Sudan University of Science and Technology

College of Graduate Studies

Molecular Detection of *Helicobacter pylori* and Human Papilloma Virus Genotype 18 in Gastric Biopsies of Patients with Gastric Problems Attending Police Hospital - Khartoum

الكشف الجزيئي عن البكتريا الملوية البوابية والفيروس البشري الحليمي نوع 18 من خزعات المعدة من المرضى الذين يعانون من مشاكل المعدة ويترددون على مستشفى الشرطة – الخرطوم

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Microbiology)

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الآيـــــة

قال تعالى:

﴿ اللَّهُ لا إِلَهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ لا تَأْخُذُهُ سِنَةٌ وَلا نَوْمٌ لَهُ مَافِي السَّمَوَاتِ وَمَا فِي الأَرْضِ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَوَاتِ وَالأَرْضَ وَلا يَئُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ ﴾

صدق الله العظيم

سورة البقرة ـ الآية (255)

DEDICATION

To my beloved father

To my love mother

To my brothers

To my friends

To my dear husband

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First of all, I am very thankful for **ALMIGHTYALLAH** the all-powerful for giving me everything. My gratitude extended to my supervisor **Dr. Wafaa Mohammed Abd-allah** for her close supervision, and stimulating suggestions. Special thanks to **Dr. Hisham Nouraldayem and Miss. Maram Mostafa Mohammed** for their support and help.

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ABSTRACT

A growing amount of evidence has shown that the Human Papilloma virus (HPV) infection and *H.pylori* directly or indirectly, can result in numerous malignant tumors.

This is a descriptive cross-sectional study conducted at the period from January 2019 to February 2020 which aimed to detect HPV-18 infection and *H.pylori* in gastric biopsy among Sudanese patients.

Gastric biopsies were collected from 100 patients suffering from gastric problems with age ranged from 15 to 80 years with mean age 42.21± 16.8 S.D.

Included in this study in which 61 (61%) of them were males and 39 (39%) were females.

The gastric disorders in this study included gastritis (57%), gastric ulcer (19%), duodenitis (14%), duodenal ulcer (1%), gastric erosion (6%), and abnormal tissue (3%).

DNA Extraction was done manual and the *H.pylori* and HPV-18 DNA were amplified for each sample using PCR and agarose gel electrophoresis was used to detect the PCR products.

PCR results yielded 34/100 (34%) positive in case of *H.pylori* and negative result with HPV-18 in all gastric biopsy samples.

Concerning gender، 20/61 (58.8%) males and 14/39 (41%) females were positive for *H. pylori*، and there was no significant association between *H.pylori* infection and gender (p = 0.749).

Regarding age groups ,the highest frequency was 12/100 (12%) among patients with age group 15- 30 years ,followed by 40-60 years (9%), 31-45 years (8%), 61-75 years (4%) and the lowest frequency at age ≥76 years (1%).There was no statistically significant difference between *H. pylori* infection and age groups (P=0.729) .

This study concluded the *H.pylori* was more frequent among patients with gastritis; there was no detected HPV-18 among patients with gastric problems while *H.pylori* was detected in rated frequency (34%), there was no significant association between age, gender, and *H.pylori* infection.

There was a significant association between gastric problems and *H.pylori* infection (P= 0.017).

الخلاصة

أظهرت كمية متزايدة من الدراسات الحديثة أن عدوى فيروس الورم الحليمي البشري نوع −18 قد يؤدي بشكل مباشر أو غير مباشر إلى العديد من الأورام الخبيثة.

هذه الدراسة وصفية مقطعية أجريت في الفترة من يناير 2019 إلى فبراير 2020 للتحقق من وجود الأحماض النووية للبكتريا الملوية البوابية وفيروس الورم الحليمي البشري نوع −18 ضمن 100 خزعة من المعدة جمعت بواسطة المنظار من المرضى السودانيين الّذين يعانون من مشاكل في المعدة و تم فحصها بواسطة تفاعل سلسلة البلمرة المتعدد.

شملت هذة الدراسة 100 مريض تراوحت أعمارهم بين 15 إلى 80 عام بمتوسط قدره 42.21 و إنحراف معياري قدره ±16.8.

شملت هذة الدراسة 61 (61%) من الرجال و 39 (39%) من النساء، و شملت ايضا مجموعة من اضطرابات المعدة تتضمن التهاب المعدة (57%)، قرحة المعدة (19%)، التهاب الاثنى عشر (14%)، قرحة الاثنى عشر (1%)، تآكل المعدة (6%)، وايضا الانسجة الغير طبيعية (3%).

تم استخراج الحمض النووي الخاص بالبكتريا الملوية البوابية و فيروس الورم الحليمي البشري − 18 يدويا، وكانت نتائج تفاعل البلمرة إيجابية للبكتريا الملوية البوابية في 100/34 (34٪) من عينات خزعة المعدة، ولم يتم العثور على الحمض النووي لفيروس الورم الحليمي البشري − 18 في جميع عينات خزعة المعدة.

فيما يتعلق بنوع الجنس، تم العثور على الحمض النووي للبكتيريا الملوية البوابية في 61/20 (58%) من المرضى الذكور و 39/14 (41%) من المرضى الاناث، كما أظهرت النتائج أيضا أنه لا يوجد إرتباط بين عدوى البكتيريا الملوية البوابية والجنس (القيمة الإحتمالية = 0.749).

فيما يتعلق بالفئات العمرية، أظهرت البكتيريا الملوية البوابية أعلى نتيجة إيجابية في الفئة العمرية 15 − 30 و هي (12 %)، تتبعها الفئات العمرية 40 −60 (9%)، 31 − 45 (8%)، 61 − 75 (4%)، و اخيرا ≤ 76 (1%) ولم يتم العثور على دلالة احصائية بين التهاب البكتريا الملوية البوابية والعمر (القيمة الإحتمالية = 0,729).

لخصت هذة الدراسة ان المرضى الذين يعانون من التهاب المعدة هم الاكثر اصابة بالبكتريا الملوية البوابية، و وضحت ايضا انه لا توجد علاقة بين الاصابة بفيروس الورم الحليمي البشري − 18 ومشاكل المعدة، بينما اظهرت هذة الدراسة وجود علاقة بين البكتريا الملوية البوابية ومشاكل المعدة بنسبة 34%، ولم يتم العثور على اتباط بين العمر، الجنس، و التهاب البكتريا الملوية البوابية.

اظهرت هذة الدراسة ايضا عدم وجود علاقة بين البكتريا الملوية البوابية ومشاكل المعدة (القيمة الإحتمالية = 0.017).

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H.pylori	*Helicobacter.pylori*
HPV	Human papillomavirus
DNA	Deoxy Ribonucleic Acid
GIT	Gastrointestinal tract
PV	Papillomavirus
ICTV	International Committee on Taxonomy of Viruses
CIN	Cervical Intraepithelial Neoplasia
PCR	Polymerase Chain Reaction
RT-PCR	Reverse-Transcriptase- polymerase chain reaction
NSAIDs	Non-steroidal anti-inflammatory drugs
Et Br	Ethidum bromid

CHAPTER I

INTRODUCTION

CHAPTER I

1. INTRODUCTION

1.1.Introduction

Gastritis is one of the commonest problems among different societies (Nisa, 2018). It is an inflammation, swelling, irritation, or erosion of the stomach lining (known as the mucosa). It can occur suddenly (acute) or gradually (chronic) (Kayaçetin and Güreşçi, 2014).

Gastritis is one of the common life-long, serious and insidious illnesses in human beings. One may estimate that more than half of the world population has this disease to some degree and extent, indicating that even many hundreds of millions of people worldwide may have chronic gastritis in a form or other. The significance of chronic gastritis as a serious disease is largely underrated in clinical practice, even though the role of gastritis in the pathogenesis of ordinary peptic ulcers and gastric cancers is obvious (Telaranta-Keerie *et al*., 2010).

Chronic gastritis appears either as non-atrophic or atrophic form; they are forms and phenotypes forms of gastritis, which represent different stages of the same Life-long disease. It has become evident that chronic gastritis can be cured with eradication of *H. pylori*, resulting in normalization of the gastric mucosa, at least in cases in which the gastritis is not developed to atrophic (atrophic gastritis) end stages (Arkkila *et al*., 2006).

Helicobacter pylori, a Gram-negative bacterium found on the luminal surface of the gastric epithelium, was first isolated by Warren and Marshall in 1983. It is an etiologic agent of peptic ulcer disease, primary gastritis, gastric mucosa-associated lymphoid-tissue lymphoma, and gastric adenocarcinoma (Makola *et al*., 2007).

The prevalence of Helicobacter pylori infection worldwide is approximately 50% Approximately 20% of persons infected with *H. pylori* develop related gastro duodenal disorders during their lifetime, but only 10% –20% of infected persons become symptomatic[6]. *H. pylori* seem to be transmitted in various ways, including oral–oral and faecal–oral routes. DNA sequence analysis of house-keeping and virulence associated genes all have illustrated the unusually high degree of genetic variability in this species. The molecular tests presently available for diagnosis, including those targeting 16s rRNA genes, are focused on *H. pylori* and considered as specific targets to confirm *H. pylori* infection, and positive amplification of *H. pylori* specific DNA may be considered as a direct evidence of the presence of the pathogen. This ribosomal gene is particular in that it

is present in all bacteria while, at the same time, it comprises nucleotide sequences that are specific to a given bacterial genus. Sequence analysis of the 16S rRNA gene has led to our current understanding of prokaryotic phylogeny and *H. pylori* 16S rRNA gene sequence analysis unambiguously differentiated the Helicobacter genus from the closely related Campylobacter genus (Mamoun *et al.,* 2015).

Human papilloma virus is non-enveloped, circular, double-stranded, and relatively small DNA tumor viruses with a genome of ~8,000 base pairs (Münger *et al.*, 2004).

HPV could be responsible for some cancers, including cancer of the esophagus, lip, and oral cavity, but a causal role for HPV has not been established (Chaturvedie 2010). Because the relationship of HPV infection and gastrointestinal disorder has implicated for patient care (Bucchi *et al*., 2016).

1.2. Rationale

The human papillomavirus (HPV) is the third pathogen investigated to association with gastritis and the oncogenic properties of HPV have been demonstrated in studies of other portions of the digestive tract (De Souza *et al*., 2018).

H.pylori infection is the most common infection associated with uncomplicated dyspepsia, heartburn, and peptic ulcer diseases, most commonly leading to upper gastrointestinal bleeding and, ultimately, to the severe complication of gastric malignancy. Ninety percent of duodenal ulcers and 70% of gastric ulcers are associated with *H. pylori* infections (Rana *et al.*, 2017).

Lately, in Sudan, most studies of gastritis and other gastric diseases causes are related to *Helicobacter pylori* but they are few researches focused on the association of viral infection with gastric problem. Only a few studies have addressed the role of HPV in the epidemiology and development of gastric cancer (Stanley *et al.*, 2012).

Therefore, this study was conducted to focus on this point and to find out possible associationbetweenHPV-18 and *H. pylori*. So the findings from this study may be as warring for seeking other causative agents of gastritis rather than *H. pylori.*

1.3. Objectives

1.3.1. General objective

To molecular detection of human papilloma virus genotype 18 and *Helicobacter pylori* in gastric Biopsies of patients with gastric problems attending police hospital, Khartoum.

1.3.2. Specific objectives

1. To detect and determine the frequency of HPV-18 among patients with gastric problems in Khartoum, Sudan by using polymerase chain reaction (PCR).
2. To detect and determine the frequency of *Helicobacter pylori* among patients with gastric problems in Khartoum, Sudan by using polymerase chain reaction (PCR).
3. To determine association between age and sex with *Helicobacter pylori* infection among the Sudanese patients with gastric problems.

CHAPTER II
LITERATURE REVIEW

CHAPTER II

2. LITERATUREREVIEW

2.1. Human papillomavirus

2.1.1. Background

Human papillomavirus (HPV) is a suspected risk factor of neoplastic transformation. Abundant evidence has demonstrated the oncogenic properties of HPV in studies on anal, oral and pharyngeal cancers, suggesting a role for the virus in the pathogenesis of cancer of other sections of the alimentary tract. (Stanley *et al.,* 2012).

2.1.2. Taxonomy

Human papillomavirus phylogenetic classification is based on the nucleotide sequence of the open reading frame encoding the major structural protein L1, as specified by the International Committee on Taxonomy of Viruses (ICTV). It is of different genera share less than 60% identical L1 nucleotide sequences PV within a genus share 60 to 70% identity, while an identity between 70% and 90% defines a species. PV subtypes show 90 to 98% and variants more than 98% L1 nucleotide sequence identity. The Papillomaviridae family presently consists of 189 PV types spread over 29 genera. HPV18 are the most common cancer-causing HPV subtypes on a global scale (Niccolai *et al.,* 2013).

So far, more than 100 different HPV genotypes have been identified based on differences in DNA sequence. These types can be classified according to various criteria, e.g. their tissue tropism, oncogenic potential and phylogenetic classification. There are 30 types are commonly found in the genital tract. These include several HPV genotypes, such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 that are known as high-risk HPV and further three genotypes (HPV 26, 53, and 66) They are widespread in nature, and have been identified in many animal species (Arbyn *et al.,* 2011).

2.1.3. Structure

HPV is a small (52–55 nm), non-enveloped virus with a circular, double-stranded DNA (deoxyribonucleic acid) viruses that measure 55 nanometers (nm) in diameter and comprise an icosahedral capsid composed of 72 pentameric capsomeres of the major capsid protein L1, partly associated with the minor capsid protein L2. Enclosed within the capsid is the viral genomic DNA which is packaged as a minichromosome (Ferlay *et al.,* 2012).

2.1.4. Pathogenesis

HPV infection occurs at the basal epithelium, although the incidence of infection is high,

most infections resolve spontaneously. A small proportion of infected persons become persistently infected; persistent infection is the most important risk factor for the development of cervical cancer. The most common clinically significant manifestation of persistent genital HPV infection is cervical intraepithelial neoplasia, or CIN. Within a few years of infection, low-grade CIN—called CIN 1—may develop, which may spontaneously resolve and the infection clear. Persistent HPV infection, however, may progress directly to higher-grade CIN, called CIN2 or CIN3. High-grade abnormalities are at risk of progression to cancer and so are considered cancer precursors. Some high-grade abnormalities spontaneously regress. If left undetected and untreated, years or decades later CIN2 or 3 can progress to cervical cancer (Satterwhite *et al*., 2013).

2.1.5. Transmission

HPV is transmitted by direct contact, usually sexual, with an infected person and occurs most frequently with sexual intercourse but can occur following non-penetrative sexual activity. Studies of newly acquired HPV infection demonstrate that infection occurs soon after onset of sexual activity. Genital HPV infection also may be transmitted by non-sexual routes, but this appears to be uncommon non-sexual routes of genital HPV transmission include transmission from a woman to a newborn infant at the time of birth(Winer *et al*., 2006).

2.1.6. Lesions associated with HPV

Ascertain subset of papilloma viruses is clearly implicated in causing oncogenic malignancies in human accounts for only a portion of these cancer cases. HPV infection is commonly at multiple mucosal sites in men and women (Feng*et al.,* 2007).

The multiplicity of infection increases among immunocompromised individuals (Junquera *et al*., 2004; Sugar *et al.,* 2006).

HPV has also been implicated in the cancers of the cutaneous squamous cells. A skin disease, while others develop in immunosuppressed individuals. The percentage of these cancers that may be attributable to HPV, however, is unclear. Other common cancers such as cancer of the breast, lung, colon, rectum, prostate, and esophagus have also been associated with HPV, although a consistent recurrent causal relationship has not been shown (Gillison and Shah, 2003).

2.1.7. Diagnosis

As HPVs cannot be cultured easily; usually, HPV detection and genotype assays are based on the detection of viral nucleic acids, mostly viral DNA. HPV-DNA is detected by target amplification methods and/or signal amplification methods. The most used target

amplification-based method is polymerase chain reaction (PCR) using conserved sequences of the HPV genome (Castillo, 2011).

Recently, real-time PCR assays have been used to determine the number of viral copies of HPV and to determine its integration status other target amplification-based methods as reverse-transcriptase (RT) - PCR assays can be applied to detect HPV mRNAs in fresh-frozen specimens or samples in which RNA is well preserved regarding signal amplification methods, these are based on an initial hybridization step of nucleic acids in the specimen with target-specific probes in liquid phase or in situ on cells or tissue slides, after which the signal (i.e., the hybridization event) is amplified and ultimately visualized with one of the various methodologies available(Castillo,2011).

2.2. *Helicobacter pylori*

H. pylori is a Gram-negative bacterium colonizing the human stomach and associated with numerous gastrointestinal disease (Kuo *et al*., 2014).

It is quite a frequent infection all over the world; more than half of the population in both developed and developing countries are infected with this microorganism (Aluze- Ele*et al*., 2015). Most of the people acquire *H. pylori* infection during their early childhood (Koupil *et al.,* 2007).It has been reported as a common cause of chronic gastritis, peptic ulcer diseases and gastric cancer in adults (Mera *et al.,* 2009).

H.pylori infections usually are symptomless and without clinical manifestation, particularly in poor communities (Pharoah *et al.,* 2002). However, signs and symptoms associated with the disease are primarily due to gastric or peptic ulcer illness or duodenal inflammation. Furthermore, other symptoms such as nausea, vomiting and abdominal pain may be attributed to other gastrointestinal diseases (Czinn 2014).

2.2.1. Transmission

The mode of transmission of *H. pylori* remains poorly understood; no single pathway has been clearly identified. The housefly has the potential to transmit *H. pylori* mechanically and thus fly excreta might theoretically contaminate food mostly in areas of the world with poor sanitation. Person-to-person contact is considered the most likely transmission route. There are 3 possible routes of transmission from the stomach of one person to another: 1) Iatrogenic: The first, and most frequent mode of transmission is iatrogenic (acquired infections) in which tubes or endoscopes that have been in contact with the gastric mucosa of one individual are used for another patient, 2) Fecal–oral: The second possible route of transmission.in which *H. pylori* has been isolated from the feces of infected young children

but isolation from adult feces has been rare. Failure to recover the bacterium from feces might be due to the toxic effect to feces, 3) Oral–oral: Studies have cultured *H. pylori* from the oral cavity; only sporadic isolates from dental plaque and saliva have been recorded (Van Duynhoven and De Jonge, 2001).

2.2.2. Diseases

H. pylori can cause many serious diseases. It frequently causes inflammation of the lining of the stomach (gastritis).Also, it can cause duodenal ulcers (in the small bowel just beyond the stomach) and it is uncommonly itis the cause of about 90% of ulcers in the duodenum and approximately 70% of stomach ulcers. Most of the remaining ulcers appear to be due to certain medications, particularly no steroidal anti-inflammatory drugs (NSAIDs) taken regularly to ease arthritis, or low-dose aspirin to help prevent heart attack or stroke in addition to some cancers of the stomach, including a rare type called lymphoma (Crowe 2019).

2.2.2.1. Gastritis

Gastritis is one of the commonest problems among different societies (Nisa, 2018). It is an inflammation, swelling, irritation, or erosion of the stomach lining (known as the mucosa). It can occur suddenly (acute) or gradually (chronic) (Kayaçetin and Güreşçi, 2014;Smith *et al*., 2017).

2.2.2.1.1. Acute gastritis

Acute gastritis is a transient mucosal inflammatory process that may be asymptomatic or cause variable degrees of epigastric pain, nausea and vomiting. In more severe cases, there may be erosion, ulceration, hemorrhage, hematemesis melena or rarely massive blood loss (Flake *et al*., 2015). Acute gastritis is often erosive and hemorrhagic neutrophils are the predominant cells in the superficial epithelium. Many cases result from drug intakes like aspirin and antacid ingestion. Acute gastritis often produces no symptoms but may cause dyspepsia, anorexia or vomiting (Park *et al*., 2010).

2.2.2.1.2. Chronic gastritis

The early phase of chronic gastritis is superficial gastritis. Chronic gastritis is classified according to the predominant size of involvement categorized as type A-refers to the body predominant form type B is the central predominant form that is *H. pylori* related. However, acute *H. pylori* infection does not produce sufficient symptoms to require medical attention, but chronic gastritis ultimately causes the affected person to seek treatment (Lanza *et al.*, 2009).

2.2.2.2. Peptic ulcer

A peptic ulcer is a broad term that includes ulcers of the digestive tract in the stomach or the duodenum. Earlier it was believed that one developed this type of ulcers due to stress and spicy food. The causative agent is an infection caused by the bacteria *H. pylori* or reaction to certain medicines like non-steroidal anti-inflammatory drugs (NSAIDs). Symptoms of peptic ulcers include abdominal discomfort and pain. Other symptoms include weight loss, poor appetite, bloating, nausea, and vomiting. Some may also experience blood in stool and vomit and black stools that indicate gastrointestinal bleeding (Kumarsunil *et al*., 2012).

2.2.2.3. Duodenitis

The strong association between antral gastritis and duodenal ulcer has been recognized for many years. duodenitis is almost invariably present in duodenal ulceration and is an even stronger predictor than gastritis being far less common in the general population than gastritis. Gastritis and duodenitis both affect the digestive tract and share the same causes including *H.pylori* infection. Gastritis is inflammation of the lining of the stomach while duodenitis is inflammation of the lining of the upper small intestine called the duodenum. The stomach and duodenum are close to each other in the body and many factors affect them in comparable ways. In addition to having the same causes the conditions share similar treatments. It can be challenging to deal with the symptoms of gastritis and duodenitis which are often uncomfortable. However, most cases do not cause long-term or severe complications and both conditions are generally easy to cure (Johnson2018).

2.2.2.4. Gastric cancer

Gastric cancer is one of the most common types of cancer and is a leading cause of cancer-associated mortality in the world the most important and common cause of gastric cancer is excessive consumption of salt, smoking, long term subjection to nitrosamines in food and drinking water, as well as microbial infections, such as it and certain viruses, including the Human papillomavirus (HPV) (Xu WG *et al.,* 2003; Backert *et al.,* 2004).

H.pylori cause chronic gastritis and progress to atrophic gastritis, intestinal metaplasia, glandular dysplasia and eventually gastric carcinoma (Fakhraei *et al.,* 2016).

2.2.3. *H. pylori* and Gastritis

Gastritis is defined as the histological presence of gastric mucosal inflammation. The broader term gastropathy encompasses lesions characterized by minimal or no inflammation. *H. pylori* infection may cause both acute and chronic gastritis. Acute non-erosive gastritis is most commonly due to *H. pylori* infection. *Helicobacter pylori*

infection induces a severe inflammatory response with gastric mucin degradation and increased mucosal permeability, followed by gastric epithelial cytotoxicity, chronic *H. pylori* infection predisposes to atrophic gastritis and autoimmune gastritis. The term Gastritis is commonly employed for any clinical condition with upper abdominal discomfort like indigestion or dyspepsia in which the specific clinical signs and radiological abnormalities are absent (Mohan, 2015).

2.2.4. Human papillomavirus and *H.pylori* infection in gastric disorder

Microbial infections have been shown to contribute to gastric tumor genesis, and gastric physiology and immunology are known to be altered by *H. pylori*. While more than half of the world's population is infected with *H. pylori*, however, only approximately 3% of these individuals will develop GC (De Souza *et al*., 2018).

2.3. Previous studies:

Fakhraei and his Colleagues (2016) in Iran conducted a study for detection of HPV DNA in gastric biopsy specimens in a high-risk region of Iran and found that; five (5%) samples were identified to be positive for HPV DNA [four male (5.7%) and one female (3.3%)],three (60%) samples were positive for HPV-16, one (20%) sample was positive for HPV-18, and one (20%) sample was positive for HPV-45.

Nazar and his Colleagues (2016) in Sudan investigated the seroprevalence of *H. pylori* among Sudanese patients with gastritis; in which seropositivity of *H. pylori* antibodies among symptomatic gastritis patients was 63.2%and most gastritis patients (32.6%) were in the age range 30-39 years. Also 27 (81.8 %) were males found positive for *H. Pylori*.

Pavlik and his Colleagues (2015) conducted a study for detection of *H. pylori* and HPV in preoperative tissue biopsies collected from malignancies in the pharyngeal area and found that *H. pylori* DNA was detected in 60 samples (85.7%), while DNA of HPV only in 42 (60%). If focused on HPV-16 as a proposed cancer inductor, it was detected in 34 samples (48.5%) only. No DNA of respective agents was detected in 7 samples (10%). There were 21*H. pylori* pathogen detections compared with only 3 of HPV.

Tongtawee and others (2016) in Thailand studied the characteristics and risk factors of *H. pylori* associated gastritis, they found that out of 300 patients were enrolled in this study, 153 were males (51%) and 149 were females (49%). They also found that among 150 positive *H. pylori* infected individuals, 95 (63.3) were have gastritis and 84 (56) were not; also found that patients demographic data and *H. pylori* infection by univariate analysis showed no association between them.

Alaa Faroge (2018) in Sudan studied Comparison between invasive and non-invasive

techniques for detection of *Helicobacter pylori* in patients with upper gastrointestinal Disorders, study include 55 patients 25 males and 30 females complain from gastric disorder and attending for Oesophago-gastro duodenscopy in Wad Madeni city, Gezira State, Sudan, their age ranged from 17 to 85 years, from September 2017 to April 2018. The invasive technique include two antrum Gastric biopsy were collected for detection of *Helicobacter pylori*by Polymerase chain reaction using primers target 16s rRNA gene. Out of 55 samples 24 (43, 6%) were positive and 31 (56.4%) were negative by Polymerase Chain Reaction.

Amna Mubarak (2019) in Sudan conducted a study for detection of *Helicobacter pylori* Cag A genein gastric lesions among Sudanese gastric disorders patients.

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 were 17 (44.7%)and ages ranges from 16 to 80 year, with mean age 56.Endoscopicbiopsy samples were collected, PCR for detection Cag A gene for *H.pylori*. histopathology 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 Cag A gene were shown as follow, 30 (78.9) samples were positive and 8 (21.1%) samples were negative. The study concludes there is no association between Cag A positive and gastric changes.

CHAPTER III
MATERIALS AND METHOD

CHAPTERIII

3. MATERIAL AND METHODS

3.1. Study design

This is descriptive, cross-sectional, hospital based study.

3.2 Study area

The study was carried out in the Police Hospital (Omar Sawi complex) to investigate the presence of HPV -18 and *H.pylori* in gastric biopsy.

3.3. Study duration:

This study was conducted in the period from January 2019 to February2020.

3.4. Study population

Gastritis patients were included in this study.

3.4.1 Inclusion criteria

Patients who attended the Police Hospital to perform intestinal endoscopy with different ages and both gender suffering from gastric problems.

3.4.2 Exclusion criteria

Patients with intestinal bleeding were excluded from this study.

3.5. Ethical considerations

Permission to carry out this study was taken from the Scientific Research Committee, Collaege of Medical of Laboratory Science, Sudan University for Sciences and Technology and Police Hospital and verbal informed consent was taken from each patient.

3.6. Sampling technique

This study based on a probability, non prometric sampling technique.

3.7. Sample size

One Hundred (n=100) samples were taken.

3.8. Method of Data collection

Data was collected through a non-self-administered questionnaire from every patient.

3.9. Sample collection

Gastric biopsy samples were collected in plain container contains tryptic Soya broth (TSB) culture media by using sterilize biopsy forceps during the gastroscopy process, and stored at - 4°C.

3.10. DNA extraction

Human papillomavirus and *H. pylori* DNA were extracted from gastric biopsy specimens using the guanidine chloride extraction method. Each sample container 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 (NH4) acetate were added to the pellet, vortexed, and incubated at 37^{o}C overnight or at 65^{o}C 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 a new tube, and 10 ml of cold absolute ethanol were added, shaken, and kept at -20^{o}C 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 of70% 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 -20^{o}C overnight (Al Rahem and Elhag, 2018).

3.11. Target amplification by Polymerase Chain Reaction (PCR) for Human papillomavirus

HPV type (18) was detected by PCR was done using Maxime PCR master mix kit and using specific primers and the sequences of the primers were:

Reversed: 5-AGT CAT ATA CCT CAC GTC GCA GTA -3

Forward: 3-TCA GTA TAT GGA GTG CAG CGT-5

Amplification was performed in a final volume of 20 µL of PCR mixture containing 0.8 µl of each primer, 10 mM of each deoxynucleotide triphosphate (dATP, dGTP, dTTP and dCTP), 10 mm Tris-HCl, 50 mMKCl, 0.1% triton X– 100, 1.5 mM MgCl2, one unit of DNA polymerase and 4 µL of template DNA. DNA amplification program was carried out as follows: Initial denaturation at 94°C for 5 minutes, each cycle with denaturation at 92°C for 30 second, primers annealing at 55°C for 40 second, primers extension at 72°C for 40 second, final extension at 72°C for 5 minutes. PCR products were detected using 1% agarose gel electrophoresis (Elmi *et al*., 2017).

3.12. Target amplification by Polymerase Chain Reaction (PCR) for *H. pylori*

H. pylori DNA was detected by PCR using a primer that targets 16s ribosomal RNA (16s rRNA).

Forward 5′ TGGCAATCAGCGTCAGGTAATG 3′

Reverse 3′ GCTAAGAGATCAGCCTATGTCC 5′

Total reaction volume of 20 µl containing, 3 µl of extracted DNA, 0.7 µl of 10 pmol/µl of each forward and reverse primers for 16S rRNA gene, in addition to 14 µl of molecular biology grade water then the mixture added to lyophilized PCRpremix™ formula.

The following conditions used to amplify *H. pylori* 16S rRNA gene with 38 cycles, Initial denaturation at 94°C for 5 minutes, each cycle with denaturation at 92°C for 30 second, primers annealing at 55°C for 40 second, primers extension at 72°C for 40 second, final extension at 72°C for 5 minutes. PCR products were detected using 1% agarose gel electrophoresis (Abu-Almaali *et al*., 2012).

3.13. Gel electrophoresis

One gram of agarose gel was weighted by sensitive balanced and dissolved in 100 ml 1 X TBE (1%) then boiled in microwave for 30 seconds. After cooling 1ul of Et Br was added and poured on tray of gel electrophoresis containg combs. Then after solidification combs was removed and 5 ul of PCR product was added in each well. In which first well contained DNA ladder (100 bp)and the agarose gel was immersed with 1X TBE buffer and connected to power supply using V 59, A 34 for 20 minutes.

After that, gel electrophoresis was estimated under ultraviolet using Gel documentation system (Göttingen- Germany) to detect presence or absence of bands for HPV-18 and *H .pylori*.

The expected size of the reaction products was (95 bp) for HPV18 and (522 bp) for *H.pylori.*

3.14. Data analysis

The obtained data were analyzed and presented using Statistical Package for Social Science (SPSS) computer software version 16.0 for Windows. Frequencies and means were calculated. Chi- square test was used to associate between qualitative variables. Statistical significance was set at *P. value* of ≤ 0.05.

CHAPTER IV

RRESULTS

CHAPTER IV

4. RRESULTS

4. Results

A total of one hundred subjects (n=100) were included in this study in which 61 (61%) of them were males and 39 (39%) were females. The age of patients was ranged from 15-76 years with an average mean of 42.21 +16.8 S.D (Table 4.1 and Figure 4.1).

The distribution of gastric disorders among patients were as follow: 57/100 (%) with gastritis, 19/100 (%) with gastric ulcer, 14/100 (%) with duodenitis, 6/100 (%) had gastric erosion, 3/100 (%) up normal tissue and 1/100 (%) duodenal ulcer as explained in figure 4.2.

All patients were negative for HPV-18by PCR as explained in figure 4.2.

Concerning Gender, there were 20/61 (58.8%) males and 14/39 (41%) females were positive for *H. pylori*and there was no significant between *H.pylori* infection and geneder (p= 0.749) (Table 4.2).

Regarding age groups, the highest frequency was 12/100 (12%) among patients with age group 15- 30years,followed by 40-60 years age group (9%), 31-45 years (8%), 61-75 years (4%) and the lowest frequency at age ≥76 years (1%).There was no statistically significant difference between *H. pylori* infection and age groups (P=0.729) (table 4.3).

The gastric disorders in this study included gastritis (57%), gastric ulcer (19%), duodenitis (14%), duodenal ulcer (1%), gastric erosion (6%), and abnormal tissue (3%).

Among the all patients included in this study, the positivity rate of *H. pylori* in patients with gastritis(14%), gastric ulcer (0%), duodenitis (0%), duodenal ulcer(9%), gastric erosion(10%), and abnormal tissue (1%).

While the negative rate was gastritis (43%), gastric ulcer (9%), duodenitis (5%), duodenal ulcer (1%), gastric erosion (5%), and abnormal tissue (3%) (Figure 4.5).

Table (4.1): Distribution of age among patients with gastric problems

Age groups	Frequency	Percentage
15-30years	34	34%
31-45years	29	29%
46 – 60years	24	24%
61-75years	8	8%
≥ 76 years	5	5%
Total	**100**	**100%**

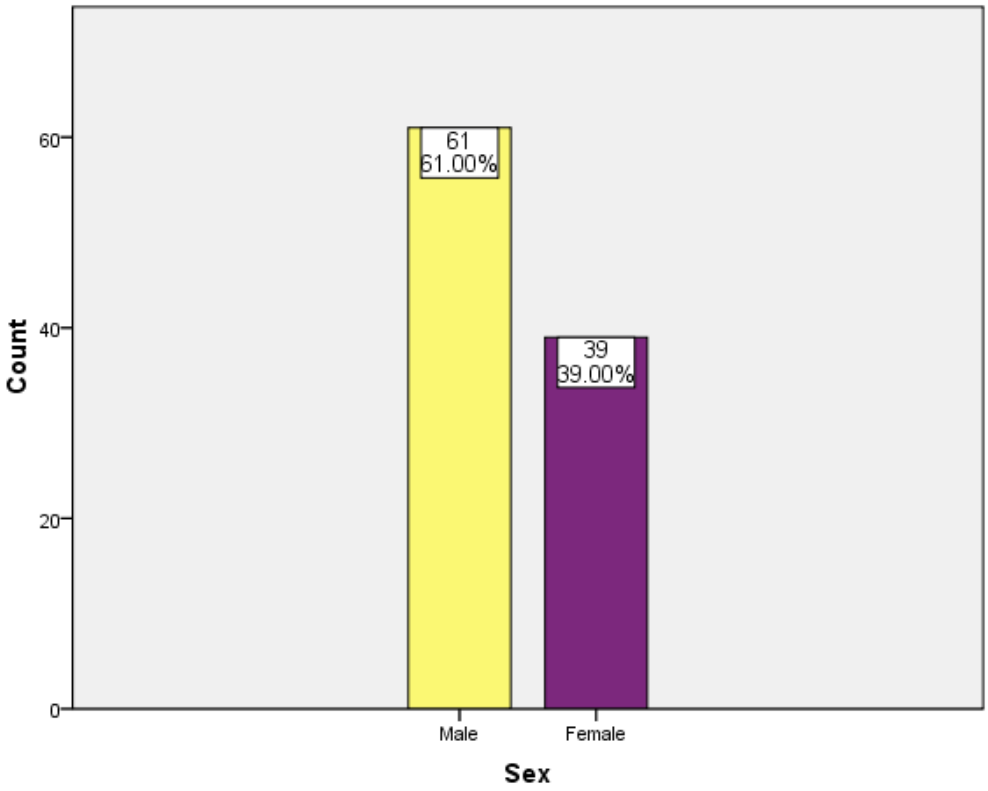


Figure (4.1): Distribution of sex among patients with gastric problems

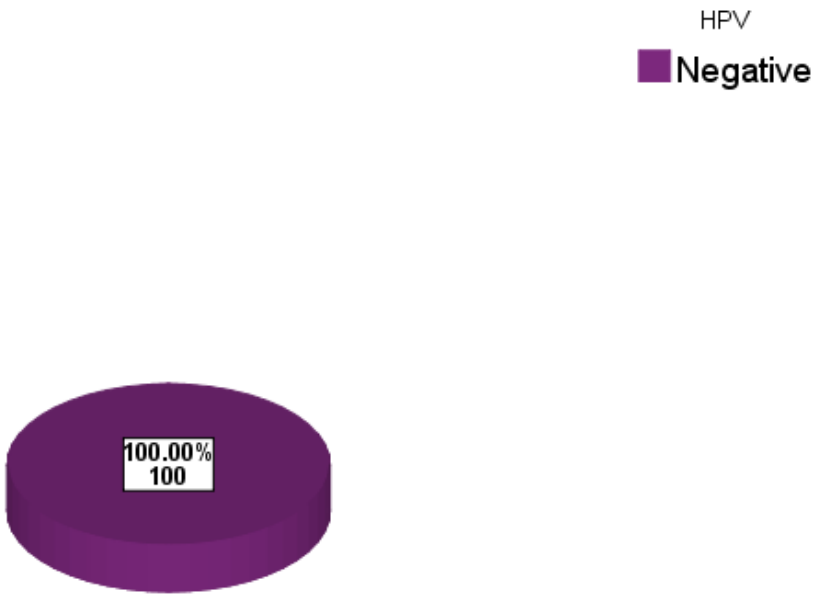


Figure (4.2): Result of HPV -18 among patients with gastric problems

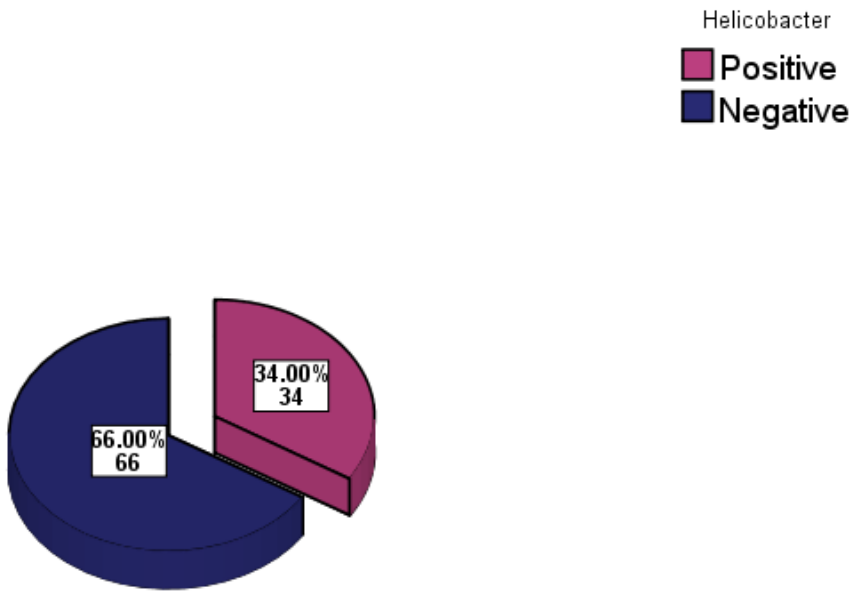


Figure (4.3): Frequency of *H. pylori* among patients with gastric problems

Table (4.2): The association between *H. pylori* and gender among patients with gastric problems

Gender	*H. pylori*				Total (%)	*P*- value
	Positive		Negative			
	No.	%	No.	%		
Male	20	58.8%	41	62.1%	61 (61%)	0.749
Female	14	41.1%	25	37.8%	39 (39%)	
Total	**34**	**34%**	**66**	**66%**	**100 (100%)**	

Table (4.3): The association between *H. pylori* and age among patients with gastric problems

Age groups	*H. pylori*				Total (%)	*P*. value
	Positive		Negative			
	No	%	No	%		
15-30 years	12	12%	22	22%	34 (34%)	0.729
31-45 years	8	8%	21	21%	29 (29%)	
46-60 years	9	9%	15	15%	24 (24%)	
61-75 years	4	4%	4	4%	8 (8%)	
≥ 76 years	1	1%	4	4%	5 (5%)	
Total	**34**	**34%**	**66**	**66%**	**100 (100%)**	

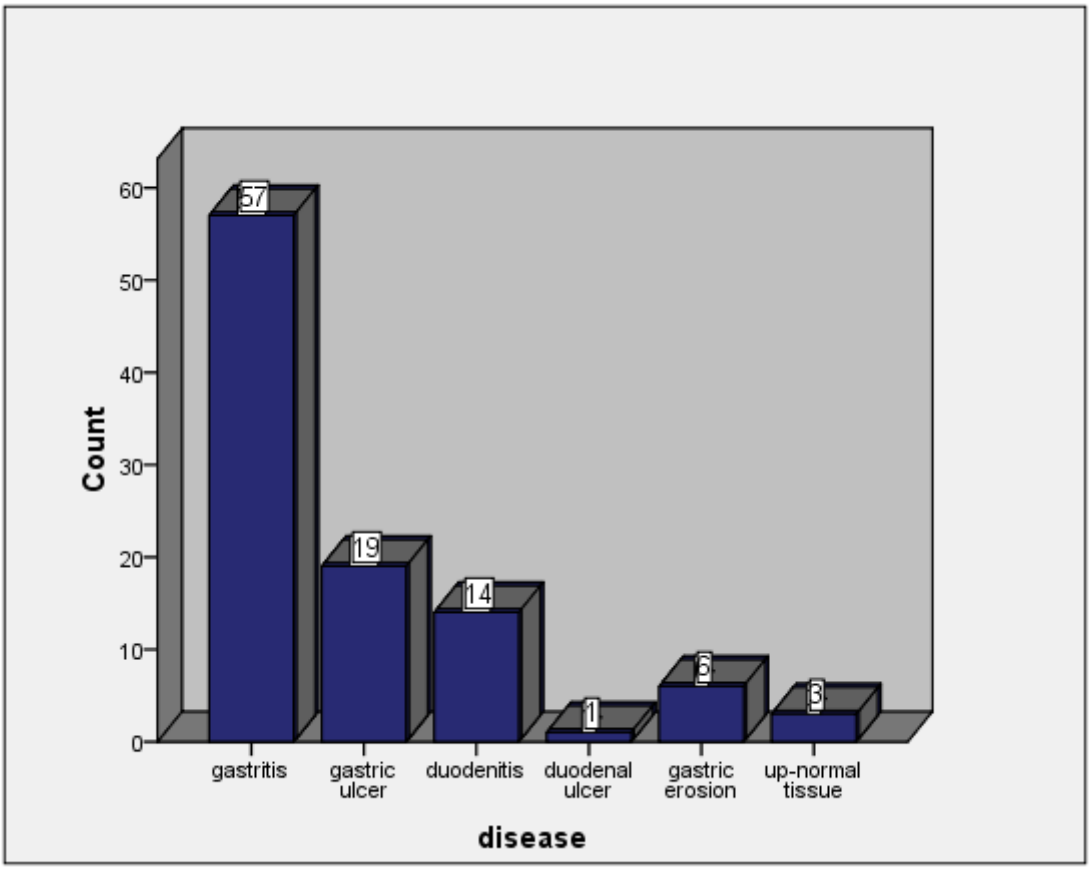
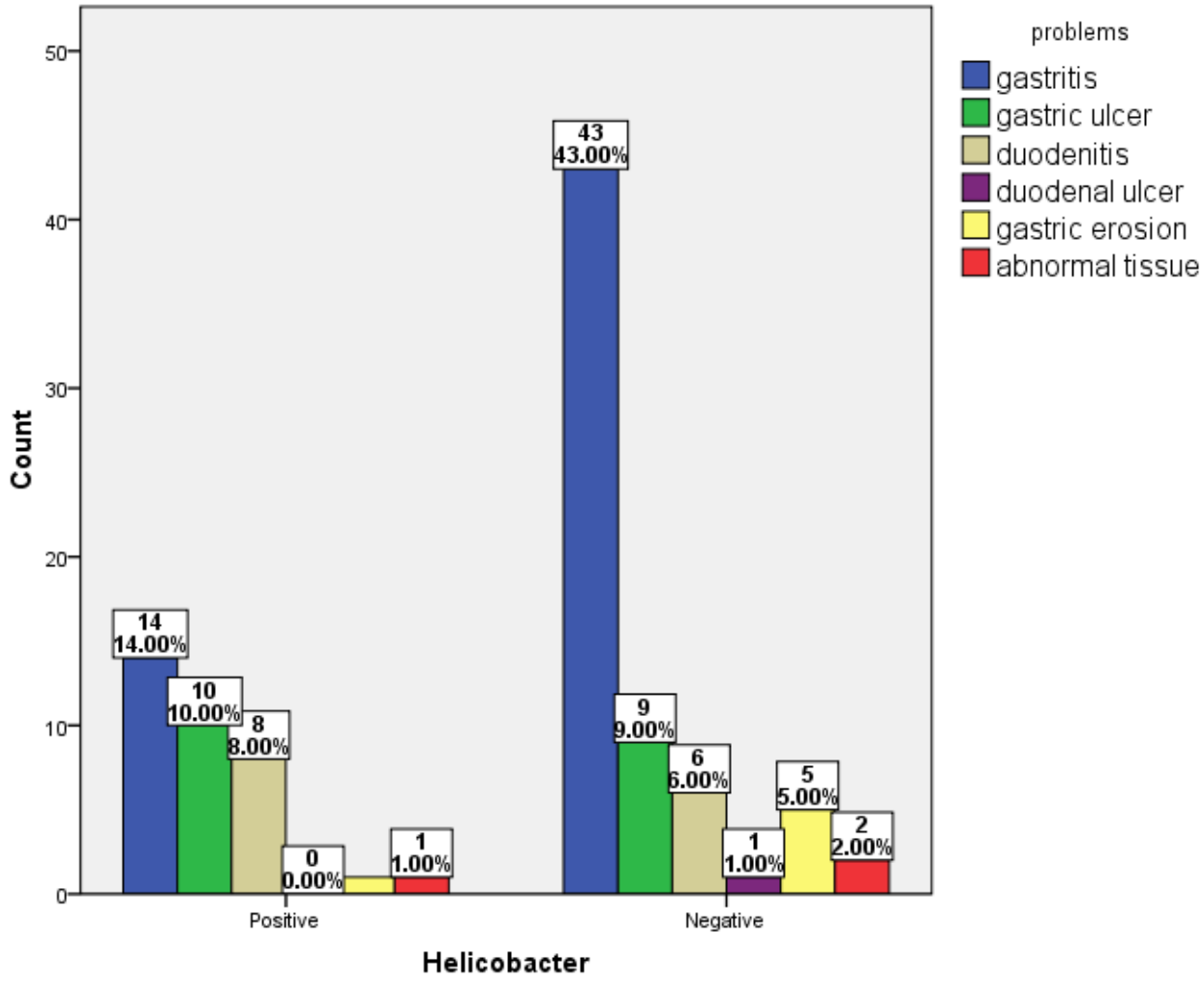


Figure (4.4): Distribution of gastric disorders among patients with gastric problems



(*P*= 0.017)

Figure (4.5): The frequency of gastric problems among *H.pylori* patients

CHAPTER V
DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

CHAPTER FIVE

5. DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

Common cause of gastritis includes infection with *H.pylori* and use of non-steroidal anti-inflammatory drugs (NSAIDs) (Melina *et al*., 2016).

Complications of gastritis may include stomach bleeding, stomach ulcers and stomach tumors. When due to autoimmune problems, low red blood cells due to not enough vitamin B12 may occur, a condition known as pernicious anemia (Varbanova *et al*., 2014).

This study showed that; no HPV-18 was detected in all patients(0%) which was disagreed with Fakhraei *et al.*(2016)in Iran, which found 5% positive samples for HPV DNA.

The variation in this result may be due to other geo types rather than HPV-18.

In this study the frequency of *H.pylori* was 34 (34%) among patients which was similar to study carried out by Correa *et al.* (2016) in Colombia (36.4%).

The present study was lower than study done in Sudan by Nazar *et al.* (2016) which found (81.8 %) were positive patients, Barvo and his colleagues (2003) in Colombia (69%), and Garg*et al.* (2012) in India (43 %).

This study was higher than study done in Sudan by Mamoun *et al*.(2015) which found (22 %) were positive patients.

These variations in results may be due to differences in sample size and hygiene of population.

In the current study, the positive frequency of *H.pylori* among males were increased (58.8.9%) than females (41.1%), and there was meaningless association between sex and *H.pylori* infection (*P*= 0.749),this was similar with the result done by Amna Mubarak (2019) in Sudan which showed that (55.4%) of positive cases were males and (44.5%) were females and disagreed with the results done by Alaa faroge (2018) in Sudan which showed that (45.5%) of positive cases were males and (54.5%) were females, Correa*et al*. (2016) in Colombia which showed that (39.6%) of positive cases were males and (34.6%) were females.

The result of this study showed that the most positive results for *H. pylori* were 12 (12.0%) patients in age group 15- 30 years, and also showed no significant association between *H. pylori* infection and the age (*P*=0.729)and that was match with Amna Mubark (2019) in

Sudan which showed that the most positive were 23 (60.5%)in age <40 and mismatched with Correa *et al.* (2016) in Colombia which showed that the most positive results were 204 (20.7%) in age group 40 – 49 years.

In the current study, the positivity rate of *H. pylori* in patients with gastritis, gastric ulcer, duodenitis, duodenal ulcer, gastric erosion, and abnormal tissue was 14%, 9%, 10%, and 1% respectively, and this disagree with yousra A *et al*. (2018) in Sudan which showed 80% of patients with gastritis, 60% duodenitis and 56 %of patients with duodenal ulcer .

5.2. Conclusion

- *H.pylori* was more frequent among patients with gastritis.
- This study concluded that, there was no detected HPV-18 among patients with gastric problems while *H.pylori* was detected in rated frequency (34%).
- There were no significant association between age, gender and *H.pylori* infection.

5.3. Recommendations

Further studies with large sample size should be done to detect other genotypes of HPV and other technique.

Further studies should be carried out for detection other genes of HPV to determine the association between HPV and *H.pylori* infection.

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APPENDICES

APPENDICES

Appendix- I

Questionnaire

Demographic data:	**No. “ “**

Name: __

- _____Telnumber:_____
- Age: _____
- Sex: ☐ Male
  ☐ Female
- Job: _____
- Education: ☐ Primary ☐ Secondary
  ☐ Graduate ☐ Post graduate
- Residence: _____
- Origin: _____
- Date.....ID number.....
- Blood group:.....
- Symptoms:
  - Abdominal pain ☐Yes ☐No
  - Nausea☐Yes ☐No
  - Recurrent Vomiting ☐Yes ☐No
  - Dysphagia
  - Difficulty in swallowing
  - Reflux symptoms: ☐Yes ☐No
  - Acidity
  - Heart pain
  - Regurgitation ☐Yes ☐No
  - Weight loss ☐Yes ☐No
  - Dyspepsia ☐Yes ☐No
  - Family history of gastric cancer ☐Yes ☐No
- Result of RUT (if done) .....

- Past history of H.pylori ☐Yes ☐No

  If yes diagnosed by:

  ☐ ICT blood test ☐ Stool Ag ☐ UBT

  ☐ Endoscopic CLO test ☐ I don't know

- Type of treatment:

  ☐ Triple ☐ Quadruple

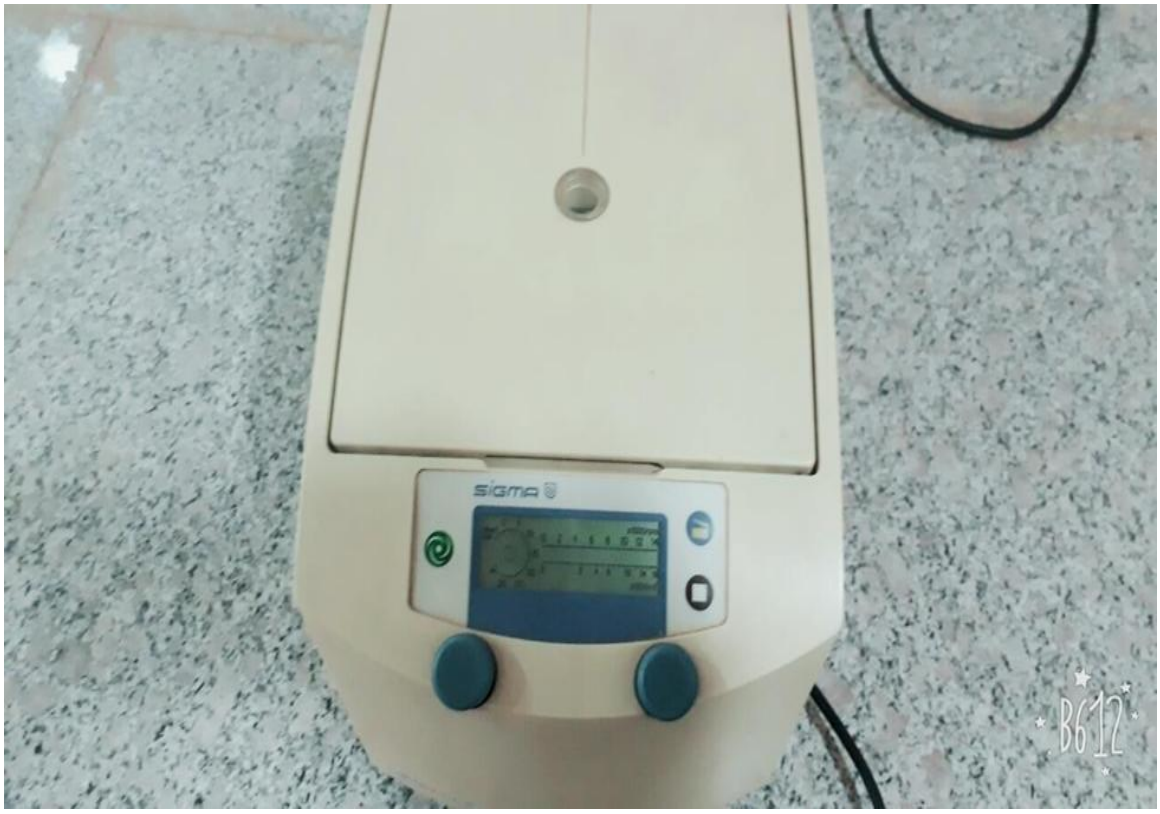
  ☐ Others: _____

- Type of antibiotic.....
- Frequency of treatment: _____
- Endoscopic findings:
- Gastritis (. . . .) Gastric ulcer (. . . .) Duodenal ulcer
- (. . . . . .) Gastric mass or abnormal tissue (. . . .) Gastric irrosion(. . .) Unexplained anamia(. . .) Idiopathic thrombocytopenic purpura
- HPV (……..)
- Location of biopsy:
- Antrum (. .) . . . . Body (. . .) . . . Fundus. (. . .) Incisura(. . .)

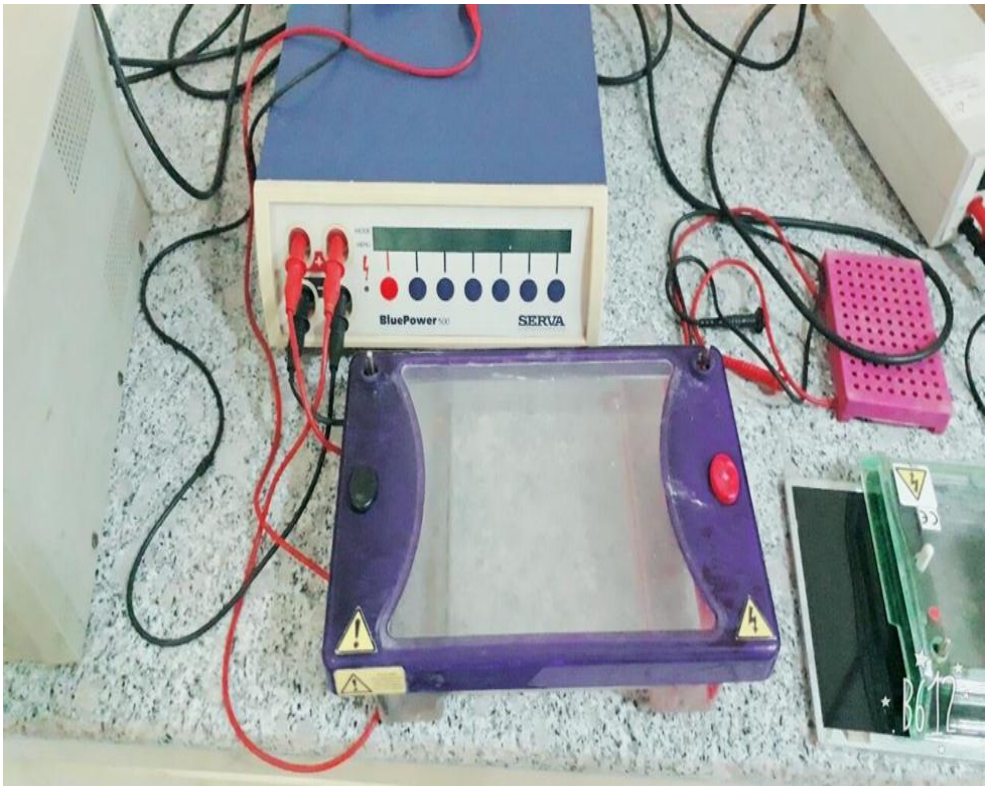
- Lab tests: _____

Appendix -11

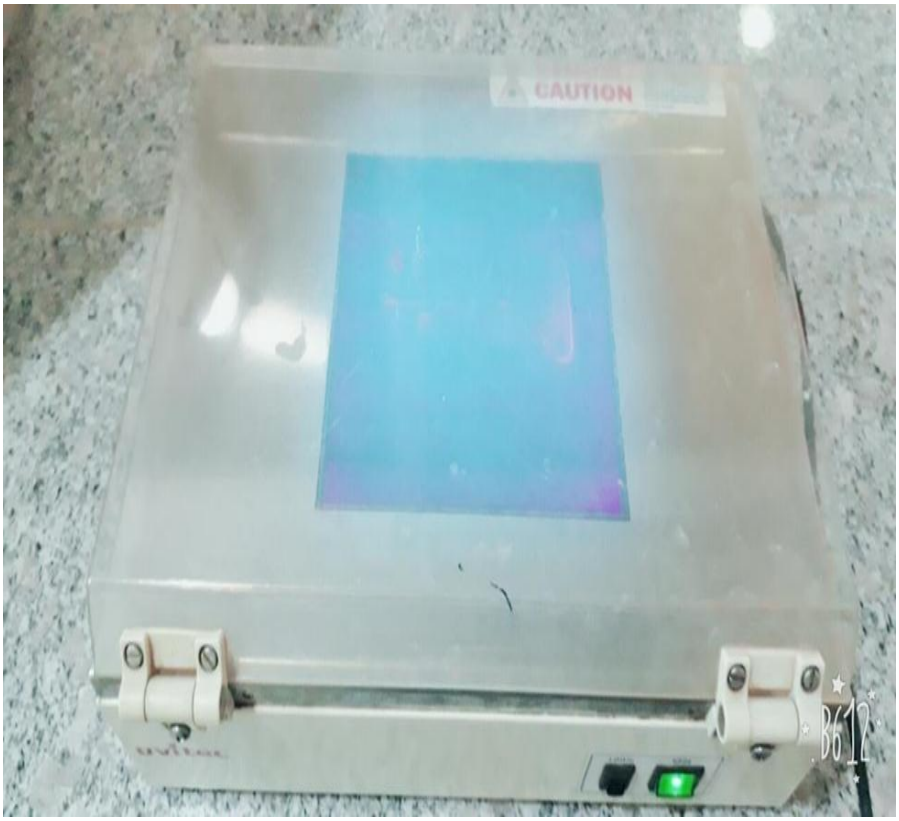
Color plates



Color plate (1): Microcentrifuge device



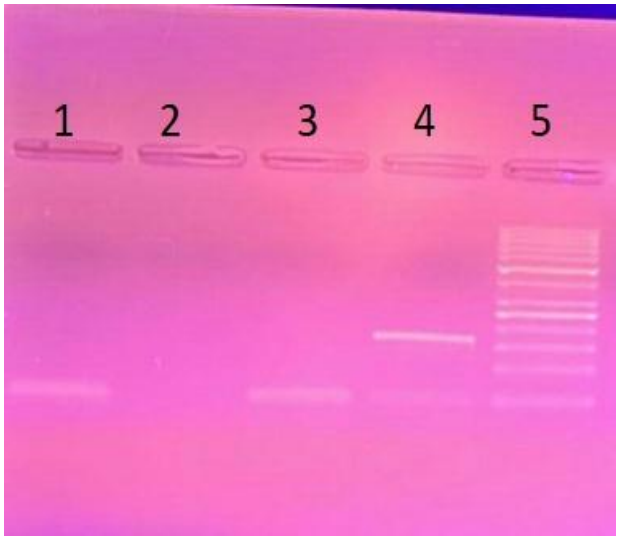
Color plate (2): gel electrophoresis and power supply device



Color plate (3): UV Light transilluminator device

Appendix-111

PCR-PRODUCT

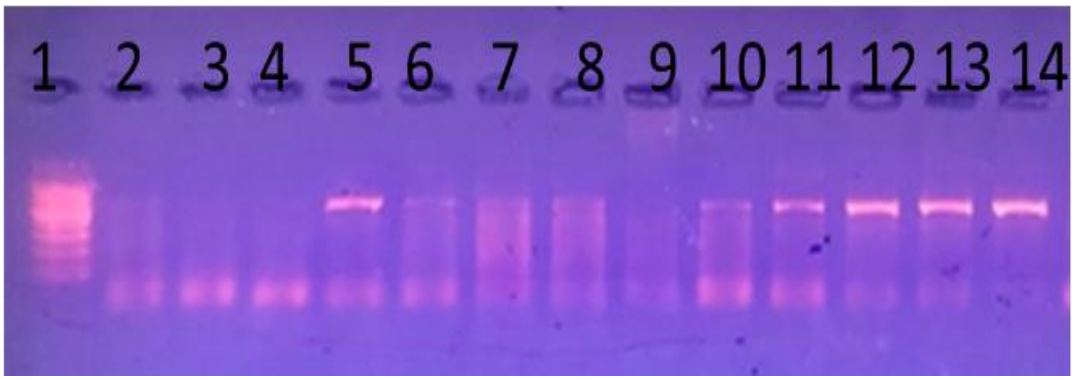


1% gel electrophoresis of amplified products of HPV-18 DNA.

Lane 5 = ladder

Lanes 1, 2, 3 = -ve samples.

Lan 4 = + ve control



Gel electrophoresis for *H.pylori*. Lane 1 DNA ladder (1500 bp).Lanes 2, 3, 4 and 9 are negative samples for 16S rRNA (532bp). Lanes 5-14 except 9 are *H. pylori* PCR positive results for 16S rRNA (532bp).