Introduction

Helicobacter pylori is a gram-negative organism that induces gastric infection which causes chronic gastritis and may lead to peptic ulcer disease (PUD), H. pylori is also associated with gastric adenocarcinoma and low-grade gastric lymphoma, referred to as mucosa-associated lymphoid tissue (MALT) lymphoma ***(Berardi et al., 2005).***

In general, the prevalence is high in developing countries and the infection is acquired at a young age, the incidence is 3-10% of the population each year in developing countries compared with 0.5% in developed countries ***(Rosenberg et al., 2010).***

There are several methods available to detect *H pylori* infection including invasive methods based on gastric biopsies and noninvasive methods like serology, urea breath tests (UBTs), and stool antigen tests ***(Nakamura et al., 2001).*** The invasive procedures (biopsies and endoscopy) usually gives the most reliable diagnosis, however, these methods are invasive, expensive, and not always applicable ***(Dzierzanowska-Fangrat et al., 2006).***

The UBTs require expensive equipment or is harmful as 14c urea breath test is not recommended in pregnancy, breast-feeding, because of the radiation involved and children should

not have this test. Because H pylori antibody titer fall very slowly even after successful eradication, this test lack of specificity and sensitivity ***(Wang et al., 2000).***

Recently, the H pylori stool antigen (ImmunoCard STAT HpSA) test has been put in the market as another noninvasive technique ***(Metz et al., 2000).***

ImmunoCard STAT HpSA is potential alternative for immediate non invasive in-office *H.pylori* diagnosis; the results are available within minutes and do not require the use of laboratory equipment ***(Calvet et al., 2010).*** The ImmunoCard STAT HpSA could be used at many situations, especially for children, pregnant women, old people and others who are not suitable for endoscopy ***(Yi-Hui Li et al., 2004).***

There are controversy in sensitivity and specificity of ImmunoCard STAT HpSA, numerous studies have shown ImmunoCard STAT HpSA to be highly sensitive and specific when applied to adult ***(Odaka et al., 2002)*** and others have shown it is less sensitive and specific ***(Calvet et al., 2010).***

Aim of the Work

The aim of this work is to evaluate a rapid Helicobacter pylori stool antigen test (ImmunoCard STAT HpSA), in detecting *H.pylori* infection in comparison to the standard gastric biopsy and histopathology.

Helicobacter Pylori

Historical Background:

Several small studies conducted in the early 20th century demonstrated the presence of curved rods in the stomach of many patients with peptic ulcers and stomach cancer ***(Egan et al., 2007).***

Helicobacter pylori was first discovered in the stomachs of patients with gastritis and stomach ulcers in 1982 by Dr. Barry Marshall and Dr. Robin Warren of Perth, Western Australia. The bacterium was classified as Campylobacter pylori; in 1989 it was included in a new genus, Helicobacter ***(Sachs et al.,2003).***

In 1987, Thomas Borody invented the first triple therapy for the treatment of duodenal ulcers. In 1994, the National Institutes of Health at USA published an opinion stating most recurrent duodenal and gastric ulcers were caused by *H.pylori*, and recommended antibiotics to be included in the treatment regimen ***(NIH Consensus Statement, 2004).***

A recent research by ***Linz et al. (2007)*** states that genetic diversity in *H.pylori* decreases with geographic distance from East Africa, the birthplace of modern humans. Their results

indicate modern humans were already infected by *H.pylori* before their migrations out of Africa, and it has remained associated with human hosts since that time**.**

Microbiology of *H.pylori*:

Genome, plasmids, and strain diversity:

The size of the two sequenced *H.pylori* genomes is approximately 1.7 Mbp. The *H.pylori* strain 26695 genome includes 1,587 genes, whereas the genome of strain J99 includes only 1,491 genes ***(Boneca et al., 2003),*** both genomes contain two copies of the 16S, 23S, and 5S rRNA genes. Many strains carry one or more cryptic plasmids, which do not seem to carry antibiotic resistance genes or virulence genes ***(Heuermann, 1995).***

H.pylori is genetically heterogeneous this results in every *H.pylori*-positive subject carrying a distinct strain ***(Kansau,1996).*** The genetic heterogeneity is possibly an adaptation of *H.pylori* to the gastric conditions of its host, as well as to the distinct patterns of the host-mediated immune response to *H.pylori* infection ***(Kuipers, 2000).***

Morphology of *H.pylori*:

H.pylori organisms are spiral, microaerophilic, gram-negative bacteria with rounded ends in gastric biopsy specimens

(Figure 1) ***(Megraud, 2003)***. However, when cultured on solid medium, the bacteria assume a rod-like shape ***(Nurgalieva, 2002)***. After prolonged culture, coccoid forms predominate ***(Sachs et al., 2003).*** By electron microscopy, coccoid forms appear as U-shaped bacilli with the ends of the two arms joined by a membrane structure **(Figure 2)** ***(Nurgalieva, 2002).***

H.pylori organisms are 2.5 to 5.0 µm long and 0.5 to 1.0 µm wide; there are four to six unipolar-sheathed flagella, which are essential for bacterial motility. Each flagellum is approximately 30µm long and approximately 2.5 nm thick ***(Nathalie and Francis, 2007).***

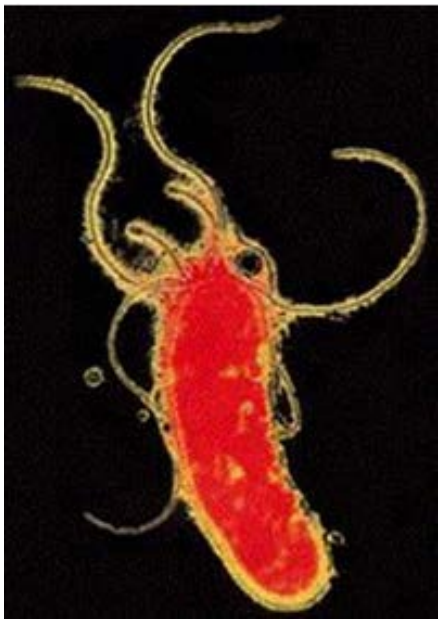

Figure (1): Morphology of *H.pylori* bacteria in vivo ***(Megraud, 2003).***

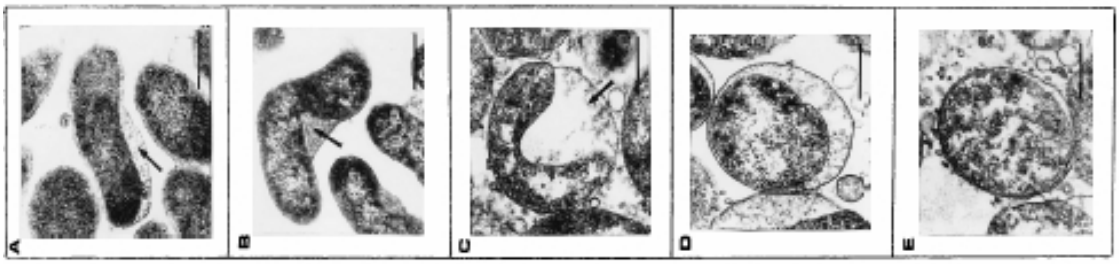

Figure (2): Morphology of *H.pylori* bacteria in vitro ***(Nurgalieva, 2002).***

The outer membrane of *H.pylori* is coated with a glycocalyx thread-like structures that link the organism to the gastric epithelial microvilli **(Figure 3)** ***(Megraud, 2003).***

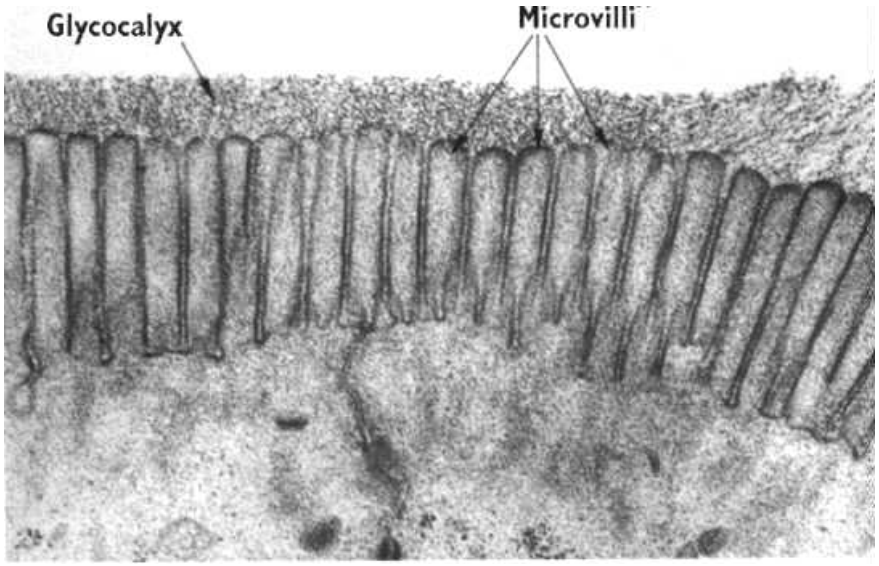

Figure (3): The outer membrane of *H.pylori* coated with a glycocalyx thread-like structures ***(Megraud, 2003).***

Pathophysiology:

a. Virulence proteins:

The role of *H.pylori* in gastro duodenal diseases has been firmly established. Infection by the organism is presumed to be from the gastric antrum and then extending down to the corpus after extensive mucosal damage ***(Akada et al., 2003)***., Disease outcome is the result of the intricate, ongoing interplay between environmental, bacterial, and host factors ***(Kuster et al., 2006).***

Intense research into *H.pylori* has led to the discovery of virulence factors such as vacA, cagA and other proteins like iceA. A combination of these is responsible for prolong and severe risk of disease associated with *H.pylori* infection ***(Asrat et al., 2004).***

These proteins have revealed many aspects of the relationships between this bacterium, the gastric mucosal surface, and the induction of disease ***(Smith et al., 2002)*** Strain-to-strain genetic variability in bacterial virulence factors does not only affect the ability of the organism to colonize and cause disease but also affects inflammation and gastric acid output ***(Kuster et al., 2006).***

The vacuolating cytotoxin, vacA, is a protein complex which is present in about 50% of all *H.pylori* strains, vacA

leads to the formation of acidic vacuoles in epithelial cells and consequently to their death following infection and colonization of an *H.pylori* strains carrying the gene ***(Haas, 2002).***

iceA is a novel gene signaling induced by contact with epithelium, there are two main allelic variants of the gene: iceA1 and iceA2 ***(Smith et al., 2002).*** The function of iceA is not yet clear but there is significant homology to a type II restriction endonuclease, the expression of iceA1 is up-regulated following contact between *H.pylori* and human epithelial cells and may be associated with peptic ulcer and other gastric related diseases ***(Arents et al., 2001).***

The well-characterized *H.pylori* virulence determinant is the cagA gene, although all *H.pylori* strains induce gastritis, cagA+ strains significantly augment the risk for severe gastritis, peptic ulcer disease, and distal gastric cancer compared to that ocurred by cagA− strains ***(Andreson, 2002).***

Initial infection by highly pathogenic strains possessing a cluster of genes known as the cag pathogenicity island result in altered expression of several genes associated with glycan biosynthesis, especially 3GlcNAc T5, a GlcNAc transferase required for the biosynthesis of Lewis antigens ***(Marcos et al., 2008).*** Resultant over-expression of 3GlcNAc T5 in human gastric carcinoma cell lines lead to increased sialyl-Lewis x

expression, a specific kind of sugar molecule that these cells display on their surface as a flag to attract immune cells to the infection site ***(Marcos et al., 2008).***

b. Gastric colonization:

H.pylori have been shown to employ multiple mechanisms to antagonize, impair, or subvert host responses. ***(Ernst et al., 2006).***

The stomach is protected by a mucosal barrier that prevents gastric secretions and other destructive agents from injuring the epithelial and deeper layers of the stomach wall. ***(Radosz-Komoniewska et al., 2005).***

The integrity of the mucosal layer is maintained by tight cellular junctions and the presence of a protective mucus layer , Prostaglandin is derived from the cell membrane lipids and serves as a chemical messenger that protects the stomach lining by improving blood flow, increasing bicarbonate secretion, and enhancing mucus production. ***(Porth, 2002).***

Gastric acidity and peristaltic muscle movement of the alimentary canal have the potential to preclude bacterial colonization of the human stomach, *H.pylori* have evolved several mechanisms to evade primary host defenses such as

acidity and peristalsis in order to establish persistent infection within the stomach, the organism elaborates a number of enzymes of which urease is one of the most important ***(Peek, 2005).***

Urease is conserved among all known Helicobacter species and is a necessary factor for the establishment of chronic infection with the organism ,two major subunits of this enzyme have been identified (ureA and ureB), this accessory protein, catalyses the cleaving of urea into ammonia and hydrogen carbonate, achieving a local neutralization of the acid pH in the cytoplasm and on the periplasm, ***(Suarez et al., 2006)*** thus, the pathogen can successfully survive in the gastric lumen (pH 1-2) for a short time before it penetrates into the bicarbonate-buffered mucus layer of the gastric mucosa, its real habitat ***(Benanti and Chivers, 2009)***, the mucus layer has a pH gradient reaching from the epithelial cell surface (pH 7) to the lumen (pH 2), and the pathogen reacts chemotactically to this gradient ***(Haas, 2002)***

Isolates that lack the ability to produce urease correspondingly fail to colonize rodent models indicating the importance of this conserved enzyme ***(Peek, 2005),*** in addition to urease, other enzymes such as catalase and oxidase are produced ***(Hovey et al., 2007).***

Motility within the gastric mucosa is aided by five or six polar flagella that are comprised of two major structural subunits: flaA and flaB, the genes encoding these two flagellar components are located at distant sites on the *H.pylori* chromosome and are transcriptionally regulated by different promoters ***(O Toole et al., 2000)***,Similar to urease production, motility is required for persistent infection, and recent data have shown that a component of the flagellar secretion apparatus, which regulates flagellar biosynthesis, also regulates urease activity, they are coupled by the FlbA gene ***(Peek, 2005).***

Other very important virulence factors are adhesins, which allows binding of the bacterium to the gastric cells, Many different molecules such as SabA, OipA, AlpA, and AlpB, show adhesion activity, including the BabA2 outer membrane protein, which is encoded by the bab (blood group antigen binding) genes ***(Maeda and Mentis, 2007)***,The BabA2 protein can bind blood antigens known as Lewis blood antigens**,** These antigens have been found both on the surface of the mucous membrane and in *H.pylori* lipopolysaccharide ***(Sheu et al., 2003).***

c. Avoidance of the immune system:

If a bacterial species persistently colonize its host, its most deficult challenge is to evade immune clearance. *H.pylori* evade immune clearance including the harsh environment in the gastric mucosa, and elicit systemic and mucosal immune responses which, however, are unable to clear the infection ***(Suarez et al., 2006).***

Multiple lines of evidence suggest that the immune response contributes to the pathogenesis associated with the infection ***(Suarez et al., 2006).*** Instead of killing the colonizing bacteria, the immune response may lead to destruction of epithelial cells and thinning of the mucosal lining leading to increased mucosal contact with luminal acid ***(Beswick et al., 2005).***

Response to gastro-duodenal infection by the organism is characterized by mucosal infiltration of lymphocytes, plasma cells, neutrophils and monocytes. Those infected with the organism have been reported to have elevated titres of IgG and IgA antibodies directed at membrane proteins (MP), flagelin, urease and lipopolysaccharide (LPS). These suggest that the infection induces a large recruitment of immune cells into the gastric mucosa. The inflammatory process is further

characterized by the production of various cytokines such as IL-2, IL-3, IL- 12, as well as IFN-γ ***(Kuipers and Michetti, 2005).***

Colonization unavoidably stimulates interleukin-8 (IL-8) expression in gastric epithelial cells. IL-8 is secreted by the host cells to attract components of the innate and adaptive immune systems to the site of infection. This polarises the immune response towards a Th1 response, further attracting inflammatory cells and T-lymphocytes ***(Suarez et al., 2006).***

An effective CD4+ T-cell response is essential to clear *H.pylori,* however this organism has been shown to inhibit CD4+ T cell proliferation and arresting IL-2 cell-cycle progression resulting in avoidance of clearance thereby staging an infection ***(Rasmus et al., 2007).***

The organism avoids recognition by producing specific bacterial factors that stimulate selective expression of host genes. *H.pylori* also synthesizes superoxide dismutase and catalase which protect it from phagocytosis, and from killing by phagocytic cells ***(Fangrat et al., 2009).*** Another mechanism through which *H.pylori* may persist is by limiting the bactericidal effects of pro inflammatory molecules, such as nitric oxide ***(Peek, 2005; Ernes et al., 2006).***

Existing data suggest that host gastric immune response to *H.pylori* can influence the clinical picture and that gastro duodenal disease may be an immunopathological consequence of a Th1-polarized response to some *H.pylori* antigens, whereas exhaustive and deregulated *H.pylori*-induced T cell-dependent B cell activation may support the onset of low-grade gastric B-cell lymphoma ***(De Jong and Enbald, 2008).***

Helicobacter pylori exposed to unfavorable conditions, such as nutrient starvation or growth inhibitors (e.g. some antibiotics, bismuth or proton pump inhibitors) transforms into unculturable coccoid forms which have been reported to survive for a long time in the environment. It is suggested that these dormant forms can be involved in the recurrence of the disease and failure of eradication therapy ***(Dzier et al., 2006).***