DETECTION OF HELICOBACTER PYLORI INFECTION IN PATIENTS WITH UPPER GASTRO INTESTINAL SYMPTOMS 63887

THESIS

Submitted for Partial Fulfillment of

The M.D. Degree in BACTERIOLOGY WIND WIND

1997/10/01 WW

Presented by Maha M. Fathy M.

M.B., B.Ch., M, S&

Under supervision of Prof. Dr. Abla Abd El Salam Haroun

Professor of Microbiology and Immunology

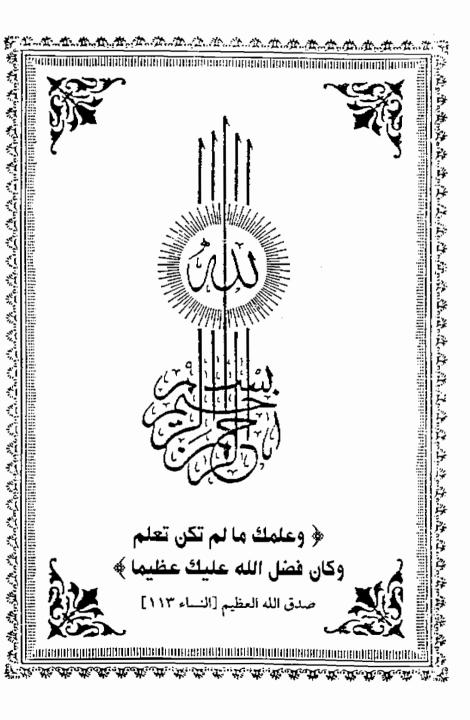
Dr. Narges Mohamed Ismail Elaish

Ass. Prof. of Microbiology and Immunology

Dr. Mohamed Reda Mahmoud El Wakeil Ass. Prof. of Tropical Medicine

Dr. Taghreed Hamed T. El Khashaab Ass. Prof. of Microbiology and Immunology







Acknowledgments

The author would like to express gratitude to her major advisor **Professor Dr. Abla Abd El-Salam Haroun**, Professor of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her helpful guidance and instructive supervision in revising the whole study.

Special thanks are due to **Dr. Narges Mohamed Ismail Elaish,** Ass. Prof. of
Microbiology and Immunology, Faculty of
Medicine, Ain Shams University, for her
valuable help, precious advise and guidance
throughout this study.

Deep thanks are due to **Dr. Taghreed Hamed T. El Khashaab,** Ass. Prof. of
Microbiology and Immunology, Faculty of
Medicine, Ain Shams University, for her
close supervision, unfailing tender advise
and continuous encouragement throughout
the whole study.

Sincere appreciations are due to **Dr. Mohamed Reda Mahmoud El-Wakeil,** Ass.
Prof. of Tropical Medicine, Faculty of
Medicine, Ain Shams University, for his
valuable advise and facilities offered
throughout this work.

Last but not least, the author would like to pay thanks to staff members of the Microbiology and Immunology Department, Tropical Medicine Department, Ain Shams, Faculty of Medicine and staff members of the Immunogenetics and Transplantation laboratory, Ain Shams Specialized Hospital.

ABSTRACT

This study was done on 90 dyspeptic patients attending the upper gastrointestinal endoscopy unit, tropical medicine department, Ain Shams University Hospital from October, 1994 till January 1996. Antral gastric biopsy specimen was taken from each patients gastrointestinal endoscopy in which detected using culture H.pulori organism was technique, Gram staining and rapid urease test (R.U.T). Also H.pylori specific IgG antibodies were detected in sera of the same patients using ELISA technique. The rate of detection of H.pylori in antral gastric biopsy specimens was 71.1% while H.pylori specific IgG antibodies were detected from 80% of sera of the patients.

The comparison between the different techniques revealed that the Gram staining had the highest performance for diagnosis of *H.pylori* infection (100% efficacy), then R.U.T (79% efficacy) culture (76% efficacy) and ELISA (67% efficacy).

The comparison between *H.pylori* positive patients and negative ones revealed no specific association between *H.pylori* and any of the presenting complaints or the endoscopic findings of the 90 patients.

CONTENTS

Part		Page
I	Introduction and Aim of Work	1
П	Review of Literature:	3
	A- Historical background	3
	B- Bacteriology	5
	1- Morphology	5
	2- Cultural characters	7
	3- Biochemical activity	7
	4- Classification	7
	C- Association of H.pylori with diseases	12
	1- Chronic gastritis	12
	2- Acute gastritis and hypochlorhydria	15
	3- Malabsorption syndromes	18
	4- Functional (or non ulcer) dyspepsia	18
	5- Peptic ulcer disease	19
	6- Gastric malignancies	20
	7- Effect of H.pylori outside the stomach	22
	D- Epidemiology	23
	1- Genetic and Environmental influences	23
	2- Prevalence by age and sex	24
	3- Ethnic differences	26
	4- Socio economic differences	27
	5- Made of transmission	28

(Continue)

	E-Virulence factors and Pathogenic mechanisms	33
	1- Spiral shape and motility	33
	2- Adaptive enzymes	34
	3- H.pylori adhesins and receptors	36
	4- The vacuolating cytotoxin and the related	
	molecules	37
	5- Toxic enzymes	41
	6- The inflammatory process	43
	7- Acid and Pepsin	48
	F- Antigenic properties and Immune response	51
	G- Diagnosis of H.pylori	57
	1- Microscopy	57
	2- Cultrure	59
	3- Urease based tests	63
	4- Serology	66
	5- DNA-Based techniques	70
	H- Treatment of H.pylori infection	72
	I- Towards a H.pylori vaccine	77
Ш	Materials and Methods	79
IV	Results	91
\mathbf{v}	Discussion	122
VI	Summary, Conclusion and Recommendations	144
VII	References	149
VIII	Arabic summary	

LIST OF TABLES

No.	Title	Page
1	Differential characteristics of Campylobacters and	9
	related taxa	
2	Clinical data of 90 dyspeptic patients	92
3	The number and percentage of different endoscopic	95
4	findings among the 90 dyspeptic patients	100
4	The number and percentage of <i>H.pylori</i> positive cases detected by different techniques among the 90	103
_	dyspeptic patients	101
5	Comparison between the number and percentage of <i>H.pylori</i> positive and <i>H.pylori</i> negative gastric biopsy specimens diagnosed by culture and Gram staining.	106
6	Comparison between the number and percentage of <i>H.pylori</i> positive and <i>H.pylori</i> negative gastric biopsy specimens diagnosed by culture and rapid urease test.	108
7	Definition of <i>H.pylori</i> status of the 90 dyspeptic patients	111
8	The performance of different techniques for diagnosis of <i>H. pylori</i> .	113
9	Sensitivity and specificity of ELISA technique in two age groups	115
10	Clinical data of <i>H.pylori</i> positive patients and <i>H.pylori</i> negative ones	117
11	H.pylori status in relation to the presence or absence of pathological endoscopic findings	118
12	The number and percentage of different endoscopic findings in the <i>H.pylori</i> positive and <i>H.pylori</i> negative patients	120

LIST OF FIGURES

No.	Title	Page
1	H.pylori from a 3-day chocolate agar culture	6
2	Section of antral mucosa showing gastric pit colonized with <i>H.pylori</i> - Warthin-Starry silver stain	6
3	The Sydney system for classification of gastritis	13
4	Association of <i>H.pylori</i> with various pathologic lesions of the upper gastrointestinal tract	15
5	The rate of different complaints among the 90 dyspeptic patients	93
6	The rae of different endoscopic findings among the 90 dyspeptic pateints	96
7	The rate of commonest endoscopic findings among the 90 dyspeptic patients	97
8	A Gram stained film prepared from antral biopsy containing the characteristic <i>H.pylori</i> like organisms	99
9	H.pylori colonies on selective Colombia agar plate	100
10	A Gram stained film prepared from the suspected H.pylori colony	101
11	The positive and negative rapid urease test	102
12	The rate of <i>H.pylori</i> positive patiets detected by different techniques among the 90 dyspeptic patients	104
13	The number and percentage of <i>H.pylori</i> positive and <i>H.pylori</i> negative gastric biopsy specimens diagnosed by culture and Gram staining	107
14	The number and percentage of <i>H.pylori</i> positive and <i>H.pylori</i> negative gastric biopsy specimens diagnosed by culture and R.U.T.	109
15	The performance of different techniques in diagnosis of <i>H.pylori</i> infection	114
16	The rate of <i>H.pylori</i> positive and <i>H.pylori</i> negative patients in different endoscopic findings.	121

I INTRODUCTION & AIM OF WORK

I- INTRODUCTION AND AIM OF WORK

Spiral organisms were first observed in the gastric mucosa by Bizzozero in 1893, almost a century later a spiral bacterium was isolated from the stomach of a patient with peptic ulcer disease (Warren and Marshall, 1983).

The bacterium was initially named Campylobacter renamed Helicobacter pylori puloridis and (H.pylori), few years later (Goodwin et al., 1989).

- Helicobacter pylori is a small spiral microaerophilic negative bacterium that colonizes the gram human upper gastrointestinal tract, primarily the stomach (Warren and Marshall, 1983). It colonizes most of the human population (Megraud, 1993). In developing countries 80% of the population are infected by the bacterium by the age of 20, while in developed countries H.pulori infection increases with the age from <20% in 30 years old people to >50% in 60 years old (Taylor and Blaser, 1991).
- Transmission of H.pylort is likely to occur by the faecal-oral route and to be more common in infants (Fiedorek et al., 1991). Infection causes a transient acute gastritis, while chronic disease occurs after a chronic infection for a period of years to decades (Morris et al., 1991).

- H.pylorl is recognised as a significant □ Now gastrointestinal pathogen for its etiologic role in non autoimmune type B gastritis, peptic ulcer disease (Peterson, 1991) and mucosa associated lymphoid tissue gastric lymphoma (Hussell et al., 1993). Additionally there is an epidemiological association with gastric carcinoma (Eurogast study group, 1993).
- ☐ The recent discovery of the infectious nature of peptic ulcer has changed its therapeutic approach using anti-H2 blockers which relieve the symptoms to antibiotic treatment to eradicate the al., 1992). Recently bacterium (Graham et immunization and immunotherapy have been investigated as a means of prevention and treatment of infection with H.pylori (Blanchard et al., 1995).
- The aim of this work is to detect the association of H.pylori infection with different upper gastrointestinal troubles and to compare different techniques for diagnosis of such infection.

II REVIEW OF LITERATURE

retrospective study of endoscopic gastric biopsy specimens described spiral bacteria in association with chronic superficial and chronic atrophic gastritis. Steer (1984) in a morphologic study of gastroduodenal mucosa in peptic ulceration described the scanning electron-microscopic appearance of bacteria in patients with ulcer-associated chronic gastritis. The organism thus identified independently by these three groups of workers was initially called Campylobacter-like organism and subsequently Campylobacter pyloridis, a grammatically incorrect name later changed to Campylobacter pylori (Marshall and goodwin, 1987).

It became increasingly clear that many of the blochemical and ultrastructural characteristics of this organism were dissimilar to these of other Campylobacter organisms, so it was assigned to the new genus Helicobacter (Goodwin et al., 1989).

B- Bacteriology

1- Morphology (Fig. 1,2)

In the stomach, *H.pylort* take the form of short spiral or S-shaped gram negative rods, 0.5-0.9µm wide and 3µm long with a wavelength of about 2.6µm. On agar cultures spiral forms are less obvious and cells appear more as singly curved rods; morphology is more typical in broth cultures.

Flagellar stains show a lophotrichate configuration with up to 7 flagella at one pole, but as detached flagella are always plentiful in smears, many cells are seen with fewer flagella. Electronmicroscopy shows flagella to be sheathed, the sheath being in the continuity with the unit membrane of the outer cell wall; they are about 30nm in diameter with a filament of 12-15nm. Some flagella have terminal bulbs. The end of the cells are truncated without a terminal concavity and the flagella are inserted through a large (90nm) disc which does not have the radial structure of the discs of C.fetus or C.jejun. H.pylori undergoes coccal transformation on exposure to air within 1-2 hours at room temperature and in this state it fails to grow on subculture (Skirrow, 1990a).