# Urea Breath Test For Detection Of Helicobacter Pylori In Egyptian Childeren

Thesis
submitted in partial fulfillment of MD degree in pediatrices

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# *ABSTRACT*

This study included (125) children selected from outpatient clinic of New Children Hospital, Cairo University. Their ages ranged from 3.5 to 10 years old. The main presentations were complaint of recurrent abdominal pain (84%), failure to thrive (1.6%) , persistent vomiting (0.8%) or combinations of them.The children underwent:

-Clinical study including history and examination.

-Urea breath test (UBT) for diagnosis of H.pylori.

-Then we proceeded to upper endoscope for antral biopsy for histopathological examination for confirmation of the diagnosis.We excluded from the study children who used antimicrobial therapy or proton pump inhibitors within one month from the study.

The results showed, UBT was positive in 84% (105 case) with a sensitivity of UBT is 100% and specificity is 71.4%. So we can conclude that UBT for establishing the diagnosis of the helicobacter pylori represents a valid strategy.

KEY WORDS

*UBT

*Helicobacter pylori

*The endoscopic biopsy

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# *LIST OF ABBREVIATIONS*

| | | |
|---|---|---|
| **DOB** | | Delta over basel.ine |
| **CDT** | | Cytolethal distending toxin |
| **EHS** | | Enterohepatic helicobacter species |
| **FISH** | | Fluorescent in situ hybridization |
| **FRET** | | Fluorescence Resonance Energy Transfer |
| **GC– MS** | | Gas chromatography–mass spectrometry |
| **H. pylori** | | Helicobacter pylori |
| **HCC** | | Hepatocellular carcinoma |
| **HCV** | | Hepatitis C virus |
| **ICP-MS** | | Inductively coupled plasma mass spectrometry |
| **IL** | | Interleukin |
| **IRMS** | | Isotope ratio mass spectrometer |
| **LCP** | | Long chain polyunsaturated fatty acids |
| **LPS** | | Lipopolysaccharide |
| **MALT** | | Mucosa-associated lymphoid tissue |
| **MM-RAP** | | Multidimensional scale Recurrent abdominal pain |
| **NASPGN** | | North American Society for Pediatric Gastroenterology and Nutrition |
| **PAC** | | PPI, amoxicillin, clarithromycin |
| **PAM** | | PPI, amoxicillin, metronidazole |
| **PCR** | | Polymerase chain reaction |
| **Pip** | | Proton inhibitor pump |

| | | |
|---|---|---|
| **PUD** | | Peptic ulcer disease |
| **RAP** | | Recurrent abdominal pain |
| **RFLP** | | Restriction fragment length polymorphism |
| **SPEM** | | Spasmolytic polypeptide expressing metaplastic |
| **SSCP** | | Single strand conformation polymorphism |
| **TIMS** | | Thermal ionisation mass spectrometry |
| **TLR** | | Toll-like receptor |
| **UBT** | | Urea breath test |
| **VacA** | | Vacuolating cytotoxin A |
| **WK** | | Week |
| **Yr.** | | Year |

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# *LIST OF TABLES*

# INTRODUTION AND AIM OF WORK

Helicobacter pylori is a spiral-shaped, Gram-negative rod and catalase-positive organism **(O'Rourke et al., 2004).**

Helicobacter pylori is the primary cause of gastric and peptic ulcer disease **(Matthews et al., 2005).**This organism has been shown to infect more than 50% of the world's population with the incidence up to 80% in developing countrieS **(Gonzalez et al., 2004).**

Acquisition of H.P at an early stage increases the risk of development of gastric cancer **(Lehmann and Beglinger, 2003).**

Helicobacter pylori can be detected at endoscopy by histology, culture, or urease test. All these biopsy based methods for detection of Helicobacter pylori are liable to sampling error because infection is patchy. In addition after partially effective eradication treatment, low levels of infection can easily be missed by endoscopic biobsy **(Robert and Marjorie, 2001).**

Carbon urea breath test is simple, robust, noninvasive, accurate and inexpensive **(Ather et al., 1994).**

With a sensitivity of 98% and specificity of 97%, the urea breath test is rapidly becoming the test of choice in detection of H.Pylori infection **(Matthews et al., 2005).**

From the above we have seen the importance of detection of Helicobacter pylori infection in childhood as a cause of many diseases even beyond the affection of the gastrointestinal tract, that worst to be investigated using noninvasive method.

So this study was conducted aiming to assess the reliability of Urea Breath Test (UBT) which is noninvasive, simple and cost-effective diagnostic method for the detection of Helicobacter pylori infection in Childhood. Comparing its specificity and sensitivity, against the Known

golden standard invasive diagnostic Endoscopy and Biopsy, as an easy way for screening and long term follow up of cases after therapy.

# *HISTORICAL BACKGROUND*

Spiral organisms had been found at first incidentally in the stomachs of the dogs in 1896 and later on the rats and cats **(Bizzozero, 1893)**. Nearly one decade ago, the pathogenesis of peptic ulcer disease as gastritis, gastric ulcer,duodenitis and duodenal ulcer was attributed to an imbalance between acid secretion and mucosal defense mechanism. In 1983, Marshall and Warren introduced a new pathogenic factor, a microrganism called H. pylori **(Coghlan et al., 1987).** Other reports confirmed these findings noting also the absence of these organisms in healthy persons **(Lunger and Neuberger, 1921)**.

During the next 30 years, there were scattered reports of these organisms being found in the human stomach especially in the patients with peptic ulcers. These reports included the demonstration of spirochetes in necropsy specimens of the stomach **(Doenge, 1938)** and in 13 of 35 gasterctomy specimens **(Freedburg and Barron, 1940).**

However, an extensive histologic study of gastric biopsy results from 1000 subjects by **Palmer (1954)**, failed to confirm the previous reports, although the more specific silver stain was omitted. He, therefore, concluded that others had been describing bacterial contamination of specimens introduced by swallowing or following death.

In 1955, Kornberg and Davies documented the presence of endogenous urease enzyme activity localized to the stomach in many species. Human studies confirmed an association between the presence of the urease enzyme ulcer disease and some patients were successfully treated with urea **(Fung et al., 1989).**

Since 1980, curved and spiral organisms have been observed by Warren in endoscopic biopsy specimens from patients with gastric and

peptic ulceration. The first successful isolation of the organisms was done by Warren at the Royal Perth Hospital in Western Australia in 1982. In 1983, he reported the findings of an unidentified curved bacillus in close contact with the gastric epithelium in biopsy specimens showing active chronic gastritis **(Warren, 1983).**

These bacteria could be cultured from antral biopsy specimens using campylobacter isolation techniques in microaerophilic atmosphere at 37 C. culture results showed organisms with a smooth coat and sheathed flagella arising from one pole of each organism. These microaerophilic bacteria resembled campylobacter by light microscope and were thus named campylobacter pyloridis. **Marshall and Warren (1984)** pointed the direction towards the probable clinical importance of campylobacter pyloridis in the genesis of gastritis and peptic ulcer disease when not associated with malignancy or non-steriodal anti-inflammatory drugs.

In September 1983, a retrospective histological study, in Birmingham, on 42% of endoscopic gastric biopsy specimens showed that these spiral bacteria were, with no doubt, associated with gastritis **(Rollason et al., 1984)**.

In the next three years the organisms had been isolated from patients with gastritis and peptic ulcer in England **(Jones et al., 1984; McNutly and Waston, 1984)**, Holland (**Langenberg et al., 1984**), USA, Canda, Japan and Peru (**Pearson et al., 1985**). However, studies on the ultra structure and fatty acid profile of campylobacter pylori (**Goodwin et al., 1985**) as well as the 16 subunit ribosomal RNA (**16 SRNA**) sequences of these organisms (**Thompson et al., 1988**) and respiratory quinines and groth characteristics clearly revealed that it did not belong to the genus campylobacter and thus because of these unique

characteristics the name of the bacterium was changed from campylobacter pyloriidis to Helicobacter pylori.

By 1988, the Perth's team had sufficient evidence to justify a new genus name "Helicobacter". The new name reflects the helical appearance of these organisms in vivo as well as the most common isolation place i.e. the pylorus (**Goodwin et al., 1989**). **Heatley and Wyatt (1995)** made a support for the name of the bacteria Helicobacter and referred the name to the morphology of the organisms, which are helical in vivo but often rode like in vitro.

The initial assertion by Marshall and Warren that this organisms, was a causative agent of gastritis and peptic ulcer disease was greated by widespread skepticism. Helicobacter pylori is now accepted to be an important etiologic agent for these conditions as well as gastric malignancies **(Cover and Blaser, 1996).**


Fig.(1).

# *MICROBIOLOGY*

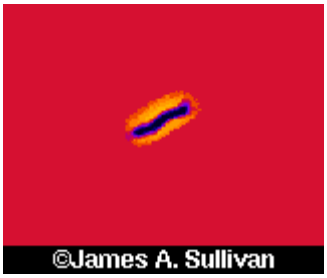


Fig.(2) Helicobacter pylori

Helicobacter pylori is a spiral-shaped, Gram-negative rod approximately 0.5 x 3.0 micrometers in size. The catalase-positive organism has 4-6 sheathed flagella attached to one pole which allow for motility.

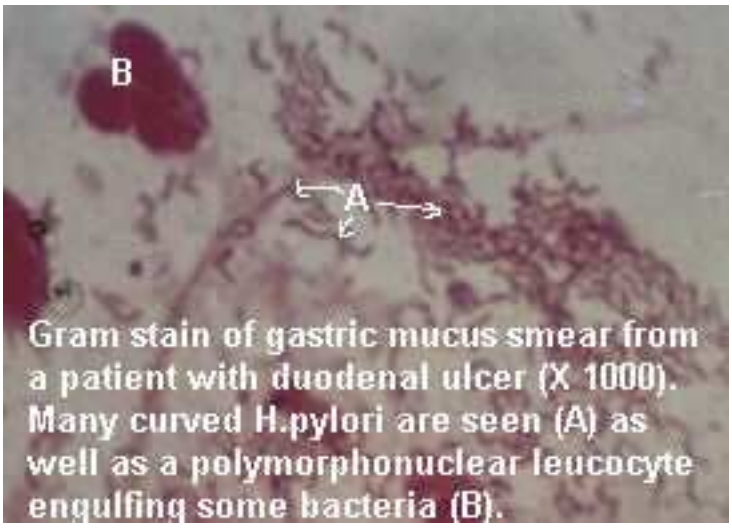


Fig. (3).**Other Helicobacters**

***Identification and Classification***

Human, primate, and pig isolates of the large tightly coiled bacterium “Helicobacter heilmannii” have been characterized by phylogenetic analysis. Fifteen isolates clustered with “Candidatus Helicobacter suis,” whereas 11 strains could not be differentiated from Helicobacter bizzozeronii, Helicobacter felis and Helicobacter salomonis.

Urease gene analysis separated these isolates into the latter three and a fourth distinct cluster containing human and feline isolates. This cluster of isolates was proposed as a unique species with the provisional name "Candidatus Helicobacter heilmannii" **(O'Rourke et al., 2004).**

Helicobacter mastomyrinus was isolated from the liver and cecum of mastomys and from cecum and feces of normal mice. Based on 16S rDNA and phenotypic traits, the bacterium was closely related to "Helicobacter muricola;" it expressed urease and cytolethal distending toxin, and caused cell distention. Livers of mastomys from which this novel Helicobacter was isolated showed mild inflammation around bile ducts and focal hepatitis with necrosis **(Shen et al., 2005).**

16S rDNA analysis may not differentiate helicobacters at the species level and does not necessarily correspond to results of polyphasic taxonomy. Gene analysis of the 60 kDa heat- shock protein (HSP60) demonstrated a higher resolution than conventional 16S rDNA for species identification of gastric and enterohepatic Helicobacter spp. **(O'Rourke et al., 2004).**

Characterization of 16S rDNA, ureaseB, and HSP60 gene sequences, DNA–DNA hybridization, as well as phenotypic analysis of Finnish canine and Feline isolates and reference strains of Helicobacter sp.flexispira taxa 2, 3, and 8, demonstrated that these strains are members of the species Helicobacter bilis **(Hänninen et al., 2005)**.

# *PATHOLOGY*

## Aetiology :

The high prevalence of Helicobacter pylori infection in the studied population is most probably due to the household living conditions **(Malaty and Graham , 1994).** favoring the fecal-oral spread of infection. **(Thomas et al. , 1992)**.

H. pylori infection is mainly acquired in early childhood and is transmissible via the fecal–oral. In support of the horizontal transmission of H. pylori among siblings, a study from Northern Ireland **(Farrel et al., 2005 )** showed that sharing a bed or bedroom with an infected parent or siblings significantly increases the risk for H. pylori infection (OR:2.5–2.9 and 3.7– 4.8, respectively).

Another study from Glasgow **(Malcolm et al., 2004)** examined the association between prevalence of H. pylori infection and social deprivation in 626 children at the time of their hospital admission. A significant association between H. pylori colonization and poor socioeconomic status was found ($p < .0001$). The prevalence of infection was significantly higher in children from the most deprived areas compared to children from intermediate and/or more affluent areas ($p < .0001$). It was estimated that over 9000 (5%) of Glasgow's children are at risk for this preventable disease.

Similar correlation was reported from Cameroon **(Ndip et al**., **2004).** In this study, H. pylori prevalence was significantly higher in children from low socioeconomic class compared to those of high socioeconomic level ( $p < .05$; OR = 2.41), it increased with age, gender and was higher in males ($P < .05$; OR = 2.67). This study highlights the importance of age, gender and socioeconomic status in