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Botany Department

Study on *Helicobacter pylori* Infection Using Circulating Antigens Detection

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Abstract

H.pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that infects the human gastric mucosa. *Helicobacter pylori* is one of the most common infective agents worldwide. It is an etiological agent of gastritis, peptic, and duodenal ulcer disease, and infection with this organism is a recognized risk factor in the development of gastric mucosa associated lymphoid tissue lymphoma and adenocarcinoma In particular, this organism has been categorized as a class1 carcinogen by the World Health Organization.

The diagnosis of *H. pylori* gastric infection can be conducted by using direct (invasive) or indirect (non-invasive) methods. Among the indirect methods, serology is a valuable tool for sero-epidemiological studies. As a result detection of circulating *H. pylori* antigens may be of interest to be included in diagnosis of *H. pylori* infection.

The assay detected *H. pylori* antigen in serum samples of 83 infected patients with a sensitivity of 92%. Serum samples of 50 non-infected individuals were used to evaluate the specificity of the ELISA. The assay showed that 46 non-infected individuals were negative by the ELISA, and this revealed 92% specificity. Statistical characteristics of ELISA as a sensitive and simple diagnostic assay of *H. pylori* based on HpCA detection in serum compared with standard culture revealed, positive predictive value of 95%; negative predictive value of 86 %;and efficiency 92 %. The diagnostic value of HpCA assessed by the area under the ROC curve. The area under ROC curve of HpCA for discriminating infected patients from those non-infected and (p value) were 0.982 ($P<0.0001$).

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Nouran Saber El-Melegy
2009

Dedication

This work is dedicated to

My Father,My Mother,My Sister,My son

And

To my dear husband

Dr-Magdy

Nouran El-melegy

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	with *H.pylori* infection	

List of abbreviations	
BabA	Blood group antigen binding adhesin
BCIP	Bromo Chloro Indolyl phosphate
BSA	Bovin Serum Albumin
C.pylori	*Campylobacter pylori*
Cag	Cytotoxin assocated protein
Cag A	Cytotoxin associated gene A
CLO	*Campylobacter* like organism
DNA	Deoxyribonucleic acid
DU	Duodenal ulcer
DU	Duodenal ulcer
EIA	Enzyme Immuno Assay
ELISA	Enzyme Linked Immunosorbent Assay
e-UBT	Endoscpic urea breath test
FMIA	Flow microparticle immunoflorescence assay
GERD	Gastro – oesophageal reflux disease
H&E	Hematoxyline and eosin
HPCA	*Helicobacter pylori* circulating antigen
IgG	Immunoglobulin G.
kDa	KiloDalton.
LPS	Lipopolysaccharide
MALT	Mucosa-associated lymphoid tissue.
MW	Molecular weight

List of abbreviations (continue)	
NASID	Nonsteroidal anti-inflammatory drugs
NBT	Nitro blue tetrazolium
NC	Nitrocellulose
ODs	Optical density
PBS	Phosphate buffer saline
PBS–T20	Phosphate buffer saline tween 20
PCR	Polymerase chain reaction
PMN	Polymorphnuclear cells
P-NPP	P-Nitro Phenyl Phosphate
PPI	Proton pump inhibitor
R_f	Relative Mobility
RNA	Ribonucleic acid
RT	Room Temperature
RUT	Rapid Urease Test
SDS. PAGE	Sodium dodecylsulfate polyacrylamide gel Electrophoresis
TBS	Tris buffer saline
TEMED	Tetramethylene Diamine
UBT	Urea breath test
Vac A	Vacuolating cytotoxin A.
WS	Warthin-starry staining

INTRODUCTION

Helicobacter pylori is a common bacterial infection in humans that is responsible for a variety of gastroduodenal pathologies, peptic and gastric ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric carcinoma **(Kabir 2003**). Several tests can be used to diagnose *H. pylori* infection; the selection of the appropriate test depends on the clinical setting **(Howde et al., 199^) .**

H. pylori infection can be diagnosed by tests requiring upper gastrointestinal endoscopy for the retrieval of a gastric biopsy specimen (microbiological culture, histological examination, and rapid urease tests). These methods have high sensitivities and specificities **(Graham et al., 2001),** yet the invasiveness and expense of direct observation of the organism have led to a search for valid and reliable noninvasive alternatives **(Westblom et al., 1999).** Non-invasive diagnostic tests for *H. pylori* infection have gained in significance**.** Although PCR, a powerful method known for its high sensitivity, can detect low numbers of *H. pylori* and has been used to follow up eradication therapy, PCR requires specialized laboratory facilities and is not generally available as a routine diagnostic test **(Kabir., 2001).**

The urea breath test has been the most widely used accurate noninvasive test, both in the pre-treatment examination of infected individuals and for early-post-treatment follow-up, and meets the requirements for such a test **(Gatta et al., 2003).** However, the performance of the test has been associated with some disadvantages. Although it is less costly than endoscopy, the urea breath test requires a specialized technician and expensive instrumentation that is not available in routine clinical