable isotope ^{13}C or the radioactive isotope ^{14}C . If *H. pylori* is present, *H. pylori* breaks down orally ingested ^{13}C - or ^{14}C labeled urea into CO_2 and ammonia. $^{13}\text{CO}_2$ or $^{14}\text{CO}_2$ diffuses into the blood, is exhaled via the lungs, and can be measured in the exhaled air. Both ^{13}C - and ^{14}C UBTs are sensitive and specific for *H. pylori* detection; however, ^{13}C is generally preferred because it is not radioactive and thus avoids the potential problems associated with the use of radioactive substances, especially in children and in pregnant women. Positive and negative UBT results tend to cluster outside of the range between 2% and 5% such that change in cutoff value within this range would be expected to have little effect on the clinical accuracy of the test . The simplicity, good tolerance, and economy of the citric acid test meal probably make its systematic use advisable. Some protocols use a test meal to delay gastric emptying and attempt to spread the urea within the stomach. Over time the choice of test meal switched from an actual test meal such as a pudding to citric acid. Most have found no significant differences between tests performed under fasting and non-fasting conditions. Thus fasting prior to testing seems to be not necessary and non-fasting may be more applicable in the routine setting (Suzuki et al, 2016).

1.3.3.3 Culture

Cultures of *H. pylori* are definitive proof of infection. However, the ability to isolate the organism from infected subjects varies widely between laboratories and makes it the most technically demanding of the *H. pylori* diagnostic tests. The bacterium loses its viability quickly when exposed to the environment, and biopsies should be cultured quickly to maximize the recovery of bacteria. Several transport media have been suggested, including normal saline, 20% glucose, Stuart's transport medium, and chocolate agar slants. *H. pylori* is recoverable from biopsy material stored in Stuart's transport medium for 24 h at 4 °C, but above 15 °C viability is poor even after 6 h. Despite the number of reports dedicated to identifying the best media, the optimal recovery method remains to be established. Selectivity is provided by the addition of different combinations of antibiotics. By definition, culture is 100% specific in diagnosing *H. pylori* infection, but unfortunately sensitivity can vary widely between centers due to local expertise. It is also possible to obtain tests sensitive to antibiotics using different techniques, including agar dilution, agar diffusion, and E-test. However, these techniques demand money, time, and personnel, and their use is limited mainly to research centers (Vaira *et al.*, 2002).

1.3.3.4 Histology

Helicobacter pylori is recognized histologically in tissue section by its characteristic morphology, position, diffuse distribution and high population density. Stains for the recognition of Helicobacter infection include Giemsa, Cresyl fast violet and Warthin-starry. Sensitivity can be improved by the use of immunostaining with a monoclonal anti-*H.pylori* antibody. A careful

histological examination of at least two biopsies is still taken as the "gold standard" for establishing *H. pylori* status (Williams, 1997).

Rationale

H. pylori infection is more frequent and is seen in younger ages in developing countries when compared to developed countries. Etiopathogenetic factors include living in crowded families, low educational level of mother, low income and infected drinking water. Intestinal parasites are more frequent in low socio-economical populations. In this study, it was aimed to determine the prevalence of intestinal parasite in patients with *H. pylori* in Khartoum state.

Objectives

General objective:

- To investigate an association (if any) between gastrointestinal parasites and *H.pylori* .

Specific objectives:

- To determine the prevalence of gastrointestinal parasites in *H.pylori* patients according to gender and age group.
- To determine the prevalence of gastrointestinal parasites in the control group according to gender and age group.
- To estimate the frequency of each parasite.

Chapter two
Materials and methods

Chapter two

Materials and methods

2.1 Study design:

It is a case control study.

2.2 Study area:

The study was conducted in Alsaaha Specialized Hospital and Yastabshiron Hospital in Khartoum.

2.3 Study population :

The study was carried out on 100 patients with *H.pylori* positive and 100 with *H.pylori* negative as the control group, the test for *H.pylori* was already done by hospitals using ICT for detection *H.pylori* Ag in stool sample .The participants were divided according to gender and age group.

2.4 Ethical consideration:

Approval was taken from Sudan University of Science and Technology, College of Graduate studies , Alsaaha Specialized Hospital and Yastabshiron Hospital administration . A verbal consent was taken from each patient.

2.5 Study duration:

The study started in June and ended in October of 2018.

2.6 Sample size:

200 stool sample were collected from individuals under study .100 samples as the study cases and other 100 samples as the study controls.

2.7 Sampling collection:

Each selected patient with *H.pylori* and healthy individuals without *H.pylori* was provided with a labeled container which is transparent and clean for fecal sample collection .

2.8 Data collection:

A questionnaire was designed to collect data on gender and age (Appendix).

2.9 Methodology:

The direct smear examination, formal ether concentration technique and the saturated sodium chloride floatation technique were used for the detection of different gastrointestinal parasites.

2.9.1 Direct smear examination:

Wet preparation was made by mixing small portion of stool taken with an applicator wooden stick with a drop of normal saline on slide and covered with cover slip and examined systematically under microscope using 10X and the high magnification 40X for observation of more details.

2.9.2 Formal ether concentration technique:

Approximately, one gram of feces was collected from different parts of the specimen and emulsified in 5 ml of formal saline in glass beaker. Further 5 ml from same solution was added and mixed. The resulting suspension

was strained through the sieve. The filtered sample was poured back into a centrifuge tube and then equal volume of ether was added. The tube was mixed for one minute and then centrifuged for 5 minutes at 2000 rpm. All upper 3 layers were discarded and the sediment was transferred into slide which was covered with cover slip and examined under microscope using 10X and 40X magnifications.

2.9.3 Saturated sodium chloride floatation technique:

Approximately, half gram of feces was collected from different parts of the specimen and emulsified in long glass tube half filled with saturated sodium chloride solution and then the tube was filled with the same solution until convex shape was formed. Carefully, a cover glass was put and air bubbles were avoided. 30 to 45 minutes after, a cover glass was taken and put on clean and dry slide and examined under microscope using 10X and 40X magnifications.

2.10 Data analysis:

The data was analyzed using statistical package for social science (SPSS) computer program version 16.

Chapter three

Result

Chapter three

Result

The results showed that out of the 100 patients with *H.pylori* , 23 were found infected with gastrointestinal parasites .This constituted an overall infection rate of 23% , while out of the 100 control group, 10 were found infected with gastrointestinal parasites . This constituted an overall infection rate of 10% (table 1) . This difference in rates was found to be statistically significant at $p= 0.013$. The overall prevalence rate among both *H.pylori* patients and control patients was found to be 16.5% (table 1). In *H.pylori* patients , the occurrence among males and females was almost close (24% and 22% respectively) , however , this difference was found to be statistically insignificant at $p= 0.841$ (table 2) . On the other hand, the occurrence of gastrointestinal parasites among males and females in the control group was found to be 9% and 11% respectively , however, this difference was found to be statistically insignificant at $p= 0.789$ (table 3). The highest occurrence rates (40% and 38%) were reported among the 1-15 and 46-60 years age groups respectively , while the lowest rate (10.7%) was reported among the 31-45 years age group for *H.pylori* patients (table 4) . These differences in rates were found to be statistically insignificant at $p= 0.132$. For the control group , the highest occurrence rate (15%) was reported among the 31-45 years age group , while the lowest occurrence rate (8%) was reported among the 16-30 years age group (table 5). This difference in rates was found to be statistically insignificant at $p= 0.528$.

The results revealed that *Entamoeba histolytica* was seen in 12% of the *H.pylori* cases followed by *Entamoeba coli* in 9% of cases and *Giardia*

lamblia in 4% (table 6). Lower rates were reported among the control group where *Entamoeba histolytica* was seen in 5% followed by *Giardia lamblia* in 3% of ontrols and *Entamoeba coli* in 2% (tabe 7).

Table 1: The overall occurrence rate of gastrointestinal parasites among patients with *H.pylori* compared to control group :

Patients	Number examined	Number positive	Occurrence
Case	100	23	23 %
Control	100	10	10 %
Total	200	33	16.5 %

P.value = .013

Table 2 : Occurrence of gastrointestinal parasites among *H.pylori* patients according to gender :

Gender	Number examined	Number positive	Occurrence
Male	46	11	24 %
Female	54	12	22 %
Total	100	23	23 %

P.value = .841

Table 3 : Occurrence of gastrointestinal parasites among control group according to gender :

Gender	Number examined	Number positive	Occurrence
Male	54	5	9 %
Female	46	5	11%
Total	100	10	10 %

P.value =.789

Table 4 : Occurrence of gastrointestinal parasites among *H.pylori* patients according to age group :

Age group	Number examined	Number positive	Occurrence
1-15	10	4	40 %
16-30	37	7	19 %
31-45	28	3	10.7 %
46-60	21	8	38 %
61-75	4	1	25 %
Total	100	23	23 %

P.value = .132

Table 5: Occurrence of gastrointestinal parasites among control group according to age group:

Age group	Number examined	Number positive	Occurrence
1-15	14	2	14.3 %
16-30	37	3	8 %
31-45	33	5	15 %
46-60	12	0	0 %
61-75	4	0	0 %
Total	100	10	10 %

P.value = .528

Table 6: Occurrence of different gastrointestinal parasites encountered among *H.pylori* patients:

GIT parasite	Number examined	Number positive	Occurrence
<i>Entamoeba histolytica</i>	100	12	12 %
<i>Giardia lamblia</i>	100	4	4 %
<i>Entamoeba coli</i>	100	9	9%

Table 7: Occurrence of different gastrointestinal parasites encountered among control group :

GIT parasite	Number examined	Number positive	Occurrence
<i>Entamoeba histolytica</i>	100	5	5 %
<i>Giardia lamblia</i>	100	3	3 %
<i>Entamoeba coli</i>	100	2	2 %

Chapter four

Discussion , conclusions and recommendations

Chapter four

Discussion

From the results, it is obvious that the overall occurrence rate of gastrointestinal parasites among patients with *H.pylori* was relatively high (23%). This rate was found to be higher than the rate reported by Ugras and Miman (2014) in Turkey (7.61%) . As far as the control group is concerned, the overall occurrence rate reported was 10% . This rate is lower than the rate among *H.pylori* patient , and higher than the rate reported by Ugras and Miman (2014). The difference in rates between the control group and patient with *H.pylori* was significant .This in our opinion, might probably reveal that there is association between the establishment of gastrointestinal parasites and *H.pylori* .

The prevalence rates in males and females with *H.pylori* and control showed insignificant statistical difference with p.value 0.841 and 0.789 respectively. these results were not in agreement with Yakoob et al (2005) who found that the occurrence of *G. lamblia* was higher in males (72%) than in females (28%) in Pakistan. The highest occurrence rates (40% and 38%) were reported among the age group 1-15 and 46-60 respectively in the *H.pylori* patients , and for control group the highest occurrence rate (15%) was reported among the 31-45 years age group. Our finding disagreed with the finding of Fadul *et al* (2016) who reported highest occurrence rate (50%) in age group >66 years old.

The results also showed that *Entamoeba histolytica* was seen in 12% of the *H.pylori* cases followed by *Entamoeba coli* in 9% of cases and *Giardia lamblia* in 4% . Lower rates were reported among the control group where

Entamoeba histolytica was seen in 5% followed by *Giardia lamblia* in 3% and *Entamoeba coli* in 2%. Our result was not in line with the finding of Goksen *et al* (2016) who reported 14.8% for *Giardia lamblia* in the *H. pylori*-positive group.

Our result proved a significant association between *H.pylori* and intestinal parasites which was in agreement with Escobar-Pardo *et al* (2011) who also found significant association between *H.pylori* and *Giardia lamblia*. However, our conclusion was in total disagreement with the finding of Ugras and Miman (2014), who reported no significant association between *H.pylori* and intestinal parasites in Turkey.

Conclusions

- Gastrointestinal parasites are more occurrence among *H.pylori* patients compared to the control group.
- Infection rate was not affected by gender.
- The highest infection rate was reported in the 1-15 and 46-60 age group among *H.pylori* patient and 31-45 years age group among the control patients.

Recommendations

Based on this work, it is recommended that :

- Further investigations are required for *H.pylori* patients with respect to gastrointestinal parasites to elucidate more on the association.
- Must treat gastrointestinal parasites in *H.pylori* patients.
- Sample size should be increased in future research to avoid errors in interpretation of results.

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Appendix

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Questionnaire

**correlation between Helicobacter pylori infection and presence
of intestinal parasites in Khartoum state**

Name :

Gender: Male

Female

Age : Years.