

Detection of Helicobacter Species in liver cirrhosis with and without Hepatitis (C) Virus Infection

Thesis

**Submitted for Partial Fulfillment of the MD Degree
In Clinical and Chemical Pathology**

By

Fatma Alzahraa Mohammed Hassan

(M.B., B. Ch, Clinical and Chemical Pathology)
Faculty of Medicine – Ain Shams University

Supervised by

Professor/ Manal Abd Elalim Abd Elsattar

Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Professor/ Mohamed Abd Elmaboud Mohamed

Professor of Internal Medicine
Faculty of Medicine – Ain Shams University

Doctor/ Hala Badr El Din Ali Othman

Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Doctor/ Hala Mahmoud Hafez

Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

**Faculty of Medicine
Ain Shams University**

2015

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(... رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ

الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ ☐

وَأَنْ أَعْمَلَ صَالِحاً تَرْضَاهُ ☐

وَأَذْخُلَنِي بِرَحْمَتِكَ فِيهِ ☐

☐ عِبَادَتِكَ الصَّالِحِينَ]

صدق الله العظيم

النمل.. آية رقم ١٩



Acknowledgement

First of all I wish to express my thanks to **ALLAH**, to the Most Merciful and the Most Grateful for His generous care throughout my life.

I cannot find enough words to express my deep feelings towards my supervisors for their great helps and guidance in producing this thesis.

My gratitude and deep appreciation to **Professor/ Manal Abd Elalim Abd Elsattar**, Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University for her guidance, valuable advice, continuous encouragement and close supervision. I have the honor to complete this work under her supervision.

I would also to extend my thanks and gratitude to **Professor/ Mohamed Abd Elmaboud Mohamed**, Professor of Internal Medicine, Faculty of Medicine – Ain Shams University, for his great efforts and help during the whole work; no words can express my deep gratefulness.

I am deeply grateful to **Doctor/ Hala Badr El Din Ali Othman**, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University for her great help and encouragement during this work,

I would like to express my sincere thanks and highest appreciation to **Doctor/ Hala Mahmoud Hafez**, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University, for her support, wise advice and precious time she had offered me.

Lastly I would like to express my thanks to everyone in the Medical Research Centre who helped me during work and my deep thanks to Doctor **Mohammed Sharaf Eldeen (lecturer)** of Molecular Biology, Medical Research Centre, Ain Shams University, for his great help during this work,



List of Contents

<i>Subject</i>	<i>Page No.</i>
List of Abbreviations	i
List of Tables	ii
List of Figures	iii
Abstract	
Introduction	1
Aim of the Work	4
Review of Literature	
Helicobacter.....	5
Helicobacter Species and Liver Diseases.....	29
Diagnosis of Helicobacter Species in Liver Disease.....	36
Treatment of Helicobacter infection.....	59
Prevention and vaccination.....	62
Patients and Methods	64
Results	80
Discussion	92
Conclusion	101
Recommendations	102
Summary	101
References	106
Arabic Summary	—

List of Abbreviations

AFP	: Alpha fetoprotein
Cdt	: Cytolethal Distending Toxin
CLO	: Campylobacter-like organism test
CLSI	: Clinical and laboratory standard institute
CPI	: Cag Pathogenicity Island
EGF	: Epidermal growth factor
EGFR	: Epidermal Growth Factor Receptor
EIA	: Enzyme Immuno Assay
HBV	: Hepatitis B virus
HCV	: Hepatitis C virus
HCC	: Hepatocellular carcinoma
HIV	: Human immunodeficiency virus
INF-γ	: Gamma interferon
ITP	: Idiopathic thrombocytopenic purpura
MALT	: Mucosa associated lymphoid tissue
MIC	: Minimal inhibitory concentration
MMP	: Matrix Metallo Proteinases
PCR	: Polymerase Chain reaction
PUD	: Peptic ulcer disease
RFLP	: Restriction Fragment Length Polymorphism
RUT	: Rapid urease test
TK	: Tyrosine Kinase
UBT	: Urea breath test

List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (1):	Habitats and phenotypic characteristics of <i>Helicobacter</i> species (a)	18
Table (2):	Virulence factors identified in <i>Helicobacter</i> species	28
Table (3):	Genes involved by point mutation or other genetic events leading to antibiotic resistance in <i>Helicobacter</i>	60
Table (4):	The grade of activity in H&E sections:	65
Table (5):	Components of PCR mixture in a final volume of 50 μ L	72
Table (6):	PCR-RFLP pattern of <i>Helicobacter</i> isolates	77
Table (7):	Descriptive data of all patients.	81
Table (8):	Data of patients in relation to liver cirrhosis activity.	82
Table (9):	Sex distribution in relation to severity of cirrhosis.....	83
Table (10):	<i>Helicobacter</i> species identified.	84
Table (11):	Distribution of <i>Helicobacter</i> infection in relation to HCV status.	85
Table (12):	Association between <i>Helicobacter</i> infection and different age groups.	85
Table (13):	Association between <i>Helicobacter</i> infection and gender.....	86
Table (14):	Association between <i>Helicobacter</i> infection and HCV infection.....	87
Table (15):	Distribution of <i>Helicobacter</i> infection and severity of liver disease	88

List of Tables (Cont.)

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
Table (16):	Association between HCV infection and severity of cirrhosis.....	89
Table (17):	Relation between combined infection and severity of cirrhosis.....	90
Table (8):	Relation between <i>H.pylori</i> infection and severity of liver cirrhosis.	91
Table (19):	Relation between identified <i>Helicobacter</i> species and severity of cirrhosis.	92

List of Figures

<i>Figure No.</i>	<i>Title</i>	<i>Page</i>
Figure (1):	Spiral shaped bacteria in vicinity of pit cells lineage	12
Figure (2):	Cocci in adjacent hepatocytes by EM	12
Figure (3):	<i>H.pylori</i> morphology.....	13
Figure (4):	Morphology of Helicobacters	13
Figure (5):	Enterohepatic Helicobacter species.....	14
Figure (6):	Transmission electron micrographs of Helicobacter sp.	15
Figure (7):	<i>H.pylori</i> gram stain	40
Figure (8):	<i>H.pylori</i> in gastric biopsys smear after Giemsa staining.....	40
Figure (9):	<i>H.pylori</i> observed on a gastric biopsy smear after acridine orange staining.....	41
Figure (10):	<i>H.heilmanni</i> observed on a gastric biopsy histological preparation (H&E) staining	42
Figure (11):	Immunohistochemical staining with anti-Helicobacter pylori specific antibodies	43
Figure (12):	Small grey translucent colonies on blood agar media (<i>H.pylori</i>).....	46
Figure (13):	Helicobacter on blood agar plate	47
Figure (14):	METAVIR Algorithm for Evaluation of Histolgoical Activity	65
Figure (15):	Helicobacter infection and liver cirrhosis activity.	84
Figure (16):	Association between Helicobacter infection and gender.....	86

List of Figures (Cont.)

<i>Figure No.</i>	<i>Title</i>	<i>Page</i>
Figure (17):	Association between Helicobacter infection and HCV infection	87
Figure (18):	Distribution of Helicobacter infection and liver cirrhosis activity.....	88

Introduction

Hepatocellular carcinoma (HCC) was the fourth cause of cancer death worldwide (*Moushira et al., 2011*). This tumour often follows chronic inflammation and long standing cirrhosis (*Pellicano et al., 2004*).

Cirrhosis of the liver is a diffuse process, characterized by fibrosis and nodule formation, which stems from hepatocellular injury. The cellular necrosis might originate from viral, toxic, metabolic and autoimmune sources. There are extensive data indicating that chronic infection can lead to cancer in various organs. Parasites such as schistosomes and liver flukes, and a bacterium, *Helicobacter pylori*, were classified as type I liver carcinogens by the International Agency for Research on Cancer in 1997. The most competing evidence comes from hepatocellular carcinoma (HCC) where chronic hepatitis B virus (HBV) infection is considered to be direct etiological factor and hepatitis C virus (HCV) infection a major risk factor for this disease (*Pellicano et al., 2004; Rocha et al., 2005*).

Chronic inflammation perse is known to be a factor of progression towards cancer (*Cassel, 1998*), though HCV itself does not appear to foster a strong inflammatory mechanism. It is thus not yet clear what factor most influence the outcome of the

liver disease. Searching for other noxae has therefore become mandatory (**Mosnier et al., 1994**).

Helicobacter infections have been implicated in the occurrence of certain liver diseases in some animal species such as *Helicobacter canis* in dogs, *Helicobacter hepaticus* and *Helicobacter bilis* in mice. In humans, *Helicobacter pylori* DNA has been detected in the liver of patients suffering from cholestatic diseases and HCC arising from non cirrhotic liver (**Pellicano et al., 2004; Shi et al., 2006**).

A study suggested that, presence of *Helicobacter* species in liver samples could possibly serves as a cofactor in the development of end-stage of liver diseases in humans. These concern have spurred considerable interest in determining the mechanism by which these extracellular bacteria and the associated inflammatory response endorse hepatic and biliary disease (**Santosh et al., 2006**).

Studies showed a high frequency of *H.pylori* and *H.pullorum* detected by PCR in the liver of patients with cirrhosis and superimposed HCC with and without associated HCV infection, suggesting that, these bacteria might play an important role in the progression of chronic HCV patients to cirrhosis and HCC (**Rocha et al., 2005**).

The mechanism by which *H. pylori* colonizes the human liver is not totally enlightened. The *H. pylori* DNA detected in the liver tissue may result from bacterial translocation from the stomach into the blood through the portal system, especially in the later stage of chronic liver disease when portal hypertension occurs. In addition, the bacteria may reach the liver by phagocytes and macrophages or circulating retrograde transfer from the duodenum. Several researchers have suggested that *H. pylori* may damage hepatocyte by a cytopathic effect (*Jose et al., 2014*).

Aim of the Work

The aim of the present study is to determine the presence of *Helicobacter* species in the liver of cirrhotic patients, with or without HCV infection.

Helicobacter

Historical Background:

Gastric spiral shaped bacteria have been observed in animals and humans for more than 100 years. The first recorded observation of gastric spiral-shaped bacteria in animals was made by Rappin in 1881 and Bizzozero in 1893. The first observation in human was made by Krienitz in 1906 (*Fox and Megraud, 2007*).

In 1982, *Campylobacter pyloridis* (later known as *Helicobacter pylori*) was successfully cultured from stomach biopsy specimens from human patients with gastritis. Subsequently, other spiral gram-negative bacteria have been observed in and isolated from the gastrointestinal tract of mammals such as cats, dogs and rodents (*Fox et al., 2000*).

Taxonomy:

Initially, many spiral gram-negative bacteria isolated from the mammalian gastrointestinal tract were grouped as *Campylobacters*. This was based on similar microscopic and ultrastructural morphologies, common microaerobic growth requirements, and similar ecologic niches. However, following extensive analysis of enzymatic activities, fatty acid profiles, 16S rRNA sequence analysis, nucleic acid hybridization and 23S rRNA analysis, the genus *Helicobacter* was formally distinguished from *Campylobacter* (*Megraud et al., 2012*). The *Helicobacter* genus belongs to class Epsilonproteobacteria, order

campylobacterales, and Helicobacteraceae family, and already involve more than 35 species. *Helicobacter Pylori* is of primary importance for medicine, however, non-*pylori* Helicobacter species have been detected in human clinical specimens and showing different organ specificity (**Boyanova, 2014**).

Clinically Significant Helicobacters:

A- Enterohepatic Helicobacter species:

These Helicobacter have been identified in liver specimens from patients with primary sclerosing cholangitis, cholangiocarcinoma, liver cirrhosis, and hepatocellular carcinoma associated with hepatitis C virus infection by genus specific PCR amplification and partial DNA sequencing (**Fox and Megraud, 2007**). It has been suggested that the presence of these Helicobacter species in liver samples could serve as a co-factor in the development of end stage of liver disease (**Pellicano et al., 2008**).

1. Helicobacter hepaticus (H.hepaticus):

It was isolated from liver, cecal and colonic mucosa of mice with chronic active hepatitis (**Rocha et al., 2005**).

H.hepaticus known to cause chronic active hepatitis and is associated with multifocal necrotic hepatitis in many strains of mice, and is responsible for the development of hepatic adenoma and hepatocellular carcinoma in mice (**Santosh et al., 2006**). This organism has been isolated from human and has