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Molecular and Bacterial Study of *Helicobacter pylori* in Gastric Ulcer Patients in Baqubah Teaching Hospital

A Thesis

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Partial Fulfillment of the Requirements for the Master Degree of
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

[نَرْفَعُ دَرَجَاتٍ مِّنْ نَّشَأٍ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ]

صدق الله العظيم

سورة يوسف {76}

Dedication

Σ To the dearest people in my life "father & mother"

For their endless love, support and encouragement.

Σ To the sisters and my husband.

Σ To my friends with whom my life shines brightly.

Σ To everyone who helped and supported me in my study.

NADIA

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Supervisor Certification

We, Certify that this thesis entitled (**Molecular and Bacterial Study of *Helicobacter pylori* in Gastric Ulcer Patients in Baqubah Teaching Hospital**) has been conducted under our supervision at College of Medicine, University of Diyala, as a partial requirements for the Master Degree of Science in Medical Microbiology.

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Summary

Helicobacter pylori is the most common causes of peptic ulcer disease, and it is one of causative agents of vitamin B12 deficiency. The infection by *H. pylori* transmitted by oral-oral, fecal-oral and gastric oral routs. The prevalence of this bacteria depend on age, sex, smoking or nonsmoking and also chronic disease such as diabetes.

The present study was conducted to determine the *H. pylori* infection and it is associated with cobalamin deficiency in patients with gastric ulcers which were diagnosed by using five different tests including, bacterial culture, rapid diagnostic test, rapid urease test, histopathology test and molecular test by detection 16SrRNA and some virulence factors like (UreA and CagA).

The study sample was 200, 127 (63.5%) males and 73 (36.5%) females age ranged from (10 to \geq 60) years were collected from Baqubah teaching hospitals, during the period from September 2018 till January 2019. From 200 individual 110 considered patients after initial diagnosis by endoscopic unit (presence symptoms) and 90 were considered control group (absence symptoms). Two types of samples were collected from each individual including gastric biopsy specimens for culture test, rapid urease test, histopathology test and polymerase chain reaction (PCR) to detect *H. pylori* infection, and blood samples were used for rapid diagnostic test to detect IgG antibodies of *H. pylori* and ELISA test to detect vitamin B12 deficiency.

The results showed that the presence of *H. pylori* in 3 patients was positive for bacterial culture, from these patients one patient appeared resistant to the antibiotic (amoxicillin, clarithromycin, tetracycline, metronidazole, ciprofloxacin, levofloxacin) but two patients showed resistance to some of these antibiotics used and sensitive to others.

In rapid diagnostic test the positive results was (109) (99.1 %), In rapid urease test the positive result from 110 patients was (86) (78.2 %), the positive results of histopathology test was 10 (9.0%), in molecular test genomic DNA was also extracted from gastric biopsies of all 200 individuals and used directly for PCR to detect *H. pylori* using 515 bp domain of (16SrRNA gene), which showed positive in 106 (96.4%) patients, and in virulence genes (UreA and CagA), the positive result of UreA 81 (73.60%) and CagA was 20 (18.20%). In addition, the extracted DNA of biopsies samples were sent for sequencing to identify the *H. pylori* strain. The result appeared that the strain of *H. pylori* that cause gastric ulcer in Baqubah city is *H. pylori* F211 which have the point mutation in 16SrRNA in cancer patients.

The incidence of infection in male was more than in female as the percentage was (70.00%) and (30.00%), respectively. Minimum age was 10 years and maximum was ≥ 60 . The highest age specific frequency in the individuals is in the age group was (40-49)&(50-59) years old. The incidence of *H. pylori* in smoking patients was 34 (30.90%), while it was 76 (69.10%) among non-smokers. In chronic diseases like diabetes the rate of infection was 59 (54.10%), hypertension in patients was percentage (1.80%), patients suffering from diabetes and hypertension was percentage (15.60%), patient with asthma and allergy (1.80%). While the rate of people who did not suffer from chronic diseases and those infected with *H. pylori* was (26.60%). The association between vitamin B12 and *H. pylori* showed that people infected with this bacterium suffered from vitamin B12 deficiency. The results showed that 110 patients with *H. pylori* infection have B12 deficiency.

The present study concluded that the PCR technique is the best method for the detection of these bacteria directly from gastric biopsy specimens, and vitamin B12 deficiency occur in patients infected with *H. pylori*.

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List of Abbreviations

Abbreviate	Key
Abs	Antibodies
Ag	Antigen
CagA	Cytotoxin associated gene activity
BabA	Blood group antigen binding Adhesion
EDTA	Ethyle Dimethyl Tetra Acitic acid
PCR	Polymerase Chain Reaction
OMP	Outer Membrane Protein
MALT	Mucosa-Associated Lymphoid Tissue
LPS	Lipoplysaccharide
P.P.I	Proton Pump Inhibitor
pH	Potential hydrogen
rRNA	ribosomal Ribonucleic acid
S	Svedberg
UBT	Urea Breath Test
VacA	Vacuolating cytotoxin activity
WHO	World Health Organization
If	Intrinsic factor
ELISA	Enzyme linked immunosorbent assay
DEHP	Di (2-ethylhexyl) phthalate

Chapter One

Introduction

1.1 Introduction

Helicobacter pylori is a gram-negative bacterium and spiral in shape, which colonizes the human stomach mucoid lining (Smyk *et al.*, 2014). It is characterized by polymorphism phenomenon and it may appear as coccoid and bacillary form (Mamoun *et al.*, 2015). It is the main cause of stomach and duodenal ulcers, which have become common in recent times due to the spread this type of bacteria, that are highly pathogenic, affect more than half of the world population (Nevine *et al.*, 2015; Mamoun *et al.*, 2015).

The incidence of this bacterium is due to the virulence genes (cag A and vacA) that are carried by particular genetic patterns of *H. pylori*, which are the most important virulence genes accompanying stomach and bowel disease (Salimzadeh *et al.*, 2015; Wang *et al.*, 2015).

To avoid the harsh condition in the gastric lumen, this bacterium has developed resistance to the stomach acid through colonization in a very narrow place of gastric lactation and secretion of the urease which break down urea located in the medium to ammonia which have the effect of the acidic acid around in the stomach lining which enables them to stay in the human stomach lifelong if not treated with antibiotics (Bakir *et al.*, 2012). Therefore, this bacterium causes many diseases such as chronic gastritis, gastric ulcers, duodenal ulcers, gastric cancer and mucosa-associated lymphoid-tissue lymphoma (Erzooki *et al.*, 2016).

The transmission of *H. pylori* may occur via oral-oral, fecal-oral, gastric-oral or iatrogenic routes (Song *et al.*, 2000). The prevalence of *H. pylori* infection varies widely according to the age, sex, race and ethnicity (Jackson *et al.*, 2009).

A number of studies have shown the highest rates of infection are associated with low socioeconomic status, family size, crowding, low level of education, poor sanitation and uncleanly water supplies (Al-Sulami *et al.*, 2012).

Diagnosis of *H. pylori* infection can be made by using several invasive or non-invasive techniques. Invasive diagnostic assays include: rapid urease test, histological examination, culture and polymerase chain reaction (PCR). Non-invasive diagnostic assays include serology tests, urea breath test and stool antigen (Behnam *et al.*, 2015).

These bacteria are characterized as being fastidious, they appear very poorly in the tissue, making it difficult to develop and considered a slow micro-organism and therefore proposes to be diagnosed directly from clinical models by using molecular techniques like (PCR) which is unique in its sensitivity and high specificity in diagnosis and accuracy in determining both the presence of infection and the genotype for these bacteria (Abu-Sbeih *et al.*, 2014).

Some studies have shown that culture assay is used as a gold standard to detect patients with active *H. pylori* infection in clinical samples (Al-Jobori *et al.*, 2011; Ramis *et al.*, 2012). A study in Iraq by Bakir *et al.* that collected 92 gastric biopsy samples, 25 *H. pylori* negative and 67 *H. pylori* positive patients. In *H. pylori* positive group, the positive rates of *H. pylori* DNA extraction in the gastric epithelial cells were increased in chronic superficial gastritis, precancerous changes and gastric cancer groups ($P > 0.01$) (Bakir *et al.*, 2012). In another study in Thi-Qar, were collected from 70 patients (35 male and 35 female). The results showed that there is a significant increasing ($p \leq 0.05$) in *H. pylori* in males (71.43 %) when compare with females patient

(24.59%) and negative result in males patient (28.57%) while the negative result in females patient are (45.71%) (Hussein *et al.*, 2015).

The detection of (CagA) in positive *H. pylori* is higher in patients who had gastric cancer compare to those with chronic superficial gastritis and atrophic gastritis ($P < 0.01$) (Bakir *et al.*, 2012). *Helicobacter pylori*-specific region of 16SrRNA sequence is high conserve among most *H. pylori* strains and allow specific detection and identification of this bacterium in biological specimens (Liu *et al.*, 2008).

H. pylori infection has a strong association with chronic infection of the stomach, that lead to impairment in the gastric acid and pepsin production, which lead to impair absorption of food and vitamin B12. Therefore, this bacterium may cause a cobalamin deficiency and it is a risk factor for gastritis ulcers (Kadhim *et al.*, 2015).

1.2 Aims of the study

The main aims of this study are to:

1- Isolation and identification of *H. pylori* from biopsies samples of patients suffering from gastric ulcer and determine the antibiotic resistance of *H. pylori*.

2- Detection of *H. pylori* by using:

- A) Rapid diagnostic test in serum patients.
- B) Rapid urease test and histopathology test in gastric biopsies samples.
- C) PCR of 16SrRNA.

3- Finding the correlation with different parameters such as gender, age, smoking, chronic disease and cancer.

4- Evaluate the rate of gastric ulcer infection with *H. pylori* and its relation with vitamin B12 deficiency.