Sudan University of Science and Technology

College of Graduate Studies

Detection of Helicobacter pylori Cag A Gene in Gastric Lesions among Sudanese Gastric Disorders Patients

الكشف عن البكتريا الحلزونية البوابية لجين Cag A في آفات المعدة لمرضى الاضطرابات المعوية السودانين

A dissertation submitted in partial fulfillment of Master degree in Medical Laboratory Sciences (Histopathology and Cytology)

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الآيـــــــــة

بسم الله الرحمن الرحيم

قال تعالى :

(يَرْفَعِ اللَّهُ الَّذِينَ آَمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

صدق الله العظيم

(الآية 11 المجادلة)

DEDICATION

To my father…

To my beloved mother…

To my grandmother…

To my aunt…

To my sister…

ACKNOWLEDGEMENTS

In The name of Allah, the most gracious and the most merciful Alhamdulillah, all praises to Allah for the strength and his blessing in completing this dissertation

Special appreciation goes to my supervisor, Dr. Mohammed Siddig Abdel Aziz, for his supervision and constant support, his invaluable help of constructive and suggestions throughout the experimental and thesis works have contributed to the success of the research.

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I would to thank very much every one help me

ABSTRACT

This descriptive case study was conducted at Faisal special hospital , Fedail hospital and Omdurman military hospital during the period from April to December 2018.

The aim of this study was to detect Helicobacter pylori CagA gene in patients suffering from gastric disorder using polymerase chain reaction (PCR).

A total of 38 (n=38) of both sexes were included for this study, patient data were collected by questionnaire .female were 21(55.3%), male were17 (44.7%) and ages ranges from 16 to 80 year, with mean age 56.

Endoscopic biopsy samples were collected, processed and stained by H&E method for histo patholigical changes, and PCR for detection CagA gene for H.pylori**.**

Histopatholog results were shown the following: inflammatory changes in 25(65.8%) samples, dysplasia in only one sample (2.6)% and no changes in 12(31.6) samples.

The result of PCR for CagA gene were shown as follow, 30(78.9) samples were positive and 8(21.1%) samples were negative.

The study conclude there is no association between CagA positive and gastric changes.

المستخلص

اجريت هذه الدراسة الوصفية في مستشفى فضيل ومستشفى الفيصل التخصصي ومستشفى ام درمان العسكري في الفترة من ابريل الى ديسمبر 2018.

هدفت الدراسة للكشف عن البكتريا الحلزونية البوابية في المرضى الذين يعانون من الاضطرابات المعويه عن طريق تقنية تفاعل البلمرة المتسلسل وصبغة الهيماتوكسلين والايوسين لمعرفة تغيرات الانسجة .

شملت هذه الدراسة الوصفية 38 مريضا من الجنسين وجمعت البيانات من المرضى عن طريق الاستبيانه.توزيع جنس المرضى كان كالاتي الذكور17 (44.7%) والاناث 21(55.3%) وتراوحت اعمارهم مابين 16-80 سنه بمتوسط عمر56 سنة.

جمعت عينات المناظير وعولجت ومن ثم صبغت عن طريق الهيماتوكسلين والايوسين لمعرفة التغيرات النسيجية وسلسلة تفاعلات البلمرة المتسلسل للكشف عن جينCagA البكتريا الحلزونية البوابية.

اظهرت النتائج التغيرات الالتهابية في 25(65.8%) عينة وفقط عينه واحدة من الانسجة التي بها نمو غير طبيعي(2.6%)و التي لم تحدث لها اي تغيرات 12(31.6%) عينة.

أما نتيجة تفاعل البلمرة السلسلي فاعطت نتيجة ايجابية في 30(78.9%) عينةو 8(21,1 %) عينة اعطت نتيجة سلبية.

خلصت الدراسة الى عدم وجود علاقة بين تغيرات الانسجة ونتيجة تفاعل البلمرة السلسلي لجين CagA.

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Chapter one

Introduction

1.1introduction

Helicobacter pylori, a gram-negative bacteria found on the luminal surface of the gastric epithelium. It induces chronic inflammation of the underlying mucosa (Kenneth,2010).

H.pylori is usually acquired during childhood through fecal-oral, gastro –oral routes, and has been shown to have a world-wide distribution (Hala, 2017).

The prevalence of H.pylori infection is high in most countries worldwide. H.pylori less frequent in northern European and north American population, about one-third of adults still be infected (Leonardo. *etal*.2014).

Infection by *Helicobacter pylori* is associated with the development of several gastro duodenal diseases, including gastritis, peptic ulcer disease (gastric ulcers and duodenal ulcers), and gastric adenocarcinoma (Pacheco ,*et al*.2008).

Helicobacter pylori CagA protein is considered to be one of the virulence factors associated with gastric cancer, the diversity of CagA important variable in determining the clinical outcome of infection by different *H. pylori* strains (Satoko,*et al*.2006).

Method of diagnosis of H.pylori is histology, culture, PCR (polymerase chain reaction),Urea breath test, Fecal antigen test, Serological test (Peter,2015).

1.2 Objectives:

1.2.1 General objective:

To detect H pylori and CagA gene in gastric biopsy among gastric disorder patient.

1.2.2. specific objectives:

1. To detect Cag A gen of H .pylori from gastric biopsy using PCR.
2. To correlate histopatholigical changes with the CagA gen PCR result.
3. To correlate between Helicobacter pylori result with (gender and age).

Chapter Two

2. Literature Review

2.1 Scientific background:

Gastric cancer is the third leading cause of cancer-related death worldwide. Despite a decrease in its incidence in some regions of the world, gastric cancer still poses a major clinical challenge because most cases are diagnosed in an advanced stage, with a poor prognosis and limited treatment options. The most common causes are Helicobacter pylori infection (Matteo and David,2015).

Worldwide, stomach cancer is the fifth most common cancer with 952,000 cases diagnosed in 2012 and 723,000 death It is more common in men and in developing countries. Less than 5% of stomach cancers occur in people under 40 years of age and 81.1% of that 5% in the age-group of 30 to 39 and 18.9% in the age group of 20 to 29 (Murtaza,*et al*.2017).

2.2.Anatomy and histology of the stomach:

The stomach has five regions: the cardia and gastro esophageal (GE) junction, (the fundus, the corpus, the antrum, and the pylorus. The fundus and corpus harbor acid-secreting glands, whereas the antrum harbors alkaline-secreting surface epithelium and endocrine, gastrin-secreting G-cells. Viewed through a laparotomy incision or a laparoscope, the GE junction is recognized at the sharp angle between the rounded dome of the fundus and the straight esophageal tube (David and Soybel, 2005).

The cardiac stomach is short and presents a simple epithelium as a layer of secreting mucus cells, which also cover the other regions of the stomach. Beneathit,a thin layer of dense connective tissue is observed . The fundic stomach follows the cardiac stomach and presents tubular-acinous glands in its first portion. In the following portion, ramified tubular glands are observed surrounded by loose connective tissue and dense septa of connective tissue. The

final portion of the stomach, the pyloric stomach, is characterized by a great development of the smooth muscle and lack of acinous or tubular glands .This portion present s numerous folding of the mucosa which are accompanied by dense connective tissue. Some loose connective tissue numerous folding of the mucosa which are accompanied by dense connective tissue (Felipe ,*et al*.2015).

2.3 Disorders of the stomach:

2.3.1.1 Benign disorders:

2.3.1.1 Adenomatous polyps:

Gastric adenomas are true neoplasms and precursors to gastric cancer, they account for 6% to 10% of all gastric polyps. Atrophic gastritis and intestinal metaplasia are frequently associated with the development of these polyps, Polyps that are greater than 2 cm and have villous histology have a higher risk of neoplastic (28%-40%) (Rafiul,*etal*.2013).

2.3.1.2 Gastro intestinal stromal tumor:

are the most common mesenchyme tumor of the digestive tract. these tumor continuum from benign to malignant and usually effect the population over 50 year , and rarely patient younger patients less than 40 and child hood (Ivan,*et* al.2008).

2.3.1.3 Gastric squamous papilloma :

papilloma is benign epithelial lesion**,** characterized by overgrowth of squamous cell and connective tissue .it reported that squamous papilloma was present in 52 year old a symptomatic female ,endoscopic biopsy use to diagnosis of squamous papilloma (Hyung,*et al*.2014).

2.3.2 Malignant disorders:

2.3.2.1 Gastric adenocarcinoma :

Adenocarcinoma accounts for 95% of all gastric cancers, Worldwide is the leading cause of cancer death. US and Europe is low risk areas and Asia is high risk areas(John ,2008)

2.3.2.2 Small cell carcinoma (SCC):

Small cell carcinoma (SCC) is a represents 0.1% of all gastric carcinomas, Incidences of SCC in the stomach have been reported in older individuals, The resent studies reported small cell carcinoma of the stomach in a 35 year old woman, it is an aggressive tumor without therapy, and is therefore associated with a poor prognosis(Steven ,*et al* .2011).

2.3.2.3 Primary non-Hodgkinlymphoma (NHL):

the stomach is a relatively rare malignant disorder, accounting for about 5% of gastric tumors. It must be distinguished from secondary involvement of the stomach by nodal lymphoma (Andrea ,*et al*.2000).

2.4 Epidemiology of gastric cancer:

Gastric cancer ranks fourth in incidence after lung, breast and colorectal and second in mortality after lung cancer among all cancers worldwide, It is estimated that in 2008 there were 989,600 new cases and 738,000 deaths from stomach cancer in the world, and approximately 70% of both new cases and deaths occurred in developing countries2,. The risk of gastric adenocarcinoma increases with age, occurring most frequently between 55 and 80 years of age and it is rare in patients under 30 years. In general, gastric cancer rates are twice as high in men as in women. The highest incidence rates in males are found in Eastern Asia between 40 and 60 per 100,000 population), Eastern Europe (around 35 per 100,000), and in some Latin American countries, with rates between 20 to 30 per 100,000 inhabitants. Some of the lowest incidence rates are found in African countries (0.6 to 3.0/100,000) (María,*et al*.2013).

2.5 Risk factors of gastric cancer :

2.5.1 Age:

One of the risk factors for contracting gastric cancer is age above 45 years of age. The number of cases involving this tumor increases with age, reaching a peak between the ages of 50 and 70 . Most deaths are recorded in the 55-75 age group **(**Jolanta, 2013).

2.5.2 Obesity:

increased body weight was associated with an increased risk of gastric cancer Research results have indicated a 2.3- fold increase of the risk of contracting gastric cancer in the cardia in obese persons in comparison to non-obese people group. **(**Jolanta**,** 2013).

2.5.3 Family history:

Early studies revealed that GC was less common in patients with blood group 0, but was frequently associated with blood group A which increases the risk by 16-20%. A positive family history of GC has been associated with an increased (three-fold) risk of GC. Interestingly, subjects with both a positive family history and infection with cagA-positive *H. pylori* strains had a 16-fold increased risk of non-cardia GC.(Compare,*etal*.2010).

2.5.4 Smoking and Alcohol:

Smoking increases the risk of developing gastric cancer significantly, from 40% increased risk for current smokers to 82% increased risk for heavy smokers .Some studies show risk with alcohol consumption as well ,Consumption of alcohol and smoking are risk factor(Murtaza, *etal* .2017).

2.5.5 Diet and gastric cancer:

Dietary factors are not proven causes but some foods including smoked food, salt and salt-rich foods, red meat, processed meat, pickled vegetables ,and bracken are associated with a higher risk of stomach cancer**,** Fresh fruits and

vegetables intake, citrus fruit intake, and antioxidant intake are associated with lower risk of stomach cancer (Murtaza ,*etal* .2017).

2.5.6 Life style :

early diagnostic strategies are the most important public health interventions in gastric cancer. Smoking and salt are strong independent risk factors for gastric cancer. Red meat and high fat increase the risk of gastric cancer, fresh fruits, vegetables and certain micronutrients (selenium, vitamin C) reduce the risk, with evidence lacking for fish, coffee and tea. daily physical activities can be protective against cancer (Lee,2013).

2.6 Diagnosis of gastric cancer:

2.6.1. Esophago gastro duodenoscopy (EGD)

is the diagnostic imaging procedure help the physician determine if a gastric lesion is present and whether the lesion has benign or malignant features, EGD is a highly sensitive and specific diagnostic test, especially when combined with endoscopic biopsy(John and Peter, 2004).

2. 6.2 Ultrasonography:

Ultrasound is the most accurate and reliable method for the preoperative staging of gastric carcinomas, and it is mandatory if a tailored therapeutic approach is planned according to stage (Ganpath,2006).

2.6.3 Computed tomography (CT):

CT technique for pretreatment evolution of gastric cancer to define extent of the preoperative staging of gastric cancer and for follow-up. A recently develop advanced CT technique that makes use of thin sections, optimal contrast material enhancement allows more accurate staging. CT may be limited in the in the identification of non enlarged lymph node metastasis, and small hematogenous metastasis (Joon ,*et al*.2006).

2.6.4 Magnetic resonance imaging (MRI):

Magntic Resonance Imaging (MRI) has been established as a valuable technique in human gastrointestinal (GI) research for analyzing gastric function . In comparison with radionuclide and ultrasound imaging methods, MRI offers improved spatial and temporal image resolution, and thus is ideal for noninvasive and reliable assessment of GI physiology. It will therefore play an important role in GI research and clinical diagnosis in the future.(Reto ,2006).

2.6.5 Histopatholigical Method:

Histollogy method regard as the gold stander for the clinical diagnosis of cancer and identification of prognostic and therapeutic targets. These techniques have evolved from era of diagnosis based on haematoxylin and eosin stained slides to the current regular evaluation of tumor.(Lei,*etal*.2012).

2.6.6 Immunohistochemistry (IHC):

This is a diagnostic tool for cell derivation of malignant tissue ,identifying prognostic marker of possible value in therapeutic target for cancer. IHC has been shown an effective adjunct to H&E diagnosis in majority tumor cases through the definitive diagnosis or confirmation of H&E section (Tanya,*etal*. 2015).

2.7 Treatment of gastric cancer:

2.7.1 Surgery:

Surgery is the only curative therapy for gastric cancer but most operable gastric cancer presents in a locally advanced stage characterized by tumor infiltration of the serosa or the presence of regional lymph node metastases. Surgery alone is no longer the standard treatment for locally advanced gastric cancer as the prognosis is markedly improved by perioperative chemotherapy (Elory ,*et al*.2017).

2.7.2 Chemotherapy:

Chemotherapy is the main standard adjuvant treatment of gastric cancer, and is indicated for patients with unrespectable or recurrent disease, or those after non-curative R2 resection, Although recent advances in chemotherapy have achieved considerable tumor regression in many cases of unrespectable/recurrent gastric cancer, these responses have not ultimately led to complete cure. The median survival time achieved in clinical trials for the disease at this stage remains to be 6–13 months. The current goal of chemotherapy therefore is to delay the appearance of disease-related symptoms and/or to prolong survival (Takeshi and

2.7.3 Endoscopic mucosal resection *(EMR):*

EMR is applied treatment of premalignant lesion and early cancer, and with low probability of lymph node metastasis. tumor with horizontal growth and diameter larger than two centimeter are difficult to remove with EMR. Its safety, efficacy with low rate of complication technique (Sukru, 2018).

2.8H. pylori is a gram negative, which colonizes the human stomach and is prevalent worldwide. It has been associated with peptic ulcer disease, gastric adenocarcinoma, and type B low-grade mucosal-associated lymphoma (Ahad ,2014).

H. pylori is endemic in Africa and Asia; In developing countries, 70%–90% of the population harbor *H. pylori* , while in the developed countries, the prevalence is lower ranging from 30% to 40%.*H. pylori* prevalence in nigeria of 81%,and 91.7% in Egypt (Ahmed,*etal*.2018).

H.pylori is a gastric bacterial pathogen that is etiologically linked to human gastric cancer. The-associated gene A (CagA) protein of H. pylori, is an oncoprotein that can induce mcytotoxinalignant neoplasms in mammal. gastric carcinogenesis progresses through mechanism in which pro-oncogenic actions of CagA are successively taken over by series of genetic alterations compiled in cancer-predisposing cells during long-standing infection with cagA-positive H. pylori (Hatakeyama, 2008).

2.8 .1 Method of diagnosis of H .pylori:

Numerous methods for detecting the presence of the bacterium in the gastric mucosa have been developed. Traditionally, diagnostic methods may be classified as invasive, which require endoscopy to obtain biopsies of gastric tissues, and noninvasive. The invasive methods include histology, urease test, culture and molecular methods, While the noninvasive methods include serology, urea breath testing, stool antigen testing and molecular methods (Warren and Marshall,1983).

2.8.1.1invasive method:

2.8.1.1.2 Histology:

Biopsies from the antrum and the corpus were obtained for histology and were fixed in 10% formalin and sent to the laboratory. Paraffin embedded and multiple 4 mm-thick histological sections were obtained from each biopsy. Preparations were stained with hematoxylin and eosin, and Giemsa evaluated by several pathologists blinded to the results of the other tests. The presence of *H. pylori* was determined but not graded (Mohammad,*etal*.2013).

2.8.1.1.3 Culture:

H. pylori culture is not a routine procedure in initial diagnosis,*H. pylori* isolation via the culture of biopsy samples is a routine second line approach. This technique IS highly specific, is not as sensitive as other tests. USED for purposes of scientific research, diagnostic approaches and for the detection of antibiotic resistance if treatment failure is suspected. (Behnam, *etal*, 2015).

2.8.1.1.4 PCR (polymerase chain reaction):

PCR applied for the detection of *H. pylori* in biopsies, it high sensitivity and specificity, simplicity, and automated procedures. any genomic material could be used as a template sample for PCR, The rapid application of the test is complemented by reasonably high (up to 95%) sensitivity and specificity genome, the selection of the target gene and PCR primer pairs influence specificity and sensitivity of the test . it is very important to design and select the PCR primers based on bioinformatics analysis of relevant genomes (Behnam,*etal*2015).

2.8.2.2 Non Invasive method:

2.8.2.2.1 Urea breath test:

The limited availability of the gold standard for diagnostics of upper gastrointestinal tract diseases (gastroscopy) is the basis for the search for new methods for the detection of Helicobacter pylori infection. The urea breath test is a method of high sensitivity and specificity. The positive result of urea breath test may be the basis for the inclusion of eradication therapy (Nawacki 2018).

2.8.2.2.2 Fecal antigen test:

This technique is easy to perform, and its accuracy may be improved by the use of monoclonal antibodies recently proposed for capturing H. pylori antigen in stool specimen. The eradication control is recommended at least 4 weeks after the end of the eradication treatment or at least 2 weeks after ant secretory treatment to obtain high sensitive result (De-Krowin, 2003).

2.8.2.2.3 Serological test:

Serology is based on the detection of specific IgG and IgA antibodies by using the ELISA method inpatients infected by H. pylori,It is not solely used in diagnosis of the infection but particularly used for epidemiological or screening studies. Serological methods are not reliable in children and fail in the diagnosis and in monitoring the success of anti-H.pylori therapy *(*Mahir,2005).

2.83 Relation between H.pylori and stomach disorder:

Since the discovery of *Helicobacter pylori (H. pylori)* by Warren and Marshall in 1982, many epidemiological studies have revealed a strong association between *H. pylori* infection and gastric cancer development. As confirmation, recent retrospective and prospective studies have demonstrated that *H. pylori*-positive patients have significantly higher risk of gastric cancer than *H. pylori*-negative patients. Careful investigations have shown more than 95% positivity for *H. pylori* infection in gastric cancer patients .(Tsutomu,etal.2008).*H. pylori* was discovered about 30 years ago , a lot of epidemiological and experimental studies have revealed a significant relationship between *H. pylori* infection and chronic/atrophic gastritis, peptic ulcer, intestinal metaplasia, gastric lymphoma or cancer development. In 2001, Uemura *et al*. confirmed that stomach cancers develop only in *H. pylori*-infected patients, but none of the uninfected group. Based on the epidemiological findings, *H. pylori* was defined as a "definite carcinogen" by the World Health Organization/International Agency for Research on Cancer (WHO/IARC) in 1994 (Takeshi ,*etal.*2014).

Chapter Three

Materials and Methods

3.1 Study design:

This is a descriptive, case study.

3.2. Study area:

This study conducted at Faisal special hospital, and Omdurman military hospital and collage of medical laboratory science- Sudan University of sciences and technology.

3.3 Study population:

Patients having gastrointestinal complaints from gastric problems.

3.4 Sample size:

Thirty –eight (n=38) biopsy samples were collected from patient suffering from gastritis, peptic ulcer, dysplasia and gastric cancer.

3.5 Data collection:

Demographical data of patients were collected by self limited administered questionnaire.

3.6 Collection and transport of specimens:

upper endoscopy was performed and multiple gastric biopsy specimen were taken from the stomach antrum ,from patient two specimen collect one for preserve and transport by normal saline for DNA extraction , other by formalin for histological examination.

3.7 Sample processing:

Samples were fixed in 10%buffered formalin , dehydration by alcohol (70%,95%,100%), clearing by xylene , infiltrated with paraffin ,sectioning and cuting by rotary microtome into section that can be placed in slide .then staining by routine stain (hematoxylin and eosion).

3.7.1. Staining:

3.7.1.1 Hematoxllin and eosin

Put the section in the xylene, Hydrate the tissue section by passing through decreasing concentration of alcohol (100%, 90%, 80%, 70%),Stain in hematoxylin for 10 minutes. Wash in running tap water ,followed by tap water wash, Stain in 1% Eosin Y for 3 minutes. Wash in tap water for 2 minutes, Dehydrate in increasing concentration of alcohols and clear in xylene and Mount in DPX.

3.7.2 Molecular techniques:

3.7.2.1 DNA extraction:

The Helicobacter pylori DNA was extracted using guanidine chloride extraction method. Each 2 ml tryptic soya broth (TSB) was centrifuged at 3000 rpm for 15-20 min. The pellet was collected and washed twice by phosphate buffer saline (PBS) to remove excess media. 2 ml of lysis buffer, 10µl of proteinase K, 1 ml of guanidine chloride and 300 µl of ammonium (NH_4) acetate were added to the pellet, vortexed, and incubated at 37oC overnight or at 65oC for 2 hr. The mixture was cooled to room temperature, and then 2 ml of pre-chilled chloroform were added, vortexed, and centrifuged at 3000 rpm for 5 min. The

upper layer of the mixture was transferred to new tube and 10 ml of cold absolute ethanol were added, shaked, and kept at -20oC for 2hr or overnight. Then the tube was centrifuged at 3000 rpm for 15-20 min., the supernatant was drained carefully, and the tube was inverted on a tissue paper for 5 min. The pellet was washed with 4 ml of 70% ethanol, centrifuged at 3000 rpm for 5 min. The supernatant was poured off and the pellet was allowed to dry for 10 min. Then it was re-suspended in 50 µl of distilled water, briefly vortexed, and kept at -20oC overnight. The extracted DNA integrity was assessed by ethidium bromide stained agarose gel electrophoresis.

3.7.2.2 Polymerase chain reaction technique (PCR):

For the last cycle was increased to 5 min to ensure complete extension of the amplified fragment. Amplification was performed in a final volume of 20 µL of PCR mixture containing 0.8 µm of each primer, 10 mM of each deoxy nucleotide triphosphate (dATP, dGTP, dTTP and dCTP), 10 mMtrisHCl, 50 mMKCl, 0.1% triton X– 100, 1.5 mM MgCl2, one unit of DNA polymerase and 4 µL of template DNA . DNA amplification was carried out as follows: Denaturation at 94°C for 5 min in the first cycle, followed by annealing for 30 sec at 65°C, Extension for 30 sec at 72°C. The extension Denaturation for 30 sec at 94°C for a total of 35 PCR cycles. Then the PCR products were resolved by 2% agarose gel electrophoresis .

Table (4): Primer sequencing :

Primers	Primer sequencing	Product base pair
F(Forward)	5ATAATGCTAAATTAGACAACTTGAGCGA3	128 BP
R(Reverse)	5 AGAAACAAAAGCAATACGATTC 3	

Table (5) :Preparation of PCR premix :

PCR reaction mixture	Volume added
Master mix	7µl
F primer	0.5µl
R primer	0.5µl
Extracted DNA	2µl
DW	10µl

Table (6): PCR protocol :

PCR steps	Temperature	Time	Cycles
Intiatial denaturating	94®C	3min	1
Denaturation	94®C	30sec	35
Annealing	53®C	30min	35
Extension	72®C	45min	35
Final extension	72®C	5min	1

3.7.2.3Preparation of agrose gel and gel electrophoresis technique:

1.5gm of a grose powder was weighted using sensitive balance ,followed by addition to clean and dry flask.100 ml of IX tris borate EDTA(TBE) added to powder(TBE prepared by weight 48.45gm of Tris Hcl,55.5gm of Boric acid and7.44gm of EDTA ,dissolved in1 litter Dw, this result in 10x TBE,1x prepared by dilution of 10ml of 10x and 90 ml DW. then the flask was putted on microwave for 1:30 sec, separated by agitations ,after complete dissolved of a agarose ,cooled ,and poured on at which comb fixed ,an a garose ,cooled gel is placed in 1xTBE filled box ,an electrical field was applied via the power supply to the rear.so DNA migrates toward the positively charged anode (red wire)

3.7.2.4 PCR product detection:

5ml amount of each PCR mixture separated in a1.5% agarose stained with ethidium bromide result was considered positive when aband size 128 was visible in the stained gel,100pb ladder considered as molecular size marker.

3.8 Data analysis:

Collected data were analyzed using the statistical package of social science (SPSS) program frequency, mean and chi square were calculated.

3.9 Ethical consideration:

All patient under study were informed about the objective of research ,the verbal consent was taken from them before enrolled under study**.**

Chapter four

4-Results

the study involved 38,previously diagnosed as gastric problem.

Table (4.1) showed frequency of age among study population between 16 and 80.most patients were less than 40 years representing 23 (60.5%) and the remaining 15 (39.5%) were more than 40 years.

Table (4.2) explains frequency of sex among study population which includes 21 (55.3%) male, 17 (44.7) female.

Table (4.3) showed the frequency of H .pylori in samples using PCR Cag A, 30 (78.9%) samples were positive and 8 (21.1%) samples were negative.

Table (4.4) showed frequency of histopathological diagnosis, 12 (31.6%) cases were revealed no change, 25 (68.8%) inflammation, 1(2.6%) dysplasia.

Table (4.5) showed relation between PCR result of CagA gen and histopathology diagnosis ,CagA positive expression was found in (10/12) in no change cases and (2/12) showed negative expression ,while in inflammation positive expression was found in (19/25) samples and (6/25) showed negative expression, while in dysplasia positive expression was found in (1/1) sample and (0/1) sample showed negative expression for CagA gen. this result showed no significant association (P.value=0.7).

Table (4.1) : Frequency age among Study population

Age group	Frequency	Percent
≤40	23	60.5
>40	15	39.5
Total	38	100.0

Table (4.2) : Frequency of sex among study population

Sex	Frequency	Percent
Male	21	55.3
Female	17	44.7
Total	38	100.0

Table (4.3) : Frequency of H.pylori CagA gene in samples using PCR

CagA	Frequency	Percent
Positive	30	78.9
Negative	8	21.1
Total	38	100.0

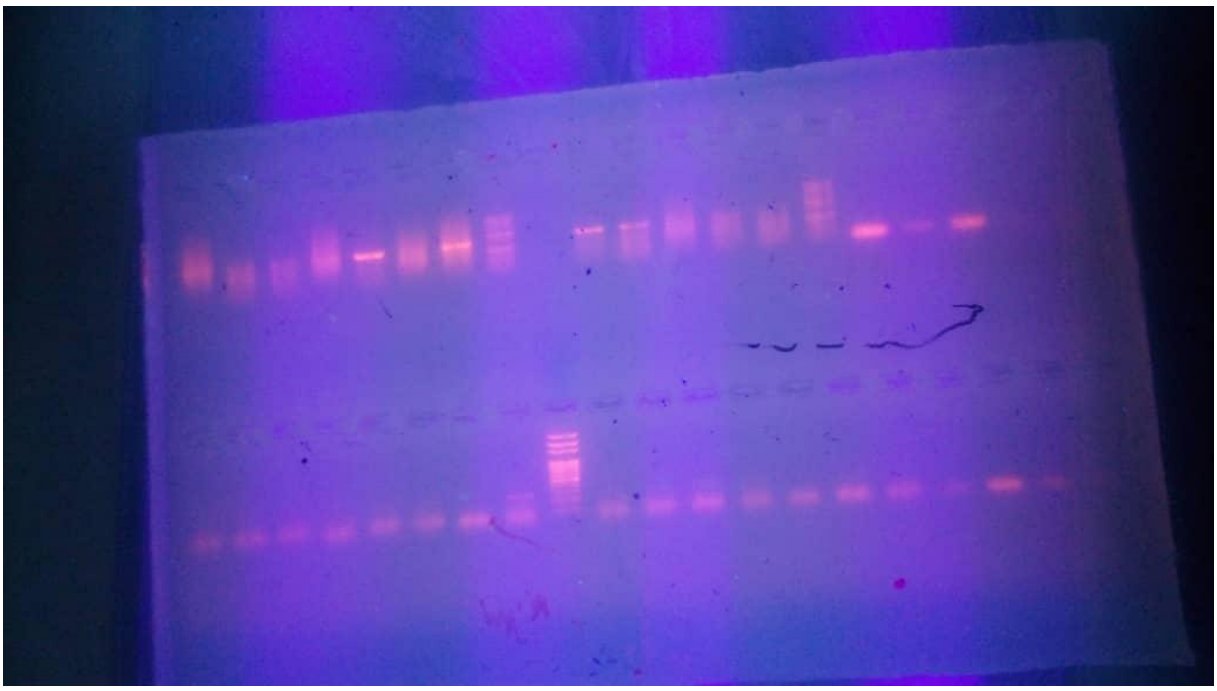
Table (4.5) :Frequency of histopathological diagnosis

Histopathological diagnosis	Frequency	Percent
No changes	12	31.6
Inflammation	25	65.8
Dysplasia	1	2.6
Total	38	100.0

Table(4.6):Relation between PCR result of CagA gene and histopathology diagnosis

Cag A gen result	Histopathological diagnosis			Total
	No change	Inflammation	dysplasia	
Positive	10	19	1	30
	26.3%	50.0%	2.6%	78.9%
Negative	2	6	0	8
	5.3%	15.8%	.0%	21.1%
Total	12	25	1	38
	31.6%	65.8%	2.6%	100.0%

P.value :0.7



Microphotograph (4.1):Gel electrophoresis of Cag A gene of H.pylori PCR Product(40X).

5. Discussion, conclusion and Recommendation

5.1 Discussion

The present study include thirty –eight samples of GIT disorder patients .concerning age group of the study population ,the study revealed that most of patients were less than 40 years more effected with gastric problem. this result is compatible with Alborazietal .(2018),who reported high prevalence of H.pylori infection in children (73%).while disagree with Yasser *etal.(*2016),who reported factor like age were not associated with H.pylori infection .also disagree with Gerham *etal* .(2015),have reported that H.pylori infection prevalence increased with age in elderly people about (70%) than children.

Concerning the sex among study population, the study revealed that most of patient were male than female,this result compatible with Marilyn *etal*. (1995), who reported disease associated with Helicobacter pylori infection was more frequency in men rather than women .while disagree with Yasser *etal.* (2016),who reported no associated between infection and sex.

The histpatholigcal diagnosis of the study population revealed that more frequent type of GIT is inflammation ,this result compatible with Justin *etal.*(1999) who reported H.pylori infected patients showed significantly have more sever gastritis. Also agree with Ke and lin.(2004),who reported H pylori is responsible for90% of case of gastritis.

The present study revealed that was no significant association between CagA gene and type of histoligical diagnosis, this result is agree with Yashio *etal.*(199),Who reported no association between CagA status and clinical outcome in patient. while disagree with Carlose *etal.*(2011). Who reported 70% of infection with CagA positive associated with progression of gastric lesion.

also disagree with Loghman *etal.*(2015),reported H.pylori Caga positive associated with sever gastric lesion.

5.2 conclusions:

On the bases study of this study we conclude: there is no association between CagA positive of H.pylori strain and gastric changes .

5.3 Recommendations:

On the base of this study we recommende :

1. Further study with large sample size is needed to accurately determine the rate of H.pylori infection in gastric biopies.
2. The result H.pylori DNA detection should be carried out in gastric disorder patients who tested negative for H.Pylori antibodies to confirm**.**

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Appendix (1):

Materials and instruments:

1-Instrument:

-Rotary microtome

-Microtome Knives

-Micro centrifuge

-Sensitive Balance

-Thermal cycle

-gel electrophoresis apparatus

-Oven

-staining racks

-stainless microtome blade

-water bath

-cover glass

2. Materials:

-Xylene

-Ethanol(100%,90%,70%,50%)

-Xylene

-Hematoxylin and Eosin

-Giemsa Stain

Acetic Acid-

-Phosphate buffer saline

-guanidine chloride

- Distilled Water

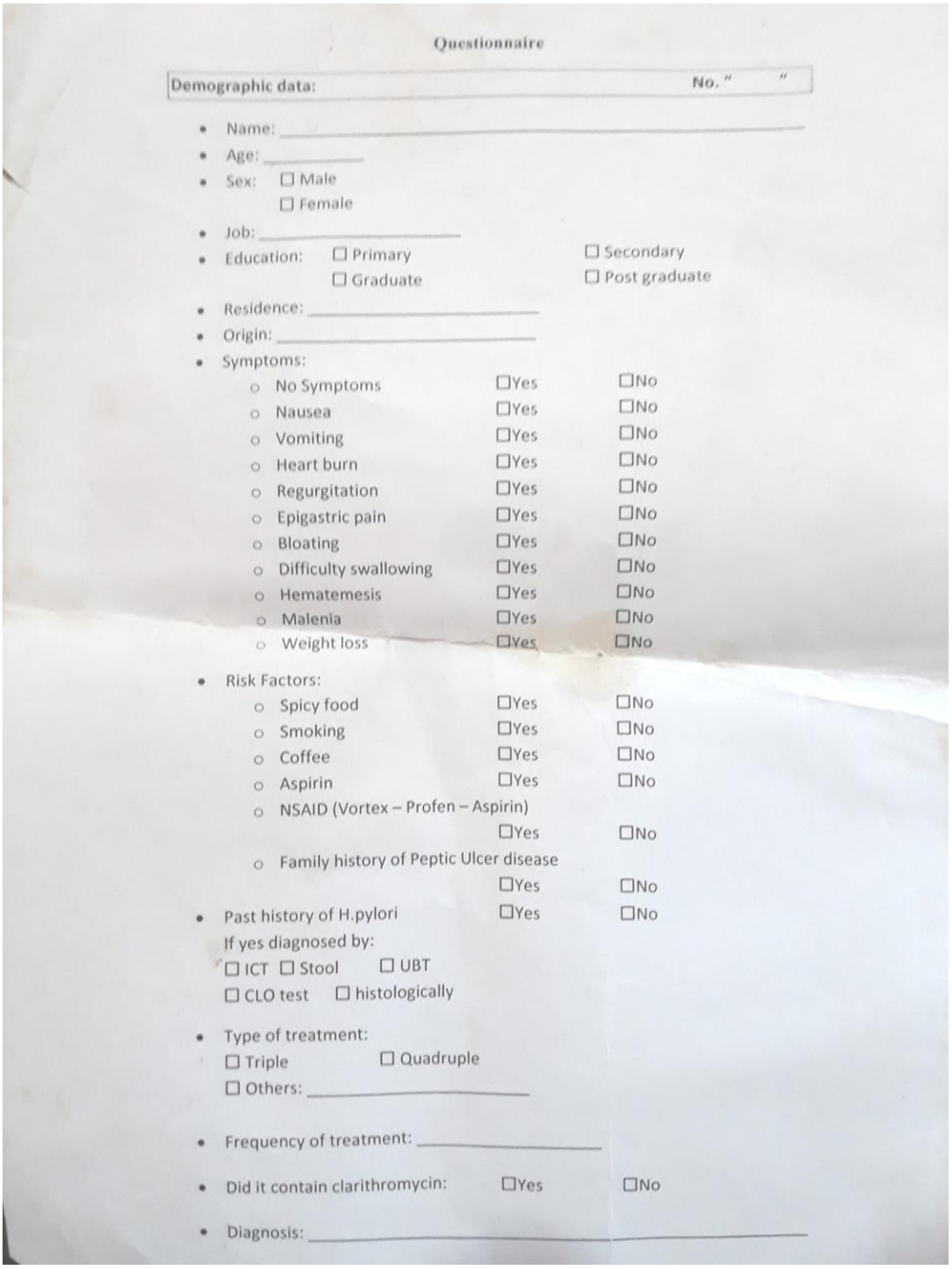
-Protenase K

-Chloroform

_Ethidium bromide

Appendix (2):

Questionnaire:



Questionnaire

Demographic data: No. " "

- Name: _____
- Age: _____
- Sex: ☐ Male ☐ Female
- Job: _____
- Education: ☐ Primary ☐ Secondary ☐ Graduate ☐ Post graduate
- Residence: _____
- Origin: _____
- Symptoms:
  - No Symptoms ☐Yes ☐No
  - Nausea ☐Yes ☐No
  - Vomiting ☐Yes ☐No
  - Heart burn ☐Yes ☐No
  - Regurgitation ☐Yes ☐No
  - Epigastric pain ☐Yes ☐No
  - Bloating ☐Yes ☐No
  - Difficulty swallowing ☐Yes ☐No
  - Hematemesis ☐Yes ☐No
  - Malenia ☐Yes ☐No
  - Weight loss ☐Yes ☐No
- Risk Factors:
  - Spicy food ☐Yes ☐No
  - Smoking ☐Yes ☐No
  - Coffee ☐Yes ☐No
  - Aspirin ☐Yes ☐No
  - NSAID (Vortex – Profen – Aspirin) ☐Yes ☐No
  - Family history of Peptic Ulcer disease ☐Yes ☐No
- Past history of H.pylori ☐Yes ☐No

  If yes diagnosed by:

  ☐ ICT ☐ Stool ☐ UBT

  ☐ CLO test ☐ histologically
- Type of treatment:

  ☐ Triple ☐ Quadruple

  ☐ Others: _____
- Frequency of treatment: _____
- Did it contain clarithromycin: ☐Yes ☐No
- Diagnosis: _____