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**Association between Helicobacter Pylori
infection and Intestinal parasite infection in
River Nile state in April --- July--- 2018**

*A thesis submitted for partial fulfillment of requirement for the degree of
B.S.C in medical laboratory Sciences (honor)*

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى :

(... يرفع الله الذين آمنوا منكم والذين
أوتوا العلم درجات والله بما تعملون
خبير)

صدق الله العظيم

سورة المجادلة ، الآية (11)

DEDICATION :

To my parents

My teachers

My friends

ACKNOWLEDGMENTS :

First of all , my gratitude to ALMIGHTY ALLAH for helping us to complete this work properly . thanks must be extended

To supervisor Dr . Elyassa Almahi for this close supervision

Valuable advices , and stimulating suggestions . his pleasant

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I also greatly acknowledge and extend my hurtful gratitude to all subjects

For their participation and cooperation .

Also special thanks to my teachers and colleagues for ultimate support throughout this study .

Abstract :

This study was conducted during the period from April to July 2018 .the aim of this study was to determine the association between Helicobacter pylori and intestinal parasite infection among all individual That was infected with intestinal parasite .

A total of 50 subjects (n=50) were included in study in all different Age . and both male and female or gender from the study participants , stool sample in clean dry container were obtained . the stool sample Tested for helicobacter pylori antigen by using immune chromatography test antigen of stool .

Out of the 50 patient tested 10 were helicobacter pylori and intestinal parasite positive , 40 patient with intestinal parasite and negative helicobacter pylori .

Also we concluded that there was very low percentage between Helicobacter pylori and intestinal parasite and was no significance (p=.91)

الخلاصة :

أجريت هذه الدراسة خلال الفترة من أبريل إلى يوليو 2018 وكان الهدف من هذه الدراسة هو تحديد العلاقة بين جرثومة المعدة و عدوى الطفيليات المعوية بين جميع الأفراد التي كانت مصابة بطفيلي معوي من 50 شخص (ن = 50) تم تضمينها في الدراسة من جميع الأعمار المختلفة لكل من الذكور والإناث المشاركين في الدراسة ، تم الحصول على عينة البراز في الحاويات الجافة النظيفة لعينة البراز التي تم اختبارها بواسطة استخدام شريط الانتجين الممنع من البراز من بين 50 مريضاً تم اختياره بنسبة 10% كانت مصابه بجرثومة المعدة و40 غير مصابة بجرثومة المعدة ومصابة بالطفيليات المعوية . كما استنتجنا أن هناك علاقة منخفضة جدا بين جرثومة المعدة والطفيليات المعوية

CHAPTER ONE

1.1. Introduction

For many years the human stomach was considered to be an inhospitable acidic environment in which bacteria could not grow. *Helicobacter pylori* is a common type of bacteria that grows in the digestive tract and has a tendency to attack the stomach lining. It infects the stomach of roughly 60 percent of the world's adult population. *Helicobacter pylori* infections are usually harmless, but they're responsible for the majority of ulcers in the stomach and small intestine. The "H" in the name is short for *Helicobacter*. "Helico" means spiral, which indicates that the bacteria are spiral shaped. *Helicobacter pylori* often infect your stomach during childhood. While infections with this strain of bacteria typically don't cause symptoms, they can lead to diseases in some people, including peptic ulcers, and an inflammatory condition inside your stomach known as gastritis. *Helicobacter pylori* are adapted to live in the harsh, acidic environment of the stomach. These bacteria can change the environment around them and reduce its acidity so they can survive.^[1] The spiral shape of *Helicobacter pylori* allows them to penetrate your stomach lining, where they're protected by mucus and your body's immune cells are not able to reach them. The bacteria can interfere with your immune response and ensure that they're not destroyed. This can lead to stomach problems. *Helicobacter pylori* is mainly transmitted by oral fecal route.^[2]

Oncogenic consequence of *Helicobacter pylori* infection

Gastric adenocarcinoma is a heterogeneous cancer. First, it is necessary to distinguish the tumors arising from the gastric proximal stomach (cardia), as most of them are not linked to *Helicobacter pylori* infection from those found in the distal part of the stomach. Among tumors from the distal stomach, on the basis of histology, it is usual to differentiate two types of cancer lesions: the intestinal type and the diffuse type according to the Lauren classification .

Intestinal type cancer is the most frequent. It corresponds to a slow evolution of the gastric mucosa which becomes atrophic; then intestinal metaplasia appears, followed by dysplasia and ultimately in situ gastric carcinoma and metastatic carcinoma .This is the so-called Correa cascade, which was described before *Helicobacter pylori* was discovered and appears late in life^[3].

1.2. Justification

- Poor hygiene among most people in river Nile .
- helicobacter pylori and intestinal parasite are same in mode of transmission
 - intestinal parasite and helicobacter pylori easy to transmitted in tropical country due to poor environment

1.3. Objectives

1.3.1. General objective

Association between Helicobacter pylori and intestinal parasite infection

1.3.2. Specific objective

- To determine which intestinal parasite have high relationship with Helicobacter pylori
- To improve diagnosis of stool samples to avoid mis-cases of Helicobacter .pylori
- To improve treatment of early cases of Helicobacter .pylori infection to avoid complication

1.4. Literature Review

Helicobacter pylori

1.4.1. Important properties

Members of the genus helicobacter are curved or spiral organism they have a rapid corkscrew motility resulting from multiple polar flagella . Helicobacter pylori the species of human significant is microaerophilic and produce urease It causes acute gastritis and duodenal and gastric ulcer. ^[4]

Helicobacter pylori and several other helicobacter species are unusual in their ability to colonize the stomach. Where low PH normally protect against bacterial infection helicobacter pylori infections are relatively common and worldwide in distribution . ^[5]

1.4.2. Pathogenesis and associated disease

Helicobacter pylori attaches to the mucus secreting cells of gastric mucosa . the production of large amount of ammonia from urea by the organism urease coupled with an inflammatory response lead to damage of mucosa loss of the protective mucus coating predispose to gastric and peptic ulcer the ammonia also neutralize stomach acid allowing the organism to survive . ^[6]

The outcome of Helicobacter reflect an interaction between strain virulence . Pro inflammatory host genotype and environment despite the presence the chronic active gastritis most infection are symptoms less and endoscopic appearance of the stomach are normal how ever in some infected person the chronic active gastritis forms launch for more series clinical outcomes such as gastric and duodenal ulcer .non ulcer dyspepsia and gastric malignancies. peptic ulceration Helicobacter pylori is actively involved in the pathogenesis of peptic ulceration unrelated to non steroidal anti inflammatory agent or the zolinger Ellison syndrome infection is virtually for ulceration and elimination of Helicobacter pylori. allows healing of ulcer without recurrence ulceration is almost always associated with recurrent of infection .

Inventorial predominant gastritis hyperacidity induced by helicobacter pylori increase gastein production promotes duodenal gastric metaplasia and this lead to colonization of the duodenum inflammation and finally ulceration. with corpus .predominant gastritis or pan gastritis host interaction lead to suppression of acid and destruction of partial cells consequently duodenal ulceration is not evident although hypoacidity can

lead to epithelial changes and gastric gland atrophy that increase the risk of gastric ulceration .

Some cases of non ulcer dyspepsia are associated with helicobacter as eradication has a small but significant effect on dyspepsia and present the development of peptic ulcer in some patient.

Gastric cancer atrophic gastritis resulting from long standing infection with helicobacter pylori .^[7]

1.4.3. Epidemiology

Most patient with these diseases show helicobacter pylori in biopsy specimen of gastric epithelium.

The natural habitat of helicobacter pylori is the human stomach and its probably acquired by ingestion. However it has been isolated from stool 'food 'water or animal ,person to person transmission probably occur because there is clustering of infection within the families the rate of infection with helicobacter pylori in developing countries is very high a finding that is accord with high rate of gastric carcinoma in the countries MALT lymphomas are B-cell tumor located typically in the stomach but they occur also where in the gastrointestinal tract as well helicobacter pylori is often found in the ' MALT ' lesion and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually B-cell lymphoma antibiotic treatment directed against the organism often causes the tumor regress .^[8]

1.4.4. Immunity

Patient infected with helicobacter pylori develop an 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 early antimicrobial treatment of helicobacter pylori infection blunts the antibody response such patient are thought to be subject to repeat infection .^[9]

1.4.5. laboratory diagnoses

The organism can be seen on gram stained smear of biopsy specimen of gastric mucosa ,it can be cultured on the same media as campylobacter but contrast to it helicobacter pylori is urease positive ,urease production is basis for non invasive diagnostic test called urea breath test in this test radio labeled urea is ingested . if the organism is present urea cleavage

the ingested urea radio labeled into co2 is evolved and radio activity is detected in the breath .

Test of helicobacter pylori antigen in stool can be used for diagnosis and confirmation that treatment has eliminated the organism .the present of IgG antibody in the patient serum also be used as evidence of the infection ^[10]

Non invasive test

Serology

Serological test mainly based on ELISA or latex agglutination detect antibodies to helicobacter pylori and are used to screen patient with dyspepsia they are less useful for screen children un reliable excluding infection in elderly patient or as test for cure in patient who have received treatment owing to variable persistence of antibody .
The accuracy of rapid bed side test of whole blood is poor .

Urea breath test

This test detect bacterial urease activity in the stomach by measuring the output of the carbon dioxide resulting from the splitting of carbon 13 or carbon14 labeled urea into carbon dioxide and ammonia ,infected patient give high reading the test has radioactivity so it is not used in children a mass spectrometer is needed to assay non radioactive carbon13 the urea breath test can not be used during or directly after antibiotic therapy .

Fecal antigen test

Stool antigen test that detect helicobacter pylori in feces are available test based on use of monoclonal antibodies are more accurate than polyclonal antibodies test and have potential to supplant serology for routine .

Polymerase chain reaction (PCR)

Detection of Helicobacter pylori using polymerase chain reaction technique . This test detects bacteria Helicobacter pylori (H. pylori) using a special technique called polymerase chain reaction (PCR). PCR detects gene sequences in bacterial DNA This test is used when an infection with helicobacter pylori is suspected A sample of saliva and stool or stomach lining cells through gastric endoscopy may be collected for this test. If you are having a gastric endoscopy, this test may be performed during

your endoscopy. However, an endoscopy is generally not performed just to collect a sample for this test.

Invasive Test

Biopsy specimen is placed into small quantity of urea solutions with a dye such as phenol red, which detects alkalinity resulting from the formation of ammonia. Most infected patients (70%) give a positive result within 2h, 90% after 24 h. Newer tests have faster reaction times and a test with monoclonal antibody promises higher sensitivity and specificity.

Histopathology and microscopy.: histopathology provides a permanent record of the nature and grading of a patient's gastritis as well as detecting helicobacter pylori. organisms can be seen in section stained with haematoxylin and eosin but more specific stains make the task easier. The bacteria can also be seen in smear of biopsy material stained with gram's stain. Fluorescein based molecular probes under development are potentially able to detect helicobacter pylori and its virulence factors.

Culture

Culture is no more sensitive than skilled microscopy of histological section,

Collection of specimen ideal patients for endoscopy should not have received antibiotics or protons pump inhibitor for one month before the test.

Mucosal biopsy specimen are taken from the gastric Antrum within 5cm of the pylorus and preferably also from the body of the stomach for maximum sensitivity, duplicate specimens are taken: one for histopathology (placed in fixative) the other for culture (placed in the neck of sterile potter made humid by adding a tiny amount of normal saline) specimen for culture must be processed as soon as possible. certainly on the same day, or placed in transport medium.

Helicobacter pylori cultured in chocolate agar or in campy Bap media under CO₂ or 5% O₂ incubation at 37°C for 3-6 day

Biopsy urease test

This is simple and cheap test that can be performed at the bedside. Biopsy specimen.

Biopsy specimen is placed into small quantity of urea solutions with a dye such as phenol red ,which detects alkalinity resulting from the formation of ammonia. Most infected patients(70%) give a positive result within 2h ,90% after 24 h .Newer tests have faster reaction times and a test with monoclonal antibody promises higher sensitivity and specificity.

1.4.6. Treatment and prevention

The concept that under lies the choice of drugs is to use antibodies to eliminate helicobacter pylori plus drug to reduce gastric acidity combination of two antibiotic is used because resistance especially to metronidazole has emerged .treatment of duodenal ulcer with antibiotic (example amoxicillin and metronidazole)and bismuth salts (pote bismol) result in greatly decreased recurrence rate ,tetracycline can be used instead of amoxicillin there is no vaccine or other specific preventive measure .^[10]

1.4.7. Intestinal parasite

Intestinal parasitic infections are amongst the most common infections throughout the world. It is estimated that 3.5 billion people are affected, and that 450 million are ill as a result of these infections, the Majority were children. ^[11]

Modes of transmission

The most common and prevalence mode of transmission among intestinal parasite may include: Oro- fecal route: It is the most common mode of transmission of the parasites. Infection is transmitted orally by ingestion of food, water or vegetables contaminated with feces containing the infective stages of the parasite. (e.g., cysts of *E. Histolytica*, and ova of *Ascars Lumbricoides*), Autoinfection: Few intestinal parasites may be transmitted to the same person by contaminated hand (external autoinfection) or by reverse peristalsis (internal autoinfection). It is observed in (*Crypto- sporidium Parvum*, *Taenia Solium*, *Enterobius Vermicularis* ,*Strongyloides Stercoralis* and *Hymenolepis Nana*), Contaminated soil and water: Soil polluted with human excreta containing eggs of the parasites can act as an important source of infection, e.g., hookworm, *Ascaris* species, *Strongyloides* species and *Trichuris* species. Water contaminated with human excreta containing cysts of *E. Histolytica* or *Giardia Lamblia*, can act as source of infection, Raw or under cooked meat: Raw beef containing the larvae of

Cysticercus Bovis and pork containing Cysticercus Cellulosae are some of the examples where undercooked meat acts as source of infection other sources of infection: Fish, crab or aquatic plants.^[12]

Rick factors

The major's risk factors that associated with intestinal parasites that most commonly associated with primary school children varied significantly with: Hand washing habits after defecation, different habits of washing fruits before eating, source of drinking water, water boiling habits, use of footwear after school hours, inadequate sanitation and hygiene. ^[13]

Major symptoms

Intestinal parasites produce a variety of symptoms in those who are affected, most of which manifest themselves in gastrointestinal complications and general weakness, gastrointestinal complications include diarrhea, nausea, dysentery, and abdominal pain and abdominal discomfort. These symptoms negatively impact with nutritional status, including decreased absorption of micronutrients , loss of appetite, weight loss, steatorhea, and intestinal blood loss that can often result in anemia(as d .latum [fish tape worm]) that absorb vitB12 and cause it is deficiency and lead to megaloblastic anemia). It may also cause physical and mental disabilities, delayed growth in children, and skin irritation around the anus and vulva. ^[14]

Lab diagnosis

Feces like other specimen received in the laboratory most be handled with care to avoid acquiring infection . fecal specimens may contain

The infected forms of parasite such us strongloyed Stercoralis Giardia Lamblia Entamoeba Histolytica cryptosporidium .

Feces specimens should be examined within a few hours of being passed . if amebic dysentery is suspected the specimen should be examined as soon as possible and kept in a warm environment (35-37C) until it is examined . ^[15]

Macroscopic examination

- Colour
- Consistency whether it is formed ,semi formed ,unformed or fluid .

- Whether it contain blood ,mucus ,pus .
- Whether contain worms .^[15]

Microscopic examination

- Wet mount by used normal saline , eosin and iodine .
- Concentration techniques .^[15]

Concentration techniques

- Formal ether techniques .
- Formal detergent techniques .
- Flootation techniques .^[15]

CHAPTER TWO

2. Methodology and material

2.1. Study design

Cross sectional study design

2.2. Study area

The study were conducted in river Nile state ,Sudan, from April to July, 2018

2.3. Study population

All individual male and female was infected by intestinal parasite during the study period .

2.3.1. Inclusion criteria

People in different age and gender that infected with intestinal parasite

2.3.2. Exclusion

All people that not infected with intestinal parasite .

2.4.sampling

2.4.1. Sampling technique

Stool sample in clean dry container tested by stool ICT antigen

2.4.2. Sample size

Collected according to questionnaire N = 50

2.4.3. Sampling collection

The sample was collected under aseptic condition in clean dry container and examined freshly within minutes .

2.5. Data collection

Personal and clinical data were collected by direct interviewing questionnaire from each subject and stool sample . and then analyses by program (SPSS).

2.6. Ethical consideration

Permission to carry out the study was obtain from the college of Health , Elshaikh Abdallah Elbadri University . All specimen donors were

examined informed for the purpose of the study before collection of the sample and verbal consent was taken from them .

2.7. ICT for detection of Helicobacter pylori antigen Helicobacter pylori antigen rapid test cassette (feces)

2.7.1. Principle

The Helicobacter pylori antigen rapid test a Cassette (feces) is a qualitative lateral flow immunoassay for detection of Helicobacter pylori antigen s in human feces specimen in this test the membrane is pre coated with anti Helicobacter pylori antibodies on the test line region of the test during testing the specimen reacts with the particle coated with anti Helicobacter pylori antibodies .the mixture migrates upward on the membrane by capillary action to react with ant-Helicobacter pylori antibodies on the membrane and generate a colored line . The presence of this colored line in the test region indicates positive .

2.7.2. Procedure

- We collect fecal specimen collect sufficient quantity of feces(1-2 ml or 1-2 g in a clean, dry container to obtain maximum antigen (if present). Best results will be obtained if the assay is performed within 6 hours after collection. Specimen collected May be stored for 3 days at 2- 8 c if not rested within 6 hours. For long term storage, specimens should be kept below -20 c.
- To process fecal specimens
- Bring the pouch to room temperature before opening it. Remove the test cassette from the foil pouch and use it within one hour. Best results will be obtained if the test is performed immediately after opening the foil pouch.
- Hold the specimen collection tube upright and open the cap onto the specimen collection tube. Invert the specimen collection tube and transfer 2full drops of the retracted specimen (approximately 80) to the specimen well of the test cassette, then start afresh following the instructions mentioned above

CAPTER THREE

Result

This is a descriptive cross sectional study that conducted in river Nile state during the period from April To July 2018, to Association between helicobacter pylori and intestinal parasite were collected data, out of the 50 patient tested 10 were helicobacter pylori and intestinal parasite positive, 40 patient with intestinal parasite and negative helicobacter pylori, after being analyzed in three program phases was result in the follow :

Table (3.1) Distribution of study group according to gender

gender	Frequency	Percent%
Male	33	66
Female	17	34
Total	50	100

The table(3. 1)and figure (3.1) show frequency of gender: the male is 33 which consist(66%) of total frequency and female frequency 17 which consist 34% of total frequency.

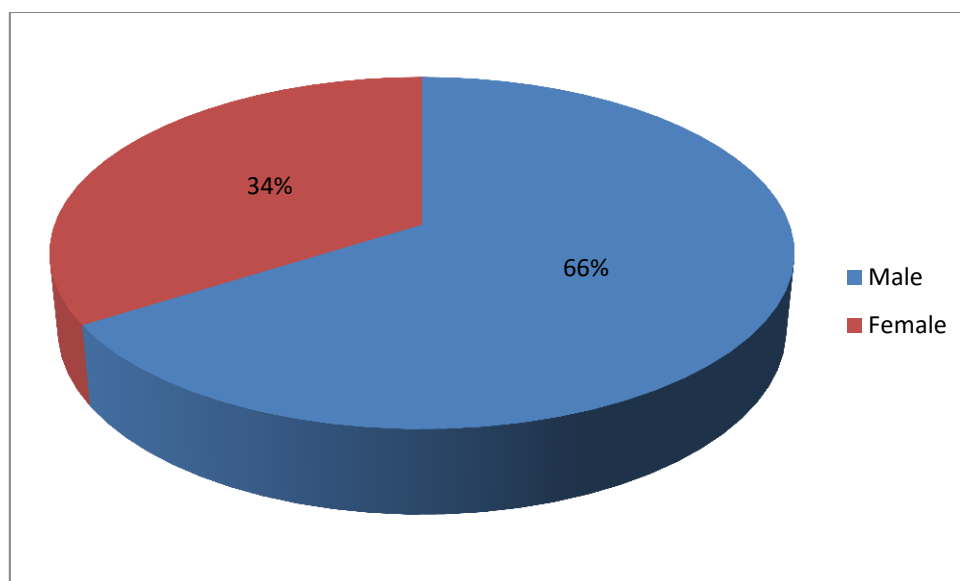


Figure (3.I): Show gender

Table (3.2) Distribution of study group according age

age	Frequency	Percent
Less than 10	1	2.0%
10_20	12	24.0%
20_30	24	48.0%
30_40	7	14.0%
More than 40	6	12.0%
Total	50	100.0%

The table(3. 2) and figure(3.3) show age distribution which group of (20 - 30) present the highest age group with percent 48%

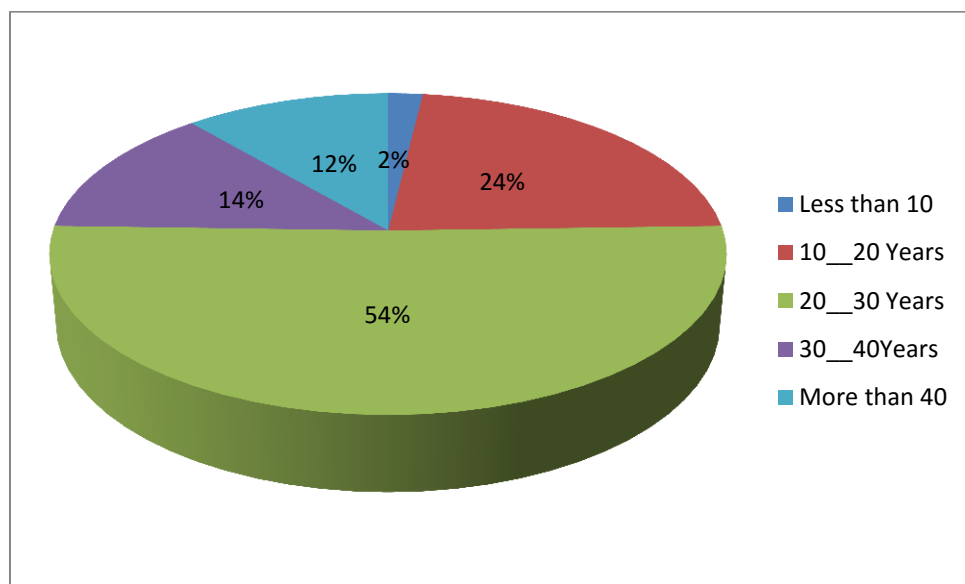


Figure (3.2): Show age

Table (3.3) Distribution of helicobacter pylori in study group

Helico bacter pylori	Frequency	Percent%
positive	10	20.0%
Negative	40	80.0%
Total	50	100.0%

The table(3.3) and figure (3.3) show distribution of Helicobacter pylori the positive frequency is 10 which consist 20% of total frequency , and the negative frequency is 40 which consist 80% of total frequency.

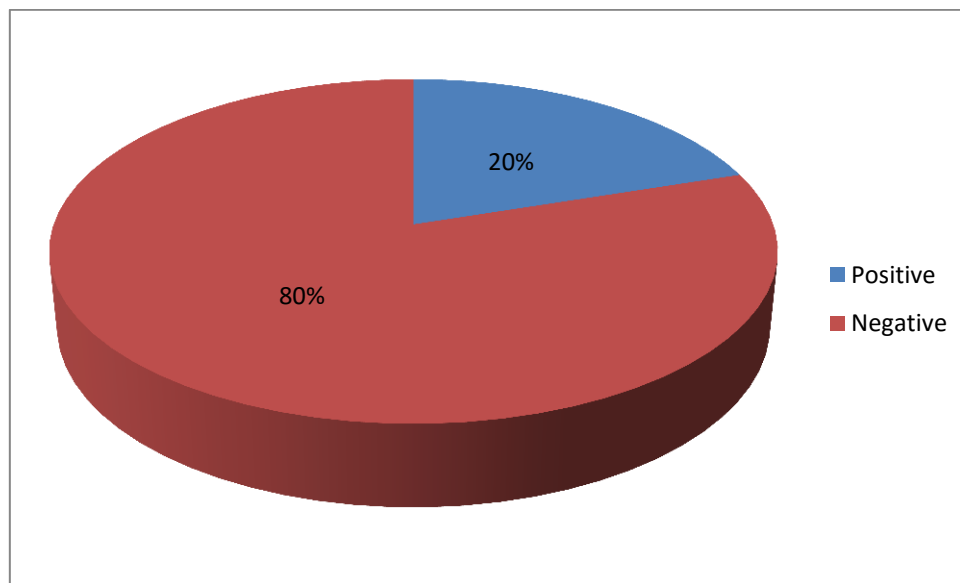


Figure (3.3): Show helicobacter pylori infection

Table(3.4) Frequency of Parasitic infection among study group

Parasitic infection	Frequency	Percent%
E. histolytica	29	58.0%
G. lambelia	18	36.0%
H.nana	3	6.0%
Total	50	100.0%

The table(3. 4) and figure(3.4) show the E.histolytica frequency is 29 which consist 58% of total frequency, the G.lambelia frequency is 18 which consist 36% of total frequency, the H.nana frequency is 3 which consist 6% of total frequency.

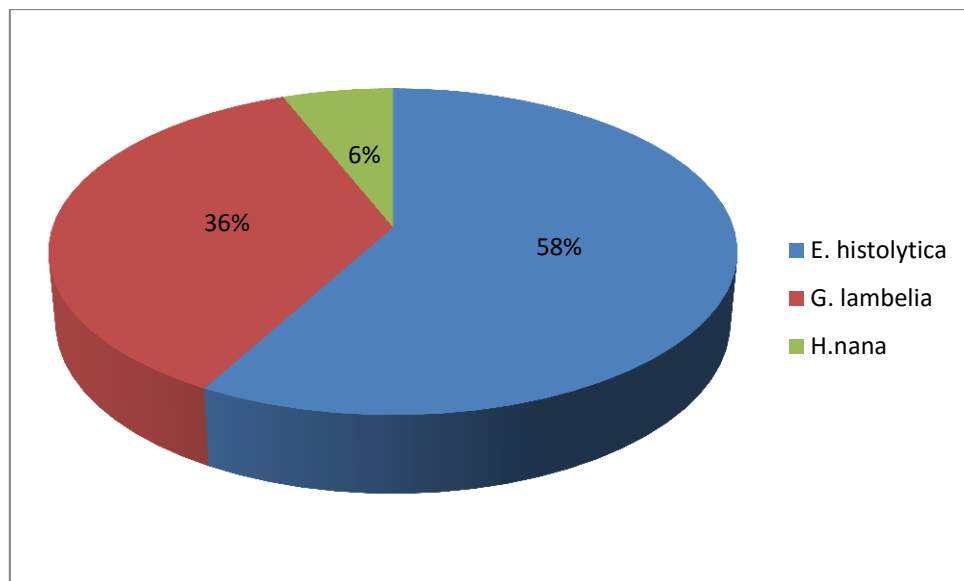


Figure (3.4): Show Parasites infection

Table (3.5) Relationship between helicobacter pylori and Parasites

Parasites infection		helicobacter pylori		Total
		positive	Negative	
E. histolytica	Count	5	24	29
	% of Total	10.0%	48.0%	58.0%
G. lambelia	Count	5	13	18
	% of Total	10.0%	26.0%	36.0%
H.nana	Count	0	3	3
	% of Total	.0%	6.0%	6.0%
Total	Count	10	40	50
	% of Total	20.0%	80.0%	100.0%

The table(3. 5) and figure(3. 5) show the E.histolytica positive percent is 10%, the G.lambelia positive percent is 10%, the H.nana positive percent is 0% .

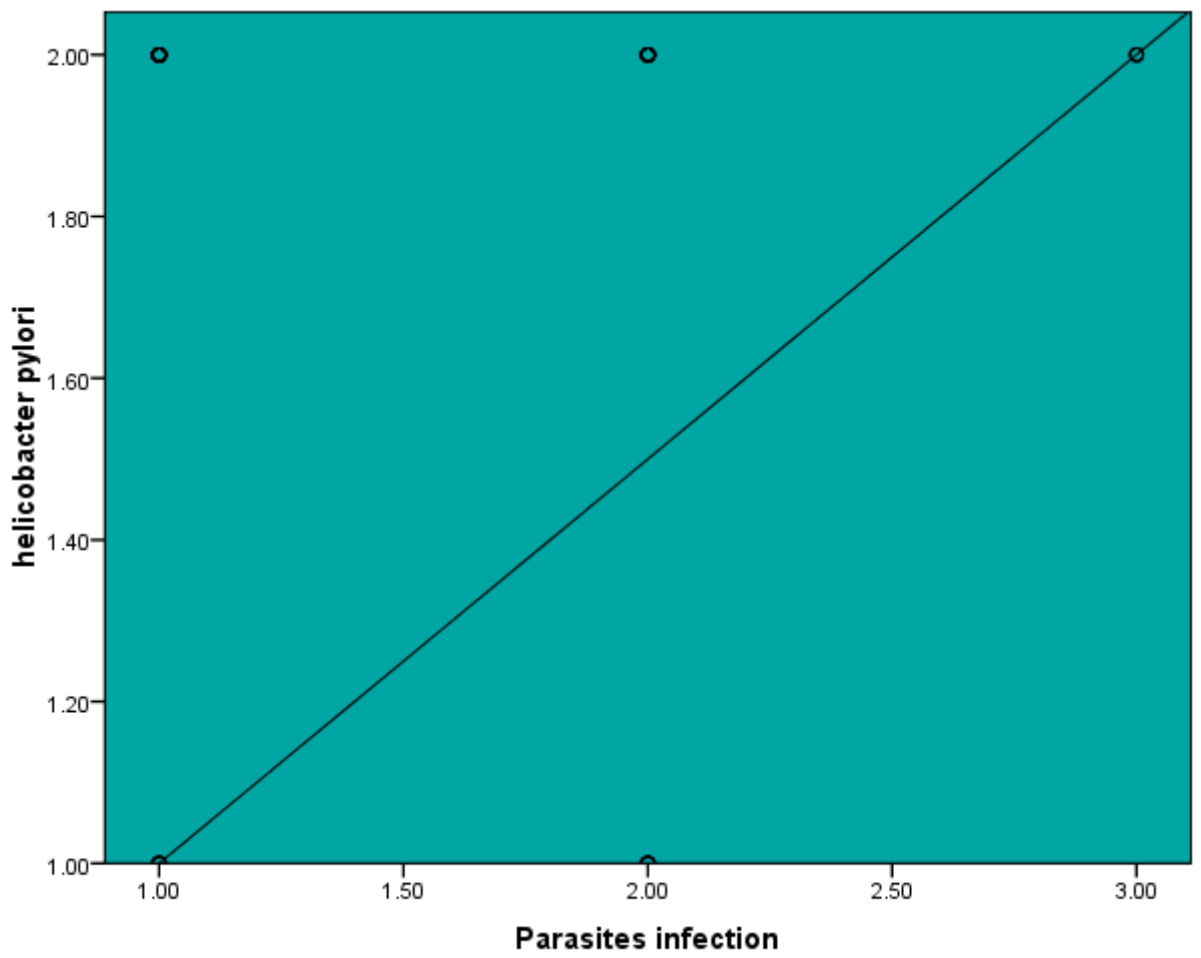


Figure (3.5) A scatter plot shows the relationship between helicobacter pylori and Parasites infection ($r=-.016$, $p=.91$)

CHAPTER FOUR

4.1. Discussion

This study was conducted on 50 patient infected with intestinal parasite to detect *Helicobacter pylori* infection, the positive *Helicobacter pylori* infection detected by using Immuno-chromatography test antigen.

The study show that distribution of gender ,male 33 (66%) and female 17 (34%)

the age between less than 10 to more than 40years and parasite infection distribution found in the study *Entamoeba Histolytica* 58% ,*Giardia lamblia* 36%and *Hemenolpsis nana* 6%.

10 patient with intestinal parasite and *Helicobacter pylori* and 40patient negative *Helicobacter pylori* and positive intestinal parasite in our study we found that *E. Histolytica* and *Giardia Lamblia* are most common intestinal parasite found with *Helicobacter pylori* among other intestinal parasite .

in previous study done by Hossein Kazeman et-al relationship between *Helicobacter pylori* infection in Ilam iran 2014

Result: a total of 130 patient with 65 positive of *Helicobacter pylori* and intestinal parasite infection based on ELIASA the parasite was found *gardia lamblia* 30.7% and *Entamoeba histolytica* 10%

In another study done by adurahaman seid et-al coinfection of intestinal parasite and *Helicobacter pylori* in hospital northeast Ethopia 2015

Result: total of 363 patient consecutive adult based on serological ICT test *Helicobacter pylori* and intestinal parasite detect in 225(70.5%) *gardia lamblia* 22.3%of isolated parasite (p=0.05)

also study done by Jorres J et-al association of intestinal parasite and *Helicobacter pylori* in children and adult from mexican community in 2003 study population include 120 children and 88adult from low socioeconomic level village determined by serum ELISA parasite infection by microscope examination

Result ;by contrast adult with intestinal parasite infection had significant lower *Helicobacter pylori* prevalence than adult without parasite 62.6%)(p=0.006)

also male and female percentage was analyzed and found that male are more infected than female ,most patient infected in age (20-30)years the relationship between *Helicobacter pylori* and intestinal parasite very weak *Helicobacter pylori*.(p=.91)

4.2. Conclusion

The study conclude that *Helicobacter pylori* infection was detected in very low value with 10 positive samples from 50 samples among whom that infected with intestinal parasite revealed that there was no significance association between *Helicobacter pylori* and intestinal parasite with [p=.91]

4.3. Recommendation.

- Recommended to increase sample size to get enough information about this topic
- Used of more advanced methods as PCR & urea breath test to detect helicobacter pylori infection
- used other more advance technique like concentration ,to detect intestinal parasite.
- We recommended by early treatment of patient infected with helicobacter pylori
- put place to reduce health education for family to control the diseases
- people must to be educated about dangerous and complication of disease
- food hygiene and water source free from parasites and microbes

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Appendix

abbreviations

E .Histolytica	Entamoeba Histolytica
ELISA	Enzymes linked immuno sorbent assay
G. Lamblia	Giardia Lamblia
H. Nana	Hymenolepis nana
H. pylori	Helicobacter pylori
ICT	Immunocomatografy test
PCR	Polymerase chain reaction

No	Material
1	Plastic container of stool
2	Slide
3	Cover glass
4	Cotton
5	Microscope
6	ICT antigen
7	Normal saline
8	Pasture pipette

Questionnaire

Name

Gender

Age

Parasite infection

H .pylori infection