

**THE ROLE OF *Helicobacter pylori* VIRULENCE  
FACTORS IN DEVELOPING STOMACH CANCER;  
A REVIEW**



**A Review Report Submitted**

**By**

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**Examination Roll No. MS- 070727**

**Session: 2006-2007**



**Submitted to**

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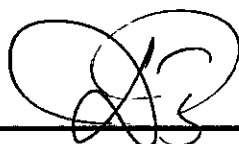
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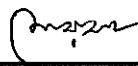
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**17<sup>th</sup> August, 2009**

# **The role of *Helicobacter pylori* virulence factors in developing stomach cancer; a review**

## **DECLARATION**

This review thesis reports the original research work of the author, except as otherwise stated. The material presented has not been submitted either in whole or in part for a degree from this or any other university. The findings of the research has not been/ will not be published any where without the concurrence of the concerned supervisor/ co-supervisor.



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Sonia Nazmir

Date: 17.08.2009

Dedicated  
To  
My Beloved Parents

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I feel the deepest of gratitude to the supreme of the universe, Almighty Allah to whom all praises go and whose mercy keeps me alive and enables me to complete this review work.

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Sonia Nazmir

17<sup>th</sup> August, 2009

## The role of *Helicobacter pylori* virulence factors in developing stomach cancer; a review

### ABSTRACT

Gastric cancer is one of the leading causes of cancer death worldwide. About 80% or more gastric cancers are stirring by infection with *Helicobacter pylori* bacterium. Prevalence of *H. pylori* infection in developing countries remains relatively constant; it is rapidly declining in the industrialized world and considerably higher in western countries. The genetic heterogeneity is possibly an adaptation of *H. pylori* to the gastric conditions of its host. Genetic heterogeneity is thought to occur via several methods of DNA rearrangement and the introduction and deletion of foreign sequences. Diversity is also seen at the nucleotide level via several mechanisms, including transcriptional and translational phase variation and mutation. The 2 genetic loci principally linked with virulence that has been most studied are the *cag* locus (*cag* pathogenicity island) and the *vacA* (vacuolating cytotoxin) gene. The portion of *vacA* that exhibits maximum diversity in an 800-bp “mid region”, can be phylogenetically divided into two families of alleles, termed m1 and m2. In particular, the “signal region”, discloses striking diversity between strains and have two main allelic families, termed s1 and s2, and the s1 type can be further subdivided into s1a, s1b, and s1c. As a result of extensive recombination among *vacA* alleles, all combinations of signal and mid regions can theoretically arise (s1a / m1, s1b / m1, s1c / m1, s1a /m2, etc). Additionally many adhesions and outer membrane proteins have their putative role in the pathogenesis of *H. pylori* infection. They are BabA (HopS), OipA (HopH), SabA (HopP), *ice A*, *hp0638* gene, AlpA, AlpB, HP-NAP, plasticity region, DupA and lipopolysaccharide. Infection with *H. pylori* strains containing *babA2*, *cagA*, and *vacA* S1 confers the highest risk of gastric cancer.

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# Chapter One

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## **Introduction**

## **Chapter One: Introduction**

### **1.1 General Information**

Cancer is the second leading cause to death (17). Cancer occurs when cells in a part of the body begin to grow out of control. There are many kinds of cancer and they all come about because of out-of-control growth of abnormal cells. A number of agents in the environment can damage DNA, increasing the mutation rate. Agents that increase the probability of cancerous transformation in a cell are known as carcinogens; examples of carcinogens are tobacco smoke, radiation, certain microbes, and synthetic chemicals in our food, water and air. Some of these carcinogens act directly on DNA while others are converted in the body into compounds that damage DNA. It is estimated that approximately 90% of all cancers are induced by environmental factors (17). Different types of cancer behave very differently. The diseases grow at different rates and respond to different treatments. Stomach cancer, also called gastric cancer starts in the stomach. Most stomach cancers start in the mucosa. The cancer can grow deeper and infect the other layers. Stomach cancers grow slowly over many years. As the cancer develops, there are usually changes that occur in the lining of the stomach. These early changes usually do not produce symptoms and are often are unnoticed. Stomach cancer can spread to different parts of the body. It can grow through the wall of the stomach and into nearby organs or it can also spread to the lymph nodes and then through the lymph system. When stomach cancer is more advanced, it will travel through the blood stream and form deposits of cancer cells in organs such as the liver, lungs, and bones. But even though it has spread to other organs, it is still called stomach cancer. Gastric cancer commonly refers to adenocarcinomas of the stomach. Adenocarcinoma is by far the most common gastric malignancy, accounting for about 95% of all cases (164). Other malignancies that may occur in the stomach include lymphomas and smooth muscle sarcomas. Although the causes of stomach cancer are not clear, researchers

have made progress in pinpointing factors that damage DNA in stomach cells. These factors include:

***Helicobacter pylori* infection:** A majority of the world's population is infected with bacteria called *Helicobacter pylori* (*H. pylori*) that live deep in the mucous layer that coats the lining of the stomach (164). Although it's not entirely clear how the bacteria are transmitted, it's likely they spread from person to person through the oral-fecal route or are ingested in contaminated drinking water. *H. pylori* infection frequently occurs in childhood and can last throughout life if not treated. It's the primary cause of stomach ulcers. Long-term infection of *H. pylori* causes inflammation that can lead to precancerous changes in the stomach lining.

**Nitrates and nitrites:** These are nitrogen-based chemicals that are added to certain foods, especially cured meats such as ham and bacon, hot dogs and deli meats. Both nitrates and nitrites combine with other nitrogen-containing substances in stomach to form N-nitroso compounds - carcinogens that are known to cause stomach cancer.

**Salted, smoked or pickled foods and red meat:** Before the advent of refrigeration, people commonly preserved food by salting, smoking or pickling. But these foods often contain large amounts of nitrites and nitrates, which can be converted in stomach into cancer-causing compounds. Countries where consumption of salted meat and fish and pickled vegetables is high - Japan and Korea are notable examples - tend to have correspondingly high rates of stomach cancer. Eating a diet high in red meat, especially when the meat is barbecued or well-done, also has been linked to stomach cancer.

**Tobacco and alcohol use:** Tobacco use can irritate the stomach lining. Alcohol has been associated with an increased risk of stomach cancer (164).

The association between *H. pylori* and gastric cancer has attracted great interest worldwide because the International Agency for Research on Cancer (IARC), a subordinate organization of the World Health Organization (WHO), identified *H. pylori* as a "group 1 (definite carcinogen)" in 1994. The IARC concluded that *H. pylori* was certainly linked to carcinogenesis on the basis of the results of epidemiologic studies (13, 124).

The association between *H. pylori* and gastric cancer may be explained by two possible mechanisms: one is based on a carcinogenesis-promoting effect of *H. pylori* itself and the other is based on the establishment of a carcinogenic environment due to long-term infection with *H. pylori*. In the second case, although *H. pylori* may have no carcinogenesis-promoting effect itself, infection causes inflammation of the gastric mucosa and chronic infection causes mucosal atrophy, resulting in intestinal metaplasia. These latter changes are considered precursors of gastric cancer (124).

## 1.2 Objectives

- To acquire knowledge about pathogenesis of *H. pylori* infection, with emphasis on its molecular biological aspects.
- To investigate recent diagnosis and treatment procedure of stomach cancer.

# Chapter Two

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## **Anatomy and Physiology of Stomach**

## Chapter Two: Anatomy and Physiology of Stomach

### 2.1 Anatomy of the human stomach

The **stomach** is an organ in the alimentary canal used to digest food. Generally, the stomach's primary function is not the absorption of nutrients from digested food; this task is usually performed by the intestine. The main job of the stomach is to break down large food molecules into smaller ones, so that they can be absorbed into the blood more easily. Latin names for the stomach include *Ventriculus* and *Gaster*; many medical terms related to the stomach start in “gastro-” or “gastric”.

The stomach is an expanded section of the digestive tube between the esophagus and the duodenum (the first part of the small intestine). It is on the left side of the abdominal cavity. The top of the stomach lies against the diaphragm. Lying beneath the stomach is the pancreas, and the greater omentum which hangs from the *greater curvature*. Two smooth muscle valves, or sphincters, keep the contents of the stomach contained. They are the esophageal sphincter (found in the cardiac region) dividing the tract above, and the pyloric sphincter dividing the stomach from the small intestine. The stomach is surrounded by parasympathetic (stimulant) and orthosympathetic (inhibitor) plexuses (anterior gastric, posterior, superior and inferior, celiac and myenteric), which regulate both the secretory activity and the motor activity of the muscles. The stomach is divided into four sections, each of which has different cells and functions (202). The sections are:

**Cardia:** Where the contents of the esophagus empty into the stomach.

**Fundus:** Formed by the upper curvature of the organ.

**Body or Corpus:** The main, central region.

**Pylorus or Antrum:** The lower section of the organ that facilitates emptying the contents into the small intestine.

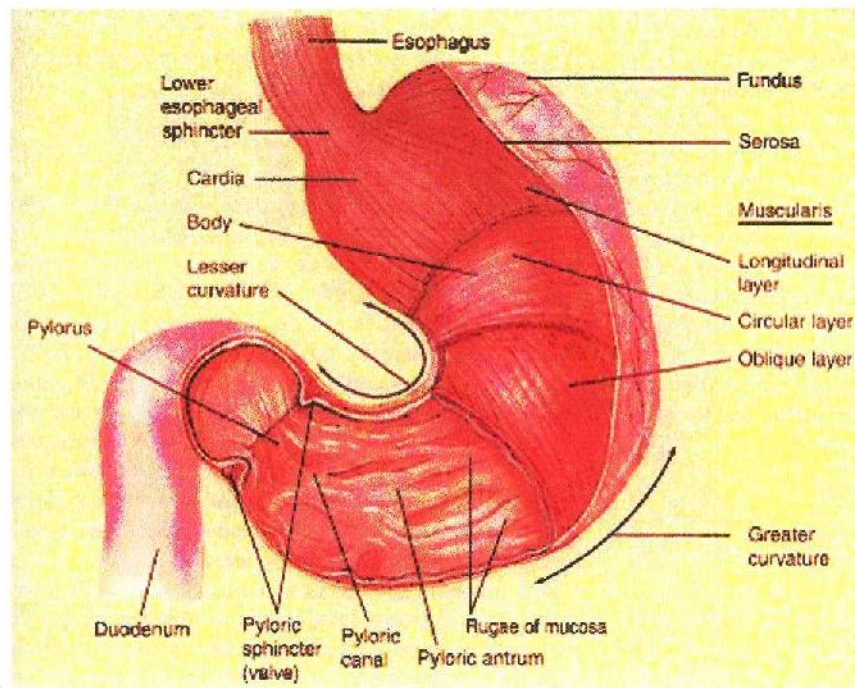


**Fig-1: *Anatomy of Stomach***

## **2.2 Histology of the human stomach**

Like the other parts of the gastrointestinal system, the stomach walls are made of a number of layers. Starting inside the (the lumen) going out, the first main layer is the mucosa. This consists of an epithelium, the lamina propria underneath, and a thin bit of smooth muscle called the muscularis mucosa. The submucosa lies under this and consists of fibrous connective tissue; it separates the mucosa from the next layer, the muscularis externa. The muscularis in the stomach differs from other GI organs in that it has three layers of muscle instead of two. Under these muscle layers is the adventitia, layers of connective tissue continuous with the omenta. The epithelium of the stomach forms deep pits, called fundic or oxyntic glands. Different types of cells are at different locations down the pits. The cells at the base of these pits are chief cells, responsible for production of pepsinogen, an inactive precursor for pepsin, which degrades proteins. The secretion of pepsinogen prevents self-digestion of the stomach cells. Further up the pits, parietal cells produce gastric acid, which kills most of the bacteria in food,

stimulates hunger, and activates pepsinogen into pepsin. Near the top of the pits, closest to the contents of the stomach, there is mucus producing cells called goblet cells that help protect the stomach from self-digestion.

The muscularis externa, as previously mentioned, is made up of three layers of smooth muscle. The innermost layer is obliquely oriented, this is not seen in other parts of the digestive system, this layer is responsible for creating the motion that churns and physically breaks down the food. The next muscle layers are the circular and then the longitudinal, which are present as in other parts of the GI tract. The antrum has thicker muscle in its walls and performs more forceful contractions than the fundus. The pylorus is surrounded by a thick circular muscular wall which is normally tonically constricted forming a functional (if not anatomically discrete) pyloric sphincter, which controls the movement of chyme into the duodenum (163, 165, and 224).

### **Stomach**

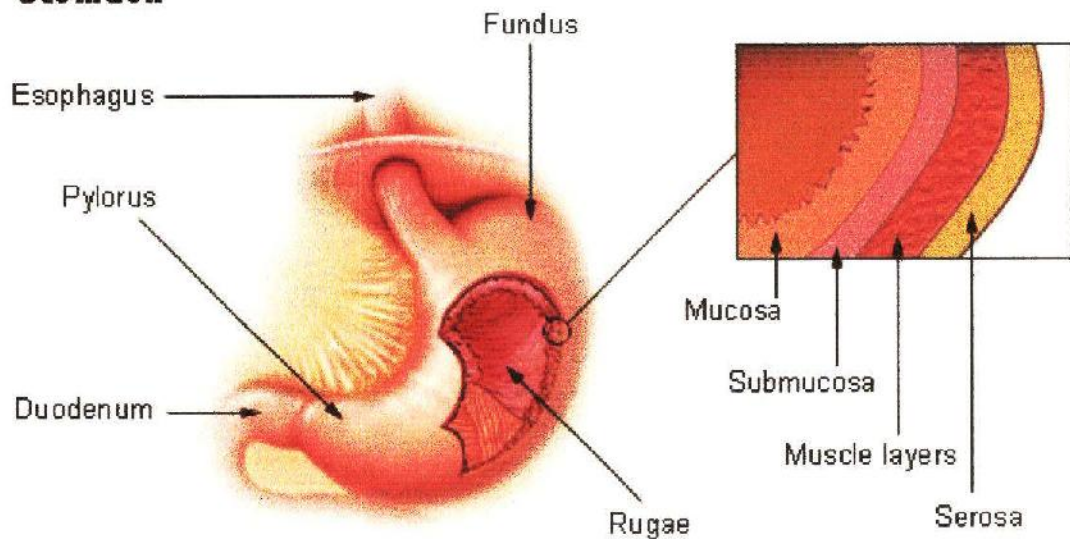


**Fig-2:** *Cross Section of Stomach*

## **2.3 Physiology of the human stomach**

### **2.3.1 Digestion and Absorption**

Digestion is the breakdown of food into smaller particles or individual nutrients. It is accomplished through six basic processes, with the help of several body fluids—particularly digestive juices that are made up of compounds such as saliva, mucus, enzymes, hydrochloric acid, bicarbonate, and bile.

The six processes of digestion involve:

- (1) The movement of food and liquids;
- (2) The lubrication of food with bodily secretions;
- (3) The mechanical breakdown of carbohydrates, fats, and proteins;
- (4) The reabsorption of nutrients—especially water;
- (5) The production of nutrients such as vitamin K and biotin by friendly bacteria; and
- (6) The excretion of waste products. The processes needed to break down food are essential to an understanding of how and when food really begins to function within the body (123, 268, and 274).

Gastric function is often classified into three phases in which secretory and motor activities are tightly coupled.

**Cephalic phase ("wake up call"):** Seeing, smelling and anticipating food is perceived in the brain and the brain informs the stomach that it should prepare for receipt of a meal. This communication is composed of parasympathetic stimuli transmitted through the vagus nerve to the enteric nervous system, resulting in release of acetylcholine in the vicinity of G cells and parietal cells. Binding of acetylcholine to its receptor on G cells induces secretion of the hormone gastrin, which, in concert with acetylcholine and histamine, stimulates parietal cells to

secrete small amounts of acid. Additionally, a low level of gastric motility is induced.

**Gastric phase ("full steam ahead"):** When a meal enters the stomach several additional factors come into play, foremost among them distension and mucosal irritation. Distension excites stretch receptors and irritation activates chemoreceptors in the mucosa. These events are sensed by enteric neurons, which secrete additional acetylcholine, further stimulating both G cells and parietal cells; gastrin from the G cells feeds back to the parietal cells, stimulating it even further. Additionally, activation of the enteric nervous system and release of gastrin cause vigorous smooth muscle contractions. The net result is that secretory and motor functions of the stomach are fully turned on - lots of acid and pepsinogen are secreted, pepsinogen is converted into pepsin and vigorous grinding and mixing contractions take place. However, there is a mechanism in place in the stomach to prevent excessive acid secretion - if luminal pH drops low enough (less than about 2); motility and secretion are temporarily suspended.

**Intestinal phase ("step on the brakes"):** As food is liquefied in the stomach, it is emptied into the small intestine. It seems to be important for the small intestine to be able to slow down gastric emptying, probably to allow it time to neutralize the acid and efficiently absorb incoming nutrients. Hence, this phase of gastric function is dominated by the small intestine sending inhibitory signals to the stomach to slow secretion and motility. Two types of signals are used: nervous and endocrine. Distension of the small intestine, as well as chemical and osmotic irritation of the mucosa is transduced into gastric-inhibitory impulses in the enteric nervous system - this nervous pathway is called the enterogastric reflex. Secondly, enteric hormones such as cholecystokinin and secretin are released from cells in the small intestine and contribute to suppression of gastric activity.

Collectively, enteric hormones and the enterogastric reflex put a strong brake on gastric secretion and motility. As the ingesta in the small intestine is processed, these stimuli diminish, the damper on the stomach is released, and its secretory and motor activities resume. Once digestion is essentially finished, waste products leave the ileum with the help of fiber, and these solids then enter the large intestine (the colon). In the colon, water is reabsorbed; some nutrients are produced by bacteria (vitamin K, biotin, vitamin B<sub>12</sub>); fibers are digested to various acids and gases; and minerals, such as potassium and sodium, are reabsorbed (when needed). Any fiber that is not broken down—and small amounts of other undigested products—are excreted in the feces.

To summarize, the brain alerts the stomach that it should expect arrival of a meal and the stomach comes out of its interdigestive quiescence and begins low level motor and secretory activity (cephalic phase). After a meal is consumed, the gastric motor and secretory activity is fully turned on (gastric phase). If the meal is at all substantial, the gastric phase is periodically suppressed by signals from the small intestine and, if gastric pH falls to very low levels, from the stomach itself. Eventually, the meal is fully liquefied and emptied, and the stomach falls back into a state of very low motor and secretory activity, where it remains until the next cephalic phase (199).

### **2.3.2 Protective Factors**

During digestion in the stomach, large proteins break down into smaller protein forms, and harmful bacteria can become inactive. Hydrochloric acid is especially important for this because it lowers the **pH** of the stomach contents below 2. Along with the uncoiling of protein in the stomach, a little carbohydrate and lipid are broken down with the help of enzymes (called amylase and lipase, respectively).

In the stomach, carbohydrates in foods turn to starch, but it is not until the chyme reaches the small intestine and becomes more neutralized that starch turns to

simple sugars that are then absorbed into the portal vein, which transports them to the liver. Also in the small intestine, lipids (mostly in the form of triglycerides) are emulsified and form monoglycerides and free fatty acids that can then go through the lymph system to the heart and bloodstream (123, 265, and 273).

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# Chapter Three

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## **Stomach Cancer**

## Chapter Three: Stomach Cancer

Stomach cancer, also known as gastric cancer, is cancer that starts in any part of the stomach. The stomach is just one of many organs located in the abdomen, the area of the body between the chest and the pelvis. It is important to differentiate among organs, because cancers and other diseases that affect them present different symptoms and are treated differently.

Stomach cancer is quite common worldwide, particularly in less developed countries. It is a leading cause of cancer-related deaths in the world. Up until the late 1930s, stomach cancer was the leading cause of cancer deaths in the United States. This is a disease that mostly affects older people. The average age at the time of diagnosis is 71. About two thirds of people with stomach cancer are older than 65. The risk of developing stomach cancer in a person's lifetime is about 1 in 100, but it is slightly higher in men than in women.

About 21,500 Americans (13,190 men and 8,310 women) will be diagnosed with stomach cancer during 2008. There will be about 10,880 (6,450 men and 4,430 women) deaths from this type of cancer in 2008 (173).

The reasons for decrease stomach cancer risk are not completely known, but may be linked to increased use of refrigeration for food storage. This led to fresh fruits and vegetables being more available and to a decreased use of salted and smoked foods. The decline of stomach cancer may be linked to the frequent use of antibiotics to treat infections in children. Antibiotics can kill the bacteria called *H. pylori* which may be a major cause of stomach cancer (210).

### 3.1 Types of Stomach Cancer

- **3.1.1 Adenocarcinomas:**

The great majority of stomach cancers are adenocarcinomas, which start in the glandular cells in the mucosa, the stomach's innermost lining. Adenocarcinomas are sometimes divided into (Lauren classification):

- *Intestinal type adenocarcinoma.* Tumor cells are described as irregular tubular structures, harboring pluristratification, multiple lumens, reduced stroma. Often, it associates intestinal metaplasia in neighboring mucosa. Depending on glandular architecture, cellular pleomorphism and mucosecretion, adenocarcinoma may present 3 degrees of differentiation: well, moderate and poorly differentiate. This type tends to occur in the distal stomach, the portion closest to the first part of small intestine (duodenum), and usually results from chronic infection with *H. pylori* bacteria or from eating a diet high in certain foods.
- *Diffuse type adenocarcinoma (mucinous, colloid):* This type can occur throughout stomach and is most often caused by genetic factors. Because its aggressiveness, this type adenocarcinoma is may extend through the stomach wall to nearby lymph nodes, eventually spreading to other organs such as the pancreas, liver and colon. Tumor cells are discohesive and secrete mucus which is delivered in the interstitium producing large pools of mucus/colloid (optically "empty" spaces). It is poorly differentiated. If the mucus remains inside the tumor cell, it pushes the nucleus at the periphery - "signet-ring cell".

Although adenocarcinomas account for about 95 percent of all stomach cancers, other forms of the disease can sometimes occur, including:

- **3.1.2 Lymphomas.** These are cancers of immune system tissue in the stomach wall. Some lymphomas are aggressive whereas others grow much more slowly. The latter, known medically as mucosa-associated lymphoid tissue (MALT) lymphomas, usually stem from *H. pylori* infection and are often curable when found in the early stages.
- **3.1.3 Carcinoid tumors.** A small percentage of stomach cancers are carcinoid tumors that originate in the stomach's hormone-producing cells.

Carcinoid tumors tend to grow less quickly and metastasize less frequently than do the more common stomach cancers.

- **3.1.4 Gastrointestinal stromal tumors (GISTs).** Scientists once thought that these rare tumors formed in the nerve or muscle cells of the intestinal tract. But now it known that they develop from cells called interstitial cells of Cajal, which are part of the autonomic nervous system. Although GISTs can be found anywhere from the esophagus to the rectum, the majority occur in the stomach. Yet GISTs are not the same as other gastric cancers, differing not only in the cells in which they originate but also in their prognosis and treatment. GISTs spread rapidly, prove fatal in a relatively short time and don't respond to treatment with the most common cancer therapies (164).



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# Chapter Four

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## **Normal Microbial Flora of Gastrointestinal Tract**

## Chapter Four: Normal Microbial Flora of Gastrointestinal Tract

### 4.1 Location: Esophagus, Stomach, Small Intestine, Large Intestine

The gastrointestinal tract is colonized with microbes at birth and remains the home for a diverse collection of organisms throughout the life of the host. Although the opportunity for colonization with new organisms occurs daily with the ingestion of food and water, the population remains relatively constant unless exogenous factors such as antibiotic treatment disrupt the balanced flora.

**Esophagus:** Oropharyngeal bacteria and yeast, as well as the bacteria that colonize the stomach, can be isolated from the esophagus. Bacteria rarely cause disease of the esophagus.

**Stomach:** Because the stomach contains hydrochloric acid and pepsinogen (secreted by the parietal and chief cells lining the gastric mucosa), the only organisms present are small numbers of acid tolerant organisms such as the lactic acid producing bacteria (*Lactobacillus*, *Streptococcus* species) and *Helicobacter*. The microbial population can dramatically change in numbers and diversity in patients receiving drugs that neutralize or reduce the production of gastric acids.

**Small Intestine:** The small intestine is colonized with many different bacteria, fungi, and parasites. Most of these organisms are anaerobes, such as *Peptostreptococcus*, *Porphyromonas*, and *Prevotella* species. Common causes of gastroenteritis (*Salmonella* and *Campylobacter* species) can be present in small numbers as asymptomatic residents; however, their detection in the clinical laboratory generally indicates diseases.

**Large Intestine:** More microbes are present in the large intestine than anywhere else in the human body. The most common bacteria include *Bifidobacterium species*, *Bacteroides fragilis*, *Eubacterium species*, *Enterococcus species*, and *E coli* (188).

#### **4.2 Most Common Microbes Colonizing the Gastrointestinal Tract**

*Acinetobacter species*

*Actinomyces species*

*Bacteroides species*

*Bifidobacterium species*

*Clostridium species*

*Cronobacterium species*

*Eubacterium species*

*Enterococcus species*

*Enterobacteriaceae*

*Fusobacterium species*

*Helicobacter species*

*Lactobacillus species*

*Peptostreptococcus species*

*Porphyromonas species*

*Propionibacterium species*

*Prevotella species*

*Pseudomonas species.*

# Chapter Five

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***Helicobacter species***

## Chapter Five: *Helicobacter species*

### 5.1 Microbiology

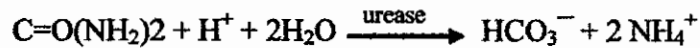
#### 5.1.1 Genus Description and Phylogeny

The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira*, and *Thiovulum*. To date, the genus *Helicobacter* consists of over 20 recognized species, with many species awaiting formal recognition (85). Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are also ureases positive. *Helicobacter* species can be subdivided into two major lineages, the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. Both groups demonstrate a high level of organ specificity.

**Gastric *Helicobacter* species:** Gastric *Helicobacter* species is a spiral shaped bacterium that have adapted to the inhospitable conditions found at the gastric mucosal surface in the stomach and duodenum (section of intestine just below stomach). It has a unique way of adapting in the harsh environment of the stomach, and it is currently thought that the stomachs of all mammals can be colonized by members of the genus *Helicobacter*.

In humans, the stomach is a highly acidic environment (maintained by the secretion of hydrochloric acid) with peptidase digestive enzymes. In fact, the stomach's interior can secrete 2 to 3 liters of gastric fluid per day, which can readily tear apart the toughest food or microorganism. Bacteria, viruses are all consumed in this chemical. It used to be thought that the stomach contained no bacteria and was actually sterile, but *Helicobacter* species changed that. The stomach is protected from its own gastric juice by a thick layer of mucus that covers the stomach lining. *Helicobacter* species takes advantage of this protection by living in the mucus lining.

Once *H. pylori* is safely ensconced in the mucus, it is able to fight the stomach acid that does reach it with an enzyme it possesses called urease (230). Urease converts urea, of which there is an abundant supply in the stomach (from saliva and gastric juices), into bicarbonate and ammonia, which are strong bases. This creates a cloud of acid neutralizing chemicals around the *H. pylori*, protecting it from the acid in the stomach.



**Urea hydrolysis: urea is broken down to ammonia and carbon dioxide**

**Enterohepatic *Helicobacter* species:** Enterohepatic *Helicobacter* species colonize the lower gastrointestinal tract, including the ileum, colon, and biliary tree of humans and other mammals. They cause persistent infections, which are associated with chronic inflammation and epithelial cell hyperproliferation that can lead to neoplastic disease, and are associated with human hepatobiliary disease (26, 230, and 260). The group of enterohepatic *Helicobacter* species consists of many different species (*H. hepaticus*, *H. bilis*, *H. muridarum*, and *H. troglodytes*) differing in morphology, ultrastructure, growth conditions, and the presence or absence of the urease virulence factor (87, 231).

### 5.1.2 Genome, plasmids, and strain diversity

The size of the two sequenced *H. pylori* genomes is approximately 1.7 Mbp, with a G + C content of 35 to 40%. The *H. pylori* strain 26695 genome includes 1,587 genes, whereas the genome of strain J99 includes only 1,491 genes (8, 36, and 245). 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 (86, 89). In contrast to other bacterial pathogens that are highly clonal (such as *Shigella dysenteriae* and *Mycobacterium tuberculosis*), *H. pylori* is genetically heterogeneous, suggesting a lack of clonality. This results in every *H. pylori*-positive subject carrying a distinct strain (129), although differences within relatives may be small. 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 (140). Genetic heterogeneity is thought to occur via several methods of DNA rearrangement and the introduction and deletion of foreign sequences (1, 78, 219, and 236). Diversity is also seen at the nucleotide level via several mechanisms, including transcriptional and translational phase variation and mutation (77, 235, and 237).

Two independent *H. pylori* isolates, strain 26695 and strain J99, have been fully sequenced and the subsequent comparison has provided a detailed look at the physical chromosomal organization (Table 1).

**Table 1: General comparative features of *H. pylori* genomes**

| Genome Feature                          | <i>H. pylori</i> |         |
|-----------------------------------------|------------------|---------|
|                                         | J99              | 26695   |
| Size (bp)                               | 1643831          | 1667867 |
| (G+C)%                                  | 39               | 39      |
| Regions of different (G+C)%             | 9                | 8       |
| <i>vacA</i> genotype                    | s1b/m1           | s1a/m1  |
| Open reading frames:                    |                  |         |
| % of genome (coding)                    | 90.8             | 91.0    |
| Predicted no.                           | 1496             | 1590    |
| No. of functionally classified genes    | 877              | 899     |
| No. of conserved genes with no function | 361              | 375     |
| No. of <i>H. pylori</i> -specific genes | 258              | 279     |
| No. of genes unique to each strain      | 89               | 117     |
| Insertion elements:                     |                  |         |
| No. of complete IS605 copies            | 0                | 5       |
| No. of partial IS605 copies             | 5                | 8       |
| No. of complete IS606 copies            | 1                | 2       |
| No. of partial IS606 copies             | 2                | 2       |

Several methods were employed to assess the macrodiversity of these two genomes. These studies showed that the majority of the genes in these two strains lie at the same relative position. Only 10 regions of the J99 genome, ranging from 1 to 83 kb, needed to be artificially transposed and/or inverted to enable the

orthologous genes to be mapped onto the 26695 genome (6, 37). To examine the conservation of gene order, each J99 predicted gene and its corresponding strain 26695 ortholog, if present, were examined to determine whether their immediate neighbors were conserved. Of the 1496 predicted genes in strain J99, 1268 (85%) have the same neighboring gene on each side in both genomes; 161 (10.4%) have the same neighbor on one side and a strain-specific gene on the other side; and 40 (2.7%) have a strain-specific gene on both sides. Only 27 (1.9%) have the same neighbor on one side and an orthologous gene out of order on the other side due to a difference in the physical structure of the two genomes.

Sequencing studies of several genes (*cagA*, *vacA*, *flaA*, *flaB*, *cysS*, *ureC/glmM*, and *trpA/B* genes from IS605) have demonstrated that the nucleotide diversity of orthologous genes between independent *H. pylori* isolates is relatively high (75, 88, and 115). However, due to the redundancy in the genetic code, there was a higher level of divergence in the genes than in the respective encoded proteins. Consistent with literature studies, no two orthologous genes between J99 and 26695 shared 100% identity whereas 41 proteins (2.9%) were 100% identical. Indeed, only eight orthologous genes (0.6%) had an identity level greater than 98%, whereas 310 proteins (22.2%) shared this level of identity (64).

The high level of genomic diversity among isolates of *H. pylori* results from a combination of a number of mechanisms. In the published *H. pylori* genome sequences some gene homologs functioning in DNA damage reversal (e.g., *Escherichia coli* homologs *mutM*, *nfo*, *usr*, *oxyR*, *ada*, and *ogt*) are absent, suggesting that *H. pylori* is not able to directly remove DNA lesions as efficiently as *E. coli*. Also, *H. pylori* lacks an *E. coli* *mutHLS*-like postreplicative mismatch repair system. Through binding to the mismatched base pairs, the *mutHLS* system performs two functions: preventing occurrence of mutations from replication errors and inhibiting homologous recombination between diverged DNA sequences. Absence of the *mutHLS* system in *H. pylori* may predict an increased mutation rate and lack of the genetic barrier for inter-strain recombination (5, 246, and 264).

The majority of *H. pylori* strains are naturally competent for DNA transformation (267). Natural transformation is a complex process involving DNA uptake followed by recombination. In *H. pylori*, a gene cluster consisting of four genes (*orf2*, *comB1*, *comB2*, and *comB3*) and the *dprA* gene (11, 228, and 229) are involved in natural competence and DNA uptake. In several well-studied naturally transformable bacteria species, specific DNA sequence are abundant throughout the genome are required for DNA uptake, which ensures that the substrate for recombination is derived from related bacteria (65, 66, and 67). But *H. pylori* does not possess such an uptake sequence. Thus, *H. pylori* might be expected to have an increased likelihood of taking up DNA from unrelated species when it is encountered. It is also possible that very small pieces of *H. pylori* DNA (from the cells of the same strain or different strains) are taken up into the cell.

After uptake the DNA may become the substrate for homologous recombination (239). Genetic recombination in *H. pylori* is so frequent that it randomizes the DNA sequences and generates linkage equilibrium (a so-called panmictic population structure) (240). *E. coli* has two recombination pathways: the primary RecBC pathway mainly serves the need of conjugational recombination, and the secondary RecF pathway functions mainly in recombinational repair (146). According to the complete genome sequences, RecBC pathway is absent in *H. pylori* and RecF is the only pathway (9).

The transcription and translation process in *H. pylori* appear to bear a lot of similarities like other gram-negative bacteria. Comparative analyses between the genomes of *H. pylori* J99 and 26695 suggest that *H. pylori* has a higher efficient mechanism of recombinational repair by natural competence for DNA uptake, lack of a DNA uptake sequence, and lack of *mutHLS* system.

### 5.1.3 Morphology

*H. pylori* is a gram-negative bacterium, measuring 2 to 4  $\mu\text{m}$  in length and 0.5 to 1  $\mu\text{m}$  in width. Although usually spiral-shaped, the bacterium can appear as a rod,

while coccoid shapes appear after prolonged *in vitro* culture or antibiotic treatment (143). These coccoids cannot be cultured *in vitro* and are thought to represent dead cells, although it has been suggested that coccoid forms may represent a viable, non culturable state (72). The organism has 2 to 6 unipolar, sheathed flagella of approximately 3  $\mu\text{m}$  in lengths, which often carry a distinctive bulb at the end (178). The flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells (50). In contrast to many other pathogens of the gastrointestinal tract, it lacks fimbrial adhesions (177).

## **5.2 Epidemiology**

### **5.2.1 Prevalence and Geographical Distribution**

The prevalence of *H. pylori* shows large geographical variations. In various developing countries; more than 80% of the population is *H. pylori* positive, even at young ages (198). The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (200). Within geographical areas, the prevalence of *H. pylori* inversely correlates with socioeconomic status, in particular in relation to living conditions during childhood (167). In Western countries, the prevalence of this bacterium is often considerably higher among first- and second-generation immigrants from the developing world (196, 251). While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world (93). The latter is thought to be caused by the reduced chances of childhood infection due to improved hygiene and sanitation. In developing countries, *H. pylori* infection rates rise rapidly in the first 5 years of life and remain constantly high thereafter, indicating that *H. pylori* is acquired early in childhood (81, 82). However, in industrialized countries the prevalence of *H. pylori* infection is low early in childhood and slowly rises with increasing age. This increase results only to a small extent from *H. pylori* acquisition at later age. The

incidence of new *H. pylori* infections among adults in the Western world is less than 0.5% per year; the higher prevalence of infection among the elderly thus reflects a birth cohort effect with higher infection rates in the past (141, 190). The active elimination of *H. pylori* from the population and improved hygiene and housing conditions have resulted in a lower infection rate in children, which is reflected in the age distribution of this lifelong-colonizing bacterium (138, 211, and 214). Overall, new infection more commonly occurs in childhood and lasts for life unless specifically treated.

### 5.2.2 Transmission and Sources of Infection

The exact mechanisms whereby *H. pylori* is acquired are largely unknown. *H. pylori* has a narrow host range and is found almost exclusively in humans and some nonhuman primates. *H. pylori* has on rare occasions been isolated from pet animals; thus, the presence of pets may be a risk factor for *H. pylori* infection (43, 44, 66, and 110). New infections are thought to occur as a consequence of direct human-to-human transmission, via either an oral-oral or fecal oral route or both. *H. pylori* has been detected in saliva, vomitus, gastric refluxate, and feces, but there is no conclusive evidence for predominant transmission via any of these products. This may be due to the fact that most research on transmission has focused on adults. It appeared that there was no clear increased risk for being a carrier of *H. pylori* among dentists, gastroenterologists, nurses, partners of an *H. pylori*-positive spouse, or visitors to a clinic for sexually transmitted diseases. As a result of these and other investigations, it is generally believed that acquisition mostly occurs in early childhood, most likely from close family members (135, 136, 209, 215, 242, and 254). Premastication of food by the parent is an uncertain risk factor for transmission of *H. pylori*. Childhoods crowding in and outside the family are all positively associated with *H. pylori* prevalence (97, 159). Several studies have reported the presence of *H. pylori* DNA in environmental water sources (72, 31, and 201), but this probably reflects contamination with either naked DNA or dead

*H. pylori* organisms. Spread via fecal contaminants is supported by the occurrence of *H. pylori* infections among institutionalized young people during outbreaks of gastroenteritis. Coupled with the extreme sensitivity of *H. pylori* to atmospheric oxygen pressure, lack of nutrients and temperatures outside the 34 to 40°C range, direct person-to-person transmission remains the most likely transmission route.

### 5.3 Pathogenesis of Infection

The primary disorder, which occurs after colonization with *H. pylori*, is chronic active gastritis. This condition can be observed in all *H. pylori*-positive subjects. *H. pylori*-induced ulcer disease, gastric cancer, and lymphoma are all complications of this chronic inflammation; ulcer disease and gastric cancer in particular occur in those individuals and at those sites with the most severe inflammation. Understanding of these factors is thus crucial for the recognition of the role of *H. pylori* in the etiology of upper gastrointestinal pathology.

#### 5.3.1 Role of *H. pylori* Virulence Factors

The natural course of infection with *H. pylori* is extremely variable. Most infected individuals remain asymptomatic, and infection with *H. pylori* is not sufficient to induce gastric cancer. Therefore, other factors — known bacterial and host cofactors, and perhaps unknown cofactors — are required. The bacterial factors that enable *H. pylori* to persist in the gastric lumen are still being studied, but considerable insight has been gained through the analysis of the *H. pylori* genome, the creation of *H. pylori* isogenic mutants, and the use of animal models.

**5.3.1.1 *cag* PAI:** A gene associated with secretion of a toxin is identified as the *cag A* (cytotoxin associated gene) and Cag A protein, which is encoded by the *cag A* gene, is a highly immunogenic protein. Cag A-positive strains of *H. pylori* are more virulent (277). The *cag A* gene is located at one end of a 40-kb DNA insertion called the *cag* pathogenicity island (*cag* PAI) and may have originated from a non-

*Helicobacter* source. The Cag A-positive strains induce morphological changes, vacuolization, and successive degeneration of *in vitro* cultured cells (127, 150).

Patients infected with Cag A- positive strains usually have a higher inflammatory response and are significantly more at risk for developing a symptomatic outcome (peptic ulcer or gastric cancer) in Western populations (142, 255, and 256), though not in Asian populations (32, 96, 103, and 191). Eighteen of the *cag* PAI-encoded proteins serve as building blocks of a type IV secretion apparatus [Type IV secretion systems (T4SS) are used not only to transport molecules toxic to host cells, but also are used to transport DNA or protein-DNA complexes. One such process is bacterial conjugation whereby two mating bacteria exchange genetic material. By facilitating conjugative transfer, type IV secretion machineries play crucial roles in the spread of antibiotic resistance genes among bacteria], which forms a syringe like structure capable of penetrating the gastric epithelial cells and facilitating the translocation of CagA, peptidoglycan, and possibly other bacterial factors into host cells (18, 51, 54, 84, 178, 179, and 225).

#### **5.3.1.2 VacA (vacuolating cytotoxin)**

Approximately 50% of all *H. pylori* strains secrete VacA, a highly immunogenic 95-kDa protein that induces massive vacuolization in epithelial cells *in vitro* (56). The VacA protein plays an important role in the pathogenesis of both peptic ulceration and gastric cancer. The activities of VacA include membrane channel formation, disruption of endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton- dependent cell functions, induction of apoptosis, and immune modulation (57, 109). There is considerable variation in vacuolating activities among strains (55, 56, 63, and 149). This is due to the sequence heterogeneity within the *vacA* gene (21, 257, and 276) at the signal region (s) and the middle region (m). The s region of the gene, which encodes the signal peptide, occurs as either an s1 or s2 type, whereas the m region, which contains the p58 cell binding domain, exists as an m1 or m2 type (257).

Vacuolating activity is high in s1/m1 genotypes, intermediate in s1/m2 genotypes, and absent in s2/m2 genotypes (21) (Fig: 3a). In line with this, *vacA* s1/m1 genotypes are more frequently associated with peptic ulceration and gastric carcinoma (21). VacA forms pores in epithelial cell membranes, thus inducing the release of urea and anions from the host cells. It also increases transcellular permeability, leading to the release of nutrients and cations (1). Interestingly, a significant part of the secreted toxin is not released into the environment but remains associated with the outer membrane of *H. pylori* (85, 121). Upon bacterial contact with host cells, these toxin clusters are transferred to the host cell surface and exert their toxic action (121) (Fig: 3b). Secreted VacA can be further processed into a 33-kDa N-terminal fragment and a 55-kDa C-terminal fragment through proteolytic cleavage. The N-terminal protein performs an essential function in the formation of anion channels, while the C-terminal protein mediates cell binding (128, 134, 185, and 249). In spite of the proteolytic cleavage, these fragments remain noncovalently associated with each other (244). Purified VacA spontaneously forms oligomeric aggregates (>900 kDa) and upon exposure to acidic pH disassembles into the active monomers that form pores in the cell membrane (59, 155, 49, and 58). Although many of the VacA-mediated effects either directly or indirectly result from membrane binding or pore formation, VacA also enters the cytosol, subsequently accumulates in the mitochondrial inner membrane, and activates endogenous mitochondrial channels, thereby inducing apoptosis (60, 88, 139, and 274). The mechanisms are described by figure 3.

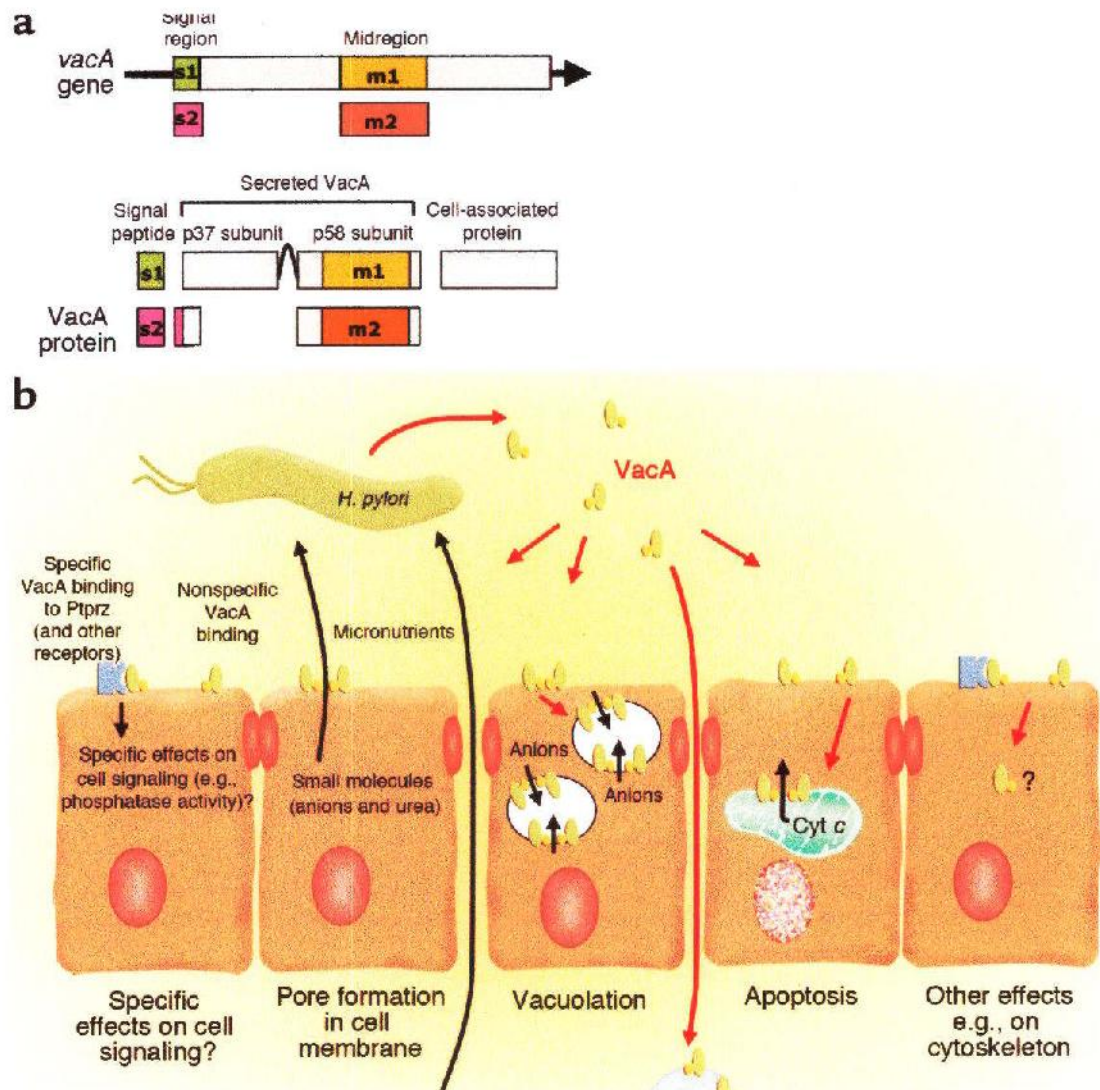


**Figure 3:** *VacA* polymorphism and function. **(a)** *VacA* polymorphism. The gene, *vacA*, is a polymorphic mosaic with two possible signal regions, *s1* and *s2*, and two possible mid-regions, *m1* and *m2*. The translated protein is an autotransporter with N- and C-terminal processing during bacterial secretion. The *s1* signal region is fully active, but the *s2* region encodes a protein with a different signal-peptide cleavage site, resulting in a short N-terminal extension to the mature toxin that blocks vacuolating activity and attenuates pore-forming activity. The mid-region encodes a cell-binding site, but the *m2* type binds to and vacuolates fewer cell lines *in vitro*. **(b)** *VacA* biological activities. Secreted *VacA* forms monomers and oligomers; the monomeric form binds to epithelial cells both nonspecifically and through specific receptor binding, for example, to Ptpz, which may modulate cell signaling. Membrane-bound *VacA* forms pores. Following *VacA* endocytosis, large vacuoles form, but, although

*marked in vitro, these are rarely seen in vivo. VacA also induces apoptosis, in part by forming pores in mitochondrial membranes, allowing cytochrome c (Cyt c) egress. Although the presence of cytoplasmic VacA is implied rather than demonstrated, yeast two-hybrid experiments show potential for specific binding to cytosolic targets including cytoskeletal proteins, consistent with observed cytoskeletal effects. Finally, VacA has suppressive effects on immune cell function.*

#### **5.3.1.3 Acid resistance**

One of the striking features of *H. pylori* is that it is able to colonize the acidic gastric environment, although the bacterium is not an acidophile. The pH of the gastric mucosa is thought to vary between 4 and 6.5, but occasional acid shocks may occur (223). *H. pylori* thus require mechanisms to protect it from acute acid shocks and mechanisms to grow at pH values around 5.5 (35, 233). Upon entry, *H. pylori* were originally thought to move rapidly toward the gastric mucus layer by chemotactic motility using the urea and bicarbonate gradients present in the gastric environment. The main component of *H. pylori* acid resistance is the urease enzyme, which converts urea into ammonia and carbamate, which spontaneously decomposes into another ammonia molecule and carbon dioxide (45). Urease activity is present in all *H. pylori* isolates, although the levels of urease activity differ significantly between strains and is dependent on the growth conditions (52, 260). The urease enzyme is also associated with the outer membrane of *H. pylori* (233). However, it is unlikely that the outer membrane urease contributes significantly to acid resistance, as the urease enzyme is rapidly inactivated at this low pH (101, 217). Both the ammonia and bicarbonate produced by urease have been implicated in the pathogenesis of *H. pylori* infection. Ammonia is thought to have a cytotoxic effect on gastric epithelial cells (230).

#### **5.3.1.4 Adhesins and outer membrane proteins**

Many bacterial factors mediate the adhesion of *H. pylori* to the gastric epithelium (70, 74, 129, 152, 173, 180, and 253). Given the narrow host range, the multitude

of adhesions probably reflects their importance for the bacterium, but it makes it extremely difficult to test for the contribution of each individual adhesin.

**(i) BabA (HopS):** The 78-kDa BabA protein probably represents the best-characterized *H. pylori* adhesion protein; it is encoded by the *babA* gene. BabA mediates binding to fucosylated Lewis b (Leb) blood group antigens on the human host cells (39, 119). There are two distinct *babA* alleles, *babA1* and *babA2*. Animal studies suggest that BabA-mediated adhesion is relevant for the colonization and pathogenesis of *H. pylori* (121, 203). BabA is thought to have a role in the virulence of *H. pylori*, as the *babA2* allele is strongly associated with peptic ulcer disease and gastric adenocarcinoma (94), but this correlation is controversial (283). Although the distribution of the *babA* alleles may be associated with more severe disease (203), the presence of the *babA2* allele is clearly linked to the *vacA* s1 and *cagA* alleles and thus again may not represent an independent disease marker (283).

**ii) OipA (HopH):** The 34-kDa OipA protein is another member of the Hop protein family that may well serve as an adhesin but was originally identified as a proinflammatory response-inducing protein (281). The gene encoding the OipA protein is present in all *H. pylori* strains. As this gene has only recently been discovered, there is currently not much data on its relevance as a disease-specific marker (280).

**(iii) SabA (HopP):** SabA mediates binding to sialic acid-containing glycoconjugates (157, 212). *H. pylori*-induced gastric inflammation and gastric carcinoma are associated with the replacement of nonsialylated Lewis antigens by sialylated Lex and sialylated Lea (157, 184, and 218). Thus, the role of SabA is probably during the chronic inflammatory and atrophic disease stages (156). Human granulocytes also carry sialylated carbohydrates on their surface, and as a consequence these cells are specifically recognized by SabA. This may promote an intimate association with the host cell that does not simply assist in evasion of the

immune surveillance but might actually allow the bacterium to control the immune response through direct transfer of CagA, VacA, and other virulence factors.

**(iv) The *hp0638* gene:** A novel putative virulence factor is encoded by the *hp0638* open reading frame. HP0638 is a member of the *H. pylori* outer membrane protein (OMP) family. The *hp0638* gene is located on the *H. pylori* chromosome approximately 100 kb from the *cag PAI*. Various studies showed that *H. pylori* contain either a functional or non-functional *hp0638* gene with functional status being regulated by a slipped strand repair mechanism in the 5' region of the gene (switch "on" is the functional status and switch "off" is the nonfunctional status) (281). The majority of strains with *hp0638* functional status "on" also possessed the *cag PAI*.

**(v) *ice A*:** Two distinct *iceA* genotypes, *iceA1* and *iceA2*, are known. *iceA1* is homologous to the *Neisseria lactamica* NlaIIIR gene encoding restriction endonuclease NlaIII, but *iceA2* shows no homology to any known gene. *iceA2* has a particular repeat structure, predicting several distinct, highly conserved protein motifs, and is preceded by a variable number of 8-bp repeats, which permit discrimination between individual strains. The function of *iceA2* remains unknown. The expression of *iceA1* is up regulated upon contact between *H. pylori* and human epithelial cells (294).

**(vi) Lipopolysaccharide (LPS):** The majority of *H. pylori* strains express LPS that contains fucosylated oligosaccharide antigens that are structurally and immunologically closely related to human blood group antigens. These bacterial antigens (Lewis antigens) display marked antigenic variation and are thought to contribute to immune evasion. The finding that Lewis antigen expression enhances bacterial internalization by epithelial cells suggests that Lewis antigen expression potentially affects the innate immune response (154).

**Table 2: Adhesins and virulence-associated proteins of *H. pylori***

| Protein/gene cluster                         | Predicted role                                                                                                                                                     | Association with <i>H. pylori</i> related disease                                                                      |
|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|
| BabA                                         | Binds to fucosylated Leb blood group antigen on cells.                                                                                                             | <i>babA2</i> allele has been implicated in peptic ulcer disease and gastric cancer                                     |
| SabA                                         | Binds to sialyl-Lex and sialyl-Lea antigens and is involved in activation of neutrophils                                                                           | None                                                                                                                   |
| SabB                                         | Binding specificity is unknown                                                                                                                                     | Absence of SabB expression via phase variation is associated with duodenal ulcers                                      |
| OipA                                         | OipA has been reported to assist in IL-8 induction, but this association is not universal                                                                          | Expression of OipA is linked to <i>cag</i> status and development of duodenal ulcers and gastric cancer                |
| AlpA and AlpB                                | Inactivation of the <i>alpA</i> and <i>alpB</i> genes results in decreased adherence to gastric epithelial cells and absence of colonization in a guinea pig model | Unknown                                                                                                                |
| HP-NAP                                       | HP-NAP is reported to activate neutrophils and is a possible adhesin to mucin; possible function in protection of <i>H. pylori</i> DNA or iron storage             | Unknown                                                                                                                |
| Plasticity region ( <i>jhp0947-jhp0950</i> ) | Unknown                                                                                                                                                            | Presence of the plasticity region is associated with development of gastric cancer, MALT lymphoma, and duodenal ulcers |
| IceA                                         | The <i>iceA1</i> allele encodes a CATG-recognizing restriction endonuclease                                                                                        | IceA1 has been associated with peptic ulcer disease, association is not universal                                      |

### 5.3.1.5 Adhesion mechanisms of *H. Pylori*

Many bacterial factors mediate the adhesion of *H. pylori* to the gastric epithelium. Given the narrow host range, the multitude of adhesins probably reflects their importance for the bacterium, but it makes it extremely difficult to test for the contribution of each individual adhesin. Thus concentrate here the adhesions mechanisms and phase variation.

#### BabA | Hop | SabA | phase variation

Bacterial adherence is an important contributor to the vigor of infection and virulence. Functional receptors for *H. pylori* adherence include fucosylated ABO blood group antigens (19, 38) and sialyl-Lewis x/a antigens (158). These receptors are recognized by the blood group antigen-binding adhesin BabA, which binds Lewis b antigen (Leb) and related ABO antigens (19, 120) and the sialic acid-binding adhesin, SabA (158). To date, BabA–Leb is the best-characterized of *H. pylori*'s several adhesin–receptor interactions (19, 93, 120, and 284).

Some *bop* genes are duplicates, and others exhibit extensive homologies at their 5' and 3' ends that should facilitate frequent recombination between them (10, 248). For example, reference strain CCUG17875 (here denoted 17875) has two *babA* genes: *babA2*, from which the BabA adhesin is expressed, and *babA1*, which is silent because of an incomplete signal peptide (120). The *babB* gene is highly homologous to *babA* at its 5' and 3' ends but not at the central region that (in *babA*) determines the specificity of receptor binding (19, 120, and 232). Other *bop* genes, *babB* included, contain repetitive sequence motifs that are prone to frame shift mutation and thereby metastable *bop* gene expression (10, 222, and 248). Most disease-associated *H. pylori* strains express the BabA adhesin, whereas many of those implicated in more benign infections do not (93, 120, and 284). Leb-nonbinding strains also usually possess silent *babA* gene sequences, which can be activated by recombination into the *babB* locus. Once at this second locus, a BabB/A chimeric adhesin is expressed at lower levels and is subject to frame shift-

based phase variation (ON/OFF switching). These changes in expression illustrate that bacterial adherence properties can be modulated by inclusion in expression loci of various strengths and plasticities. *H. pylori* populations use these mechanisms for quantitative modulation of adhesive properties during cycles of periodic activation and deactivation.

Abilities to cycle between non adherence and adherence to epithelial surfaces and to dynamically change adherence strength is likely to help *H. pylori* establish and maintain chronic infections in the face of human diversity, immune responses, and disease progression (19, 158). Adherence promotes delivery of toxins and other effector molecules to target tissues and improves access to nutrients that leach from tissues when damaged (211), but it can be a mixed blessing in also exposing *H. pylori* to the brunt of bactericidal host responses. In principle, flexibility in adherence might have been achieved by classical regulatory mechanisms by using, for example, membrane sensors and transcription repressors or activators to monitor and cope with differences in the gastric milieu.

Expression of the BabA adhesin is believed to be regulated at the level of translation because of the lack of the translational start in *babA1* (120). The *babA1* gene is transcriptionally silent, most likely because of four additional nucleotides between the -10 and -35 sites of *pbabA1*, which should diminish the strength of the promoter. The repetitive (A)<sub>n</sub> sequences between the -10 and the -35 sites should also be prone to slippage mutations and thereby allow changes in levels of transcription of downstream genes. Northern blot analysis also detected shorter distinct *babA* and *babB* transcripts in cells with functional *bab* genes. It is tempting to speculate that transcript cleavage may also be used to help fine-tune the level of expressed adhesin and Leb binding. The amount of expressed *babA* and *babB/A* transcript closely matched the level of expressed protein and the Leb-binding capacity. This feature probably reflects the strength of the promoters for which the *babB* promoter is weaker relative to that of *babA2*. Thus, BabA is more strongly expressed from the original *pbabA2* compared with the *babB* promoter, which is in

line with the idea of recombinations as mechanisms of generating subclones with altered levels of gene expression.

Synthesis of the adhesin encoded by the *babB/A* chimera is also prone to switch OFF and ON at frequencies at nearly  $10^{-2}$ , unlike that from the original *babA* gene. By flexible ON/OFF shifts in adherence, the bacterial population will contain cells that closely attach to the epithelium and others that better escape inflammation responses. Infection with *H. pylori* induces changes to mucosal glycosylation patterns, with up-regulation of inflammation association sialyl-Lewis antigens (158). Both sialylated and fucosylated antigens reciprocally change expression levels when fucosylation is down-regulated during early phases of infection but then again increase during persistent infection. Furthermore, higher infection loads of *H. pylori* were seen in patients with higher densities of Leb (108, 227). These results suggest that *H. pylori* infection promotes distinct shifts in mucosal glycosylation patterns and, in accord, selection of *H. pylori* clones with optimum adherence phenotype (36).

## 5.4 Immune Response

If a microbe is to persist in a vertebrate host, its biggest challenge is to avoid clearance by the immune system. Transient *H. pylori* colonization has been documented in both primates and humans (68, 197). However, usually, following *H. pylori* acquisition, there is rapid host recognition in the form of both innate and acquired immune responses, including generation of specific local and systemic antibodies (192).

### 5.4.1 Role of antibodies in protective immunity

Based on analogy with other mucosal infections, it was initially assumed that a protective immune response against *H. pylori* would predominantly be mediated by antibodies. Reports on a good correlation between the presence of *H. pylori*

antibodies in the milk of Gambian mothers and the absence of *H. pylori* infection in their breast-fed children (151, 245) supported this assumption.

#### **5.4.2 Immune modulation**

*H. pylori* activate the acquired immune system, as indicated by both humoral and cellular recognition of its antigens (29) although it has evolved to substantially downregulate and avoid acquired immune effectors. Recognition by the acquired immune system requires antigen presentation, and *H. pylori* interfere with both uptake and processing of antigens, partially through a VacA effect (168). *H. pylori* also suppress T cell proliferation and activation and induce selective T cell apoptosis, again partially through specific VacA effects on signaling (90, 266). It evades host adaptive responses by mimicry of the gastric epithelial fucosylated (Lewis) antigens, and by antigenic variation of surface proteins including a critical pilus molecule, CagY (16).

As this high-frequency antigenic variation occurs through mutation (usually slipped-strand mispairing) and intragenomic recombination between homologous sequences (200, 247), these genetic mechanisms are important contributors to immune evasion. Finally, *H. pylori* can also suppress less specific immune mechanisms such as phagocytosis (5, 293). The relative contributions of the different host manipulation and evasion strategies to *H. pylori* persistence are not established, possibly differing in individual hosts, but the existence of these varied mechanisms implies that immune surveillance of the gastric lumen is powerful, and that bacterial survival requires its subversion.

#### **5.4.3 Activation of the innate immune response**

Innate immune system recognition of microorganisms involves Toll-like receptors (TLRs) that discriminate pathogen-associated molecular patterns (147). TLR stimulation triggers proinflammatory signaling through NF- $\kappa$ B activation, and *H.*

*pylori* has evolved to minimize such stimulation and chemokine expression in the cells of the gastric mucosa.

The intracellular peptidoglycan, transferred into the cytoplasm by *cag* PAI-mediated contact between the epithelial cell and the bacterium, may be a key activator of the innate response against *H. pylori* (262). This intracellular peptidoglycan is recognized by Nod1, a member of the recently discovered Nod family. Two members of this protein family, Nod1 (also known as CARD4) (262) and Nod2 (CARD15), are involved in the recognition of bacterial peptidoglycans and seem to act as intracellular (Nod1) and extracellular (Nod2) receptors for gram-negative bacteria in epithelial cells (48, 123).

Thus, at least for *cag* PAI-positive *H. pylori* strains, the Nod1-mediated signaling seems to be a key factor in the recognition and subsequent activation of the proinflammatory response in gastric epithelial cells (262). However, only some of the *H. pylori* cells in the mucus layer actually attach to the gastric epithelial cells (40, 282); thus, differences in adhesion mediated by *H. pylori* properties and differences in host cell receptors may well influence the degree of inflammation mediated by *H. pylori* infection. This may have an important effect on the subsequent development of gastritis and disease.

#### **5.4.4 Resistance to phagocytosis and modulation of dendritic cell activity**

Although the gastric mucosal recognition of *H. pylori* is positively affected by the presence of the *cag* PAI, it does not involve CagA. NF- $\kappa$ B activation, and consequently IL-12 induction by macrophages/monocytes, even seems to be completely independent of *cag* PAI (64); in contrast to epithelial cells, however, it does involve CD14 and TLR4 (28, 156, and 235). *H. pylori* infection results in upregulation of MIP-3  $\alpha$  gene expression in gastric epithelial cells, thus inducing an influx of monocytic cells into the lamina propria of the gastric mucosa. These cells may be functionally impaired, as *H. pylori* is capable of inhibiting phagocytosis by macrophages through an as-yet-unknown pathway (3, 4, 205, and 206). This not

only results in reduced anti-*H. pylori* activity of the macrophages but more importantly results in decreased and altered processing of *H. pylori* antigens by activated macrophages (dendritic cells). Since the activation of B and T cells is dependent on the presentation of *H. pylori* antigens by dendritic cells, this is of crucial importance for the outcome of the immune response. Although the exact mode whereby *H. pylori* mediates this effect is unknown, the dendritic cell-specific surface receptor C-type lectin ICAM-3-grabbing nonintegrin (DC-SIGN; CD209) may be involved (91, 259). DC-SIGN is known to act as a receptor for some pathogens, such as human immunodeficiency virus type 1, and can also act as a ligand for *H. pylori*, as binding of *H. pylori* to monocytic cells can be blocked with anti-DC-SIGN monoclonal antibodies (15). DC-SIGN recognition seems to require the presence of the Lewis blood group determinants on the LPS, as only *H. pylori* variants that carry the Lewis blood group determinants (Le<sup>+</sup>) bind to DC-SIGN (34). This leads to a Le<sup>+</sup>-specific blocking of Th1 cell activation and IL-6 production (15). Lewis antigen is prone to phase variation; hence, there will always be a significant proportion of Le<sup>-</sup> variants within an isolate, and as this fraction increases, the inhibition of the Th1 cell response is reduced. Interestingly, *H. pylori* preferentially induces IL-12—not IL-6 or IL-10, as most other extracellular bacteria tend to do—in human dendritic cells (99).

#### 5.4.5 Regulatory T cells

IL-10-producing T cells seem to be crucial in the control of *H. pylori*-induced inflammation and enable the bacterium to persist in the gastric mucosa. For example,

*H. pylori* is unable to persist in IL-10 knockout mice (50, 126). CD25<sup>+</sup> regulatory T cells probably play a key role in this, as mice lacking CD25<sup>+</sup> cells developed more severe gastritis while having reduced bacterial loads in the gastric mucosa (204). Similarly, removal of CD25<sup>+</sup> cells from *H. pylori*-positive volunteers resulted in increased in vitro proliferation and gamma interferon production, indicating that

*H. pylori* infection results in downregulation of the immune response through interaction with regulatory T cells (234).

# Chapter Six

## **Diagnosis and Treatment**

## Chapter Six: Diagnosis and Treatment

Various tests have been developed for the detection of *H. pylori*. The available tests are generally divided into invasive tests, based on gastric specimens for histology, culture or other methods, and noninvasive tests, based on peripheral samples, such as blood, breath samples, stools, urine, or saliva for detection of antibodies, bacterial antigens, or urease activity. Additionally molecular diagnosis is an advanced procedure for identification of *H. pylori*. The choice of a specific test for an individual patient depends on local experience and the clinical setting (152, 250, and 291). In research protocols, a combination of two methods is often applied.

**6.1 The symptoms of stomach cancer:** The following are the most common symptoms of stomach cancer. However, each individual may experience symptoms differently. Symptoms may include:

- indigestion or heartburn (burning sensation)
- discomfort or pain in the abdomen
- nausea and vomiting
- diarrhea or constipation
- bloating after meals
- loss of appetite
- weakness and fatigue
- vomiting blood or blood in the stool

### 6.2 Physical diagnosis procedure

In addition to a complete medical history physical examination procedures for stomach cancer may include the following:

**6.2.1 Fecal occult blood test** - checks for hidden (occult) blood in the stool. It involves placing a very small amount of stool on a special card, which is then tested in the physician's office or sent to a laboratory.

## 6.3 Molecular Diagnosis of Stomach Cancer

**6.3.1. Polymerase-Chain-Reaction (PCR):** The PCR process is based on the heat-stable DNA polymerase mixed with a DNA template, a synthetic DNA oligonucleotide primer that anneals to the template, and the four deoxynucleoside triphosphates to make a reaction mixture. After a short synthesis period, a new copy of DNA results from elongation of the annealed primer. Because of the thermal stability of the *Taq* polymerase, this process can be repeated for many cycles with only a slight loss of enzyme activity during each cycle.

In 2005, Shiho Yamazaki et al used a simple PCR-based method for genotyping of *vacA* gene in the University of Fukui, Fukui, Japan. Two different areas of Japan were selected as sources for strains. Fukui is a typical rural prefecture located on the central Japanese mainland (Honshu), while Okinawa consists of islands in the southwestern part of Japan. A total of 220 *H. pylori* clinical isolates (115 Fukui strains and 105 Okinawa strains) were obtained during upper gastroduodenal endoscopy and gastric biopsy specimens from each patient were inoculated and cultured for 3 to 5 days at 37°C under microaerobic conditions (5% O<sub>2</sub>, 5% CO<sub>2</sub>, 90% N<sub>2</sub>). *H. pylori* cells were harvested from each plate, transferred into a brucella broth liquid culture medium containing 10% fetal calf serum, and cultured for 24 h with agitation. DNA from each *H. pylori* isolate was extracted from the pellet of the bacterial suspension, suspended in TE buffer and stored at 4°C until PCR amplification was performed.

Genotyping of the *vacA* gene was performed by PCR amplification in the s-region (the signal peptide-encoding region) and the m-region (middle region) using the primers. PCR products were separated by 2% agarose gel electrophoresis and examined under UV illumination. The outcome of the study is given below by tabular form.

**Table 3: Oligonucleotide primers used in typing of *H. pylori vacA* alleles**

|     | Primer              | Sequence <sup>a</sup>                                                        | Size and location of PCR product                           |
|-----|---------------------|------------------------------------------------------------------------------|------------------------------------------------------------|
| s1a | vacA s1a-F<br>VA1-R | 5'-CTC TCG CTT TAG TAG GAG C-3'<br>5'-CTG CTT GAA TGC GCC AAA C-3'           | 213 bp (843–1055) <sup>b</sup>                             |
| s1b | SS3-F<br>VA1-R      | 5'-AGC GCC ATA CCG CAA GAG-3'                                                | 187 bp (869–1055) <sup>c</sup>                             |
| s1c | vacA s1c-F<br>VA1-R | 5'-CTC TCG CTT TAG TGG GGY T-3'                                              | 213 bp (843–1055) <sup>c</sup>                             |
| s2  | SS2-F<br>VA1-R      | 5'-GCT AAC ACG CCA AAT GAT CC-3'                                             | 199 bp (433–631) <sup>d</sup>                              |
| m1a | VA3-F<br>VA3-R      | 5'-GGT CAA AAT GCG GTC ATG G-3'<br>5'-CCA TTG GTA CCT GTA GAA AC-3'          | 290 bp (2741–3030) <sup>b</sup>                            |
| m1b | VAm-F3<br>VAm-R3    | 5'-GGC CCC AAT GCA GTC ATG GAT-3'<br>5'-GCT GTT AGT GCC TAA AGA AGC<br>AT-3' | 291 bp 2741–3031) <sup>c</sup>                             |
| m2  | VA4-F<br>VA4-R      | 5'-GGA GCC CCA GGA AAC ATT G-3'<br>5'-CAT AAC TAG CGC CTT GCA C-3'           | 352 bp (976–1327) <sup>e</sup><br>(2284–2635) <sup>d</sup> |

<sup>a</sup>-Y, C or T.

<sup>b</sup>- Nucleotide positions in the *vacA* gene of *H. pylori* 60190 (GenBank accession no. HPU05676).

<sup>c</sup>- Corresponding to nucleotide positions of *H. pylori* 60190.

<sup>d</sup>- Nucleotide positions in the *vacA* gene of *H. pylori* Tx30a (GenBank accession no. HPU29401).

<sup>e</sup> -Nucleotide positions in the *vacA* gene of *H. pylori* 87–203 (GenBank accession no. HPU05677).

A potential advantage of PCR is that it may enable the diagnosis of *H. pylori* to be made noninvasively by detecting *H. pylori* DNA in nongastric fluids such as saliva. However, PCR methods hold great promise in the detection of genetic differences between *H. pylori* strains for research and epidemiologic studies. The disadvantage of PCR method is-it's a time consuming process for diagnosis (284).

**6.3.2. Reverse Transcription-PCR (RT-PCR):** Between January 2000 and January 2002 Takashi Marutsuka et al. have taken a study to establish an ultra-rapid quantitative reverse transcription-PCR (RT-PCR) protocol that enables the diagnosis of cancer spread during operation. In this study, they established a combined system of an ultra-rapid RT-PCR using a fully automated mRNA extractor and a real-time one-step RT-PCR system with hybridization probe format for detection of peritoneal free cancer cells. This new method enabled to obtain the results of RT-PCR with approximately 70 min after sampling. Furthermore, researchers carried out multiple markers RT-PCR assay for a combination of CEA and CK20 to eliminate false-positive results and to improve specificity. They observed 63 patients with gastric carcinoma. All had histologically proven gastric carcinoma and underwent a potentially curative operation with extended lymph node dissection (D2). No patients had received preoperative radiation therapy or chemotherapy. No patients had an intestinal perforation. The lymph nodes were cut into two equal parts under sterile conditions to prevent RNA contamination between specimens. One-half of the lymph node was fixed in 10% buffered formalin for routine histological examination, and the other half was frozen in liquid nitrogen for the RT-PCR assay. mRNA was extracted using the MagNA Pure LC system (Roche) according to the manufacturer's instructions. In brief, the isolation procedure is based on magnetic bead technology. Real-time, one-step, no-nested RT-PCR for CEA mRNA and CK20 mRNA was realized.

**Table 4: Oligonucleotide primers for CEA mRNA and CK20 mRNA Amplification**

| Amplification Type | Primer Sequences                                    | Probe Sequences for Identification                                  |
|--------------------|-----------------------------------------------------|---------------------------------------------------------------------|
| CEA                | 5'-GACGCAAGAGCCTATGTATG<br>5'-GGCATAGGTCCCGTTATTA   | 5'-CCCAGACTCGTCTTACCTTTCGG-FL<br>5'-LC-AGCGAACCTCAACCTCTCCTGC-P     |
| CK20               | 5'-GAAGTCGATGGCCTACACAA<br>5'-AACGGGCCTTGGTCTCCTCTA | 5'-CTTTGGCCTCTTGAAGGTTCTTCTG-FL<br>5'-LC-GCCATGACTTCATACTTCTGCCTCAP |

*In sequences, FL means fluorescein, LC means LC Red 640 labeling, and P means phosphate group to block extension.*

Reverse Transcription-PCR has potentiality for screening a small amount of tumor cells in circulating blood, bone marrow, lymph nodes, and peritoneal lavage fluid. Therefore, the RT-PCR technique is suitable for detecting minute quantities of free cancer cells. However, RT-PCR has its own drawbacks, such as the following: (a) results of RT-PCR are not available during surgery because of the time-consuming gene amplification and subsequent data analysis; and (b) results of RT-PCR involve, even in low frequency, false-positive results exist by DNA contamination or pseudogenes (162).

**6.3.3. DNA microarray technique and Laser Capture Microdissection:** The DNA microarray technique is a powerful tool for identifying novel genes that participate in the development and progression of gastric cancer (286). An array is an orderly arrangement of samples. It provides a medium for matching known and unknown DNA samples based on base pairing rules and automating the process of identifying unknowns.

There are two major application forms for the DNA microarray technology:

- 1) Identification of sequence (gene/gene mutation).
- 2) Determination of expression level (abundance) of genes.

Laser microdissection is an innovative technique which offers researchers a simple, reliable, rapid and accurate tool for precise and contamination-free procurement of cell groups from tissue sections under direct visualization. In laser capture microdissection, a laser microbeam of near-infrared power (at a temperature below 70°C) does not cut the tissue, but rather melts a thermoplastic ethyl vinyl acetate membrane and the melted membrane sticks to the selected cells, which can then be lifted and secured in a microfuge tube containing the appropriate extraction solution. The surrounding tissue remains unchanged. Specified lesions or cell types in complex tissues can easily be sampled without damage to the morphology of the selected zone and the surrounding tissue (268).

In 2006 Long Wang et al., using the combined methods of laser microdissection (LMD), P27-based RNA amplification, and cDNA microarray for evaluation the differentially expressed genes in primary carcinoma cells and lymph node metastatic cells in 36 of 58 patients. After P27-based amplification, the expression profiles of tumor cells of primary gastric cancer and their corresponding metastatic lymph nodes in 36 patients were observed by Scatter plots of cDNA microarray and analyzed. Scatter plots of cDNA microarray analysis performed by primary carcinoma cells and lymph node metastatic cells from 58 patients with gastric cancer were labeled by amplified aRNA from primary gastric carcinoma cells (Cy3) and metastatic carcinoma cells (Cy5) respectively, and hybridized to the cDNA microarray using laser microdissection in combination with microarray (268).

Analysis of the cDNA microarray data showed that 49 genes (including 31 with unknown function) were over-expressed in primary carcinoma cells from 36 patients. On the other hand, 37 genes (including 9 with unknown function) were significantly suppressed in primary carcinoma cells from 36 patients. The up-regulated genes were related to cell adhesion, cytoskeleton, cell defense and metabolism. Meanwhile, the down-regulated genes included those associated with cell development, cell cycle, signal transduction, adhesion, cell defense, gene expression and cell metabolism (268).

**6.3.4. Molecular Markers:** Molecular markers are used to improve the quality of histopathological diagnosis by analyzing genetic and epigenetic alterations. Understanding the structure and function of gastric cancer-associated genes is fundamental for establishing methods of gastric cancer diagnosis and quantification of an individual's risk of the disease (286). More and more molecular diagnostic markers are being identified to aid in the diagnosis of gastric cancer. The function of molecular markers is given below-

**a) Diagnosis of genetic instability:** MSI is defined as the presence of replication errors in simple repetitive microsatellite sequences due to DNA mismatch repair deficiency. MSI is detected in both gastric adenoma and intestinal metaplasia, which are precancerous lesions associated with well-differentiated gastric cancers. It is classified as high frequency (MSI-H), low-frequency (MSI-L), or stable (MSS) (272). MSI has been recognized as one of the earliest changes in carcinogenesis and results in genomic instability (166). MSI is present in a subset of gastric cancers (106), ranging from 13% to 44% of gastric tumors (70); in particular, MSI-H occurs in 10% to 16% of gastric cancers (73). Furthermore, hypermethylation of CpG islands in the promoter region of the *hMLH1* gene is associated with decreased hMLH1 protein expression and often occurs in gastric cancer cases with MSI-H, indicating that epigenetic inactivation of this gene in association with promoter methylation may underlie MSI (148). MSI in gastric cancer cases is also

associated with antral tumor location, intestinal-type differentiation, and a better prognosis (68, 70). MSI-H-positive gastric cancers are clinicopathologically distinct; thus, it may be valuable to identify subgroups of gastric cancers (102). The MSI technique is promising for the early detection of cancer: rather than searching for a specific gene, it may be used as a screening tool (48). Additional genes with simple, tandem repeat sequences within their coding regions that have been found to be specifically altered in gastric cancers displaying MSI include *BAX*, *hMSH3*, *hMSH6*, *E2F-4*, TGF- $\beta$  receptor II, and insulin-like growth factor receptor II, which are known to be involved in the regulation of cell-cycle progression and apoptotic signaling (71, 83, and 125).

Techniques of detecting MSI are promising for the early diagnosis of tumors before they become microscopically obvious.

**b) Analysis degree of malignancy:** The group of activated oncogenes consists primarily of various growth factors and growth factor receptors. For example, in previous studies, the *c-met* protooncogene, which encodes a tyrosine kinase receptor for the hepatocyte growth factor, was overexpressed in 50% of both diffuse- and intestinal-type gastric cancers (10, 46, 105, 287, and 292). Tumors having overexpression of *c-met* have tended to display increased invasiveness and be poorly differentiated (including scirrhous tumors). Also, *c-met* is amplified and/or overexpressed but not mutated in several tumors, suggesting that overexpression of normal *c-met*, rather than structural alteration is responsible for activation of the tyrosine kinase receptor (187, 197). Furthermore, rearrangement of the *tpo* and *c-met* genes has been reported in gastric cancer and identified in a small subset of gastric cancers (289).

The *c-erbB2* (*HER-2/neu*) gene, another protooncogene, is a transmembrane tyrosine kinase receptor. *HER-2/neu* protein overexpression or gene amplification is associated with approximately one-fourth of all gastrointestinal tract

malignancies (47, 213). HER-2/neu overexpression also appears to be linked with advanced rather than early disease with limited invasion.

Other oncogenes, such as cyclin E, *c-erbB3*, *K-sam*, *Ras*, and *c-myc*, have also been found to be amplified and overexpressed in gastric carcinomas (106, 137, 176, 204, and 290). In particular, *K-sam*, which belongs to the fibroblast growth factor receptor family, is frequently overexpressed in diffuse-type gastric cancers, due to amplification of it (33, 130). Additionally, more enhanced expression of the *Ras* gene was found in intestinal-type gastric cancers than in diffuse-type cancers (61) and in advanced rather than in early gastric cancer (181, 182, and 279). Expression of *c-myc* has also been found to correlate with the disease stage, depth of invasion, and peritoneal dissemination (175, 188).

**c) Differential Diagnosis:** Inactivation of tumor suppressor genes due to mutations and/or loss of heterozygosity (LOH) are also a frequent event in gastric carcinogenesis. For example, inactivation of p53 and p16 has been reported in both diffuse- and intestinal-type gastric cancers. Mutation or LOH of the *p53* gene has been reported in up to 80% of gastric cancer cases independent of the histological subtype, and predominately in cases of metastatic disease (42, 53, 79, 80, 114, 121, 122, and 263). However, *p53* mutations have also been identified in early dysplastic and metaplastic lesions (about 10%) (132, 207, and 278), and some studies have reported that *p53* alterations have a crucial role in early intestinal-type gastric carcinogenesis, likely acting at the transition step between metaplasia and dysplasia, and that the alterations are mainly associated with tumor progression in diffuse-type cancer (207).

Adenomatous polyposis coli (*APC*) gene mutations seem to occur more frequently in intestinal-type gastric cancers (41, 208, 261, and 172). Up to 60% of intestinal-type gastric tumors and approximately 25% of adenomas have mutation and/or LOH of the tumor suppressor gene *APC* (104, 113, 116, 241, and 288). These alterations are rare in diffuse-type carcinomas but may be associated with signet-

ring cell carcinomas (171). Somatic mutation of the *APC* gene plays an important role in the pathogenesis of gastric adenoma and dysplasia, but has a limited role in the progression of it to adenocarcinoma (144). In addition, the APC protein is important for the degradation of  $\beta$ -catenin (117, 170). Specifically, it binds to  $\beta$ -catenin, whose free concentration within the cell is tightly regulated and kept at a low level. After interaction with the transcription factor lymphoid enhancer factor-1 (LEF-1),  $\beta$ -catenin translocates into the nucleus, where it regulates gene expression (193).  $\beta$ -catenin mutations have also been detected in intestinal type gastric tumors, but are absent from diffuse-type tumors (45, 186). In intestinal-type gastric cancers, the accumulation of  $\beta$ -catenin protein may result from impaired degradation of the  $\beta$ -catenin protein due to alterations of the  $\beta$ -catenin and *APC* genes (62, 69, and 118).

**d) Survivin Protein:** Survivin is a protein that is highly expressed in a vast number of malignancies, but is minimally expressed in normal tissues. It plays a role as an inhibitor of cell death in cancer cells, thus facilitating the growth of these cells. In the case of gastric cancer, survivin is over-expressed in tumor cells and plays a role in the carcinogenesis process. Therefore, the detection of survivin in body fluids could serve as a diagnostic marker that allows the early detection of malignancy. Several studies on gastric cancer have indicated that there is a relationship between survivin expression and the ultimate behavior of the carcinoma. Since the expression pattern of survivin is selective to cancer cells, it plays a role as an "ideal target" for cancer therapy. Currently, several pre-clinical and clinical trials are ongoing to investigate the effects of interfering with survivin function in cancer cells as a biologic therapy. Survivin is a potentially significant protein in the diagnosis, prognosis and treatment of gastric tumors (269).

**6.4 Treatment for stomach cancer:** Specific treatment for stomach cancer will be determined by physician based on-

- Age, overall health, and medical history
- Extent of the disease
- Tolerance for specific medications, procedures, or therapies
- Expectations for the course of the disease

Treatment for stomach cancer may include:

- 6.4.1 Surgery:** Surgery may be necessary to remove cancerous tissue, as well as nearby noncancerous tissue. The most common operation is called gastrectomy. If part of the stomach is removed, it is called a subtotal or partial gastrectomy. If the entire stomach is removed, it is called a total gastrectomy.
- 6.4.2 External radiation (external beam therapy):** External radiation precisely sends high levels of radiation directly to the cancer cells. The machine is controlled by the radiation therapist. Since radiation is used to kill cancer cells and to shrink tumors, special shields may be used to protect the tissue surrounding the treatment area. Radiation treatments are painless and usually last a few minutes. External radiation may be used to ease (palliate) symptoms such as pain or blockage.
- 6.4.3 Chemotherapy:** Chemotherapy is the use of anticancer drugs to treat cancerous cells. In most cases, chemotherapy works by interfering with the cancer cell's ability to grow or reproduce. Different groups of drugs work in different ways to fight cancer cells. The oncologist will recommend a treatment plan for each individual.

**6.4.4 Laparoscopy:** Laparoscopy is another procedure used to stage some patients with stomach cancer. This involves a medical device similar to an endoscope. A laparoscopy is a minimally invasive surgery technique with one or a few small incisions, which can be performed on an outpatient basis, followed by rapid recovery. Patients who may receive radiation therapy or chemotherapy before surgery may undergo a laparoscopic procedure to determine the precise stage of cancer. If specialist needs to examine stomach in more detail, in order to look further signs of the spread of cancer, may have a small operation called a laparoscopy. This procedure is carried out under a general anaesthetic. In this procedure, physician will insert a thin viewing tube with a camera on the end (a laparoscope) through a small cut (incision) in the lower part of stomach in order to examine it. In some cases, specialist may need to examine more than one area of stomach, and may need to make more than one incision.

Sometimes, several of these treatments may be combined to treat stomach cancer (153).



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# Chapter Seven

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## **Correlation of Stomach Cancer and *H. pylori***

## Chapter Seven: Correlation of Stomach Cancer and *H. pylori*

The exact causes of stomach cancer are unclear to researchers. Nevertheless, several risk factors have been identified. They include:

- ❖ Age.
- ❖ Gender.
- ❖ Ethnicity (Stomach cancer is more common among Hispanics, African Americans, Asian, and Pacific Islanders).
- ❖ Tobacco and alcohol abuse (The risk of developing stomach cancer is higher in smokers).
- ❖ Type A blood (people with type A blood have a higher risk of developing stomach cancer).
- ❖ Family history (the risk of developing stomach cancer increases when there is a family history of stomach cancer).
- ❖ Diet (a diet rich in smoked food, salted meat and fish, and pickled vegetables increases the risk of developing stomach cancer).
- ❖ Obesity (overweight or obese people are more likely to develop stomach cancer).
- ❖ Previous stomach surgery (people that had part of their stomach removed are at a higher risk for developing stomach cancer).
- ❖ Medical conditions (*H. pylori* Infection, Pernicious Anemia, Menetrier Disease), genetic mutations (people that carry a mutation of the breast cancer genes [known as BRCA1 and BRCA2], have a higher risk for developing stomach cancer).
- ❖ Geographic location (stomach cancer is more common in Japan, China, Southern and Eastern Europe, and South and Central America. It is less common in Northern and Western Africa, Melanesia, South Central Asia, and North America) (183).

In this chapter, mainly the author was interested to investigate the correlation of stomach cancer and *H. pylori*. So, few of the research studies have been focused in this section.

### **7.1. *H. pylori* and Gastric Cancer**

Numerous studies have suggested a positive correlation between positive serological tests for *H. pylori* and gastric cancer. In 1996, the International Agency for Research on Cancer classified *H. pylori* as a Group 1 carcinogen, because *H. pylori* infection substantially increases the risk of gastric cancer development. Cancer is a several-phase process; long-term exposure to a potential mutation-inducing process considerably increases the risk of cancer development. Cell proliferation on a prolonged inflammation-affected site allows for the development of continuous mutation. As gastric cancer takes several years to develop, subjects with *H. pylori* infection acquired in early childhood are at higher risk of developing the cancer.

In 2001, Aleksandar et al. undertook a study to determine the presence of *H. pylori* infection in the gastric mucosa of cancer patients relative to patients with other clinical diagnoses (gastric ulcer, duodenal ulcer). In addition, they wanted to examine a possible correlation between *H. pylori* infection and gastric cancerogenesis in both intestinal-type and diffuse-type adenocarcinoma.

**Patients and Methods:** The study involved two treatment groups with a total of 117 patients. The first group comprised patients with various clinical diagnoses who underwent endoscopy. Following the pathologist's assessment, a total of 203 biopsy specimens of the gastric mucosa were taken and analyzed using microbiological and histopathological methods in order to identify pathological processes in the mucosa, as well as the presence of *H. pylori* infection. The second treatment group comprised 74 patients with gastric cancer, who were examined for the gastric cancer type, *H. pylori* infection, and the cancer localization in the

antrum, corpus, and ulceration border of gastric ulcer patients, and from the infiltration- adjacent region, i.e., the endoscopy-presented unaffected mucosa of cancer patients. The microbiological analysis included: preparation of direct microscopic smears stained according to Gram and *H. pylori* cultivation. For the latter, they used a solid selective medium. *H. pylori* was identified on the basis of morphologic appearance of the cells, morphology of the colonies, and urease, catalase, and oxidase tests.

**Results:** In order to determine the presence of *H. pylori* infection and to verify gastritis, 203 biopsy specimens of the gastric mucosa (one or more per patient) were taken and analyzed histopathologically. Distribution of *H. pylori* positive tests by clinical diagnoses is given in Table 8. The presence of *H. pylori* was confirmed at a high rate for the patients with various clinical diagnoses. The rate was also high for the patients who declined endoscopy (84.61%).

**Table 8. *H. pylori* in clinical gastric disease**

| Diagnosis                   | No. of patients | Helicobacter pylori test |       |          |       |
|-----------------------------|-----------------|--------------------------|-------|----------|-------|
|                             |                 | Positive                 |       | Negative |       |
|                             |                 | No.                      | %     | No.      | %     |
| Gastric ulcer               | 32              | 27                       | 84.37 | 5        | 15.62 |
| Duodenal ulcer              | 31              | 27                       | 87.09 | 4        | 12.90 |
| Gastric cancer              | 8               | 7                        | 87.50 | 1        | 12.50 |
| Pericious anemia            | 6               | 5                        | 83.33 | 1        | 16.66 |
| Resected stomach            | 19              | 14                       | 73.68 | 5        | 26.31 |
| Benign epithelial tumors    | 8               | 6                        | 75.00 | 2        | 25.00 |
| Negative endoscopic finding | 13              | 11                       | 84.61 | 2        | 15.38 |
| Total                       | 117             | 97                       | 82.90 | 20       | 17.09 |

There are no statistically significant differences in the prevalence of *H. pylori* positive tests between the patients with gastric ulcer and those with gastric cancer (Fisher exact test:  $p=1.00$ ;  $p>0.05$ ). No statistically significant difference in the

prevalence of positive tests for *H. pylori* was established between the patients with duodenal ulcer and those with gastric cancer (Fisher exact test:  $p=1.00$ ;  $p>0.05$ ).

Positive tests for *H. pylori* were also analyzed relative to the colonization site in the stomach. The bacterium was found in the antrum of 78.35% patients and in the corpus of 61.95% patients.

**Table 9. Localization of intestinal-type and diffuse-type gastric cancer and *H. pylori***

| Localization                      | Intestinal-type gastric cancer |       | Diffuse-type gastric cancer |       |
|-----------------------------------|--------------------------------|-------|-----------------------------|-------|
|                                   | No.                            | %     | No.                         | %     |
| The number of cancer patients     | 52                             | 70.27 | 22                          | 29.72 |
| <i>H. pylori</i> positive finding | 37                             | 71.15 | 15                          | 68.18 |
| Localization in the antrum        | 20                             | 38.46 | 10                          | 45.45 |
| Localization in the corpus        | 19                             | 36.53 | 8                           | 36.36 |
| Localization in the fornix        | 13                             | 25.0  | 4                           | 18.18 |

The second group comprised 74 patients with gastric cancer (Table 9); 27-75 years of age (mean age, 51.10 years). Of a total number of the patients examined for adenocarcinoma, 46 cases (62.16%) were registered in males, whilst 28 cases (37.82%) were registered in females. Intestinal-type adenocarcinoma was diagnosed in 52 patients, of whom 37 (71.15%) were *H. pylori* positive. Diffuse-type adenocarcinoma was diagnosed in 22 patients who showed a lower rate (68.18%) of *H. pylori* incidence. No statistically significant difference was revealed in the frequency of *H. pylori* positive findings between the patients with intestinal-type and those with diffuse-type gastric cancer ( $\chi^2= 0.07$ ;  $p= 0.798$ ;  $p>0.05$ ).

**Discussion:** Aleksandar et al. examined the presence of *H. pylori* in the gastric mucosa of 117 patients who underwent endoscopy due to disorders in their upper epigastrium. They confirmed the presence of *H. pylori* in 84.37% patients with gastric ulcer, and 87.09% patients with duodenal bulb ulcer. These patients served as controls to *H. pylori* positive patients with gastric cancer. Out of 117 examined patients who underwent gastroscopy, 8 were found to have gastric cancer. In 87.50% of them *H. pylori* was detected in the mucosa surrounding gastric cancer. It is well known that this microorganism does not colonize the cells of the cancer, but when accompanied with gastric cancer it helps maintain the colonization of the non-neoplastic gastric epithelium.

**Conclusion:** *H. pylori* infection has a significant role in the development of gastric adenocarcinoma. No statistically significant difference in the incidence of *H. pylori* positive tests exists between the patients with intestinal-type adenocarcinoma and those with diffuse-type adenocarcinoma (2).

## **7.2. Analysis of *H. pylori* Genotypes and Correlation with Clinical Outcome.**

The *H. pylori* genotypes and the geographic distribution are linked to the severity of peptic ulcer disease (PUD). *H. pylori* appears to be one of the most genetically diverse bacterial species, as evidenced by the presence, among different strains, of nonconserved DNA fragments such as the *cagA* gene in the *cag* pathogenicity island and allelic variation within the *vacA* gene. Such diversity was found to affect the function and antigenicity of virulence factors associated with bacterial infection and, ultimately, disease outcome. Turkey's geographic location, which has been under continuous influences from both Asian and Western countries, has made it an ideal site for epidemiological studies on *H. pylori* infection and genotyping. The prevalence of gastric cancer in Turkey is much lower than that in certain Asian countries (e.g., Japan) according to the World Health Organization world cancer report (annual rates of 4,440 cases [Turkey] versus 115,294 cases [Japan]/100,000 individuals); however, it is not known whether the difference is due to host,

environment, or bacterial factors or a combination of these factors. In 2003 Huseyin Saribasak, Barik A. Salih, Yoshio Yamaoka, and Ersan Sander were aimed to detect whether the *cagA* and *vacA* genotypes of the Turkish strains were of the genotypes typically observed in Western countries or in Asia and to determine their association with clinical outcomes.

**Patients and Methods:** Seventy-three patients (42 males, 31 females; mean age, 46 years; age range, 17 to 80 years) (22 with gastritis, 33 with PUD, and 10 with gastric cancer) were enrolled in this study. Three biopsy specimens—for *Campylobacter*-like organism (CLO) test, culture, and PCR—were taken from the antral part of the stomach of each patient. Patients had received no treatment for *H. pylori* infection. Informed consent was obtained from all patients and approved by an ethics committee at Fatih University.

**Results:** Patients were considered infected with *H. pylori* when the gastric biopsy specimens gave positive results in any one of the objective tests: CLO test, culture, or PCR. Sixty-five (89%) of 73 patients were *H. pylori* positive. Among these, 49 (75%) were positive by the CLO test, 48 (74%) were positive by culture, and 65 (100%) were positive by PCR. PCR amplification of DNA sample directly from gastric biopsy specimens detected the *cagA* gene in 51 (78%) of 65 patients, including 12 (55%) of 22 patients with gastritis, 5 (100%) of 5 patients with gastric ulcer, 25 (89%) of 28 patients with duodenal ulcer, and 9 (90%) of 10 patients with gastric cancer (Table 5). All *cagA* genotypes detected were type 2a, which was typically observed in strains from Western countries and showed a significant association with PUD ( $P < 0.00001$ ) and gastric cancer ( $P < 0.001$ ). PCR amplification of DNA samples isolated from culture material (single colony or pool of colonies) gave similar results to the DNA isolated directly from gastric biopsy specimens.

**Table 5. *H. pylori* *cagA* and *vacA* s1a genotype correlation with PUD**

| Patient group <sup>a</sup> | No. (%) positive for: <i>cagA</i> | No. (%) positive for: <i>vacA</i> s1a |
|----------------------------|-----------------------------------|---------------------------------------|
| G ( <i>n</i> = 22)         | 12 (54)                           | 18 (82)                               |
| GU ( <i>n</i> = 5)         | 5 (100)                           | 5 (100)                               |
| DU ( <i>n</i> = 28)        | 25 (89)                           | 23 (82)                               |
| GC ( <i>n</i> = 10)        | 9 (90)                            | 8 (80)                                |
| Total ( <i>n</i> = 65)     | 51 (78)                           | 54 (83)                               |

<sup>a</sup> Abbreviations: G, gastritis; GU, gastric ulcer; DU, duodenal ulcer; GC, gastric cancer; *n*, number examined.

The *vacA* s1a genotype was detected in 54 (83%) of 65 patients: 18 (82%) of 22 with gastritis, 5 (100%) of 5 with gastric ulcer, 23 (82%) of 28 with duodenal ulcer, and 8 (80%) of 10 with gastric cancer (Table 6). The *vacA* s1a genotype had no significant association with PUD. No other *vacA* s1 subtype was detected in this study except for one s1b subtype in a patient with gastritis. The *vacA* s2 genotype was found in three (5%) of the patients. The *vacA* m1 genotype was detected in 8 (12%) patients, while the m2 genotype was detected in 28 (43%) of the 65 patients. All m1 genotypes were m1a genotype, and those of the m2 genotype were m2a genotype (Table 6). Both genotypes have been thought to be specific to strains from Western countries. Table 5 shows the association between the *cagA* and *vacA* s1a expression and PUD. The expression of the *cagA* gene was strongly associated with that of the *vacA* s1a genotype ( $P < 0.0001$ ) in patients with gastric ulcer, duodenal ulcer, and gastric cancer. No significant association was found between *cagA* and *vacA* m1 or m2 genotypes and PUD. Multiple *vacA* genotypes were significantly more prevalent in PUD and gastric cancer patients than in patients with gastritis (Table 6).

**Table 6. *H. pylori* vacA s and m regions subtyping in dyspeptic patients**

No. of patients with subtyping result

| Patient group <sup>a</sup> | s1a | s1b | s2 | M | N | m1a | m2a | M  | N |
|----------------------------|-----|-----|----|---|---|-----|-----|----|---|
| G (n = 22)                 | 18  | 1   | 1  | 2 | 0 | 2   | 12  | 8  | 0 |
| GU (n = 5)                 | 5   | 0   | 0  | 0 | 0 | 0   | 2   | 3  | 0 |
| DU (n = 28)                | 23  | 0   | 1  | 3 | 1 | 3   | 10  | 15 | 0 |
| GC (n = 10)                | 8   | 0   | 1  | 1 | 0 | 3   | 4   | 3  | 0 |
| Total (n = 65)             | 54  | 1   | 3  | 6 | 1 | 8   | 28  | 29 | 0 |

<sup>a</sup> Abbreviations: G, gastritis; GU, gastric ulcer; DU, duodenal ulcer; GC, gastric cancer; n number examined; M, multiple infections (either s or m subtypes); N, negative (either s or m subtypes).

PCR-RFLP analysis performed on specimens from 30 randomly selected subjects revealed three different patterns following digestion of the amplified *cagA* gene with the HaeIII enzyme (Table 7). These patterns had no significant association with clinical outcomes (220, 221).

**Table 7. RFLP-PCR patterns of *cagA* gene in a representative group of patients<sup>a</sup>**

|         | No. of patients |   |    |    |       |
|---------|-----------------|---|----|----|-------|
| Pattern | G               | G | DU | GC | Total |
| No cut  | 4               | 1 | 5  | 1  | 11    |
| I       | 4               | 1 | 7  | 1  | 13    |
| II      | 0               | 0 | 4  | 0  | 4     |
| III     | 1               | 1 | 0  | 0  | 2     |
| Total   | 9               | 3 | 16 | 2  | 30    |

### **7.3. Diversity of *H. pylori vacA* and *cagA* Genes and Relationship to VacA and CagA Protein Expression, Cytotoxin Production, and Associated Diseases**

In 1997 Rudi et al. had taken a research study and the objectives of this study were (i) to characterize the *vacA* alleles and analyze differences in the *cagA* gene that are present in German *H. pylori* strains and (ii) to correlate differences found within these genes with VacA and CagA protein expression, in vitro cytotoxicity, and clinical outcome.

#### **Results:**

**Strain collection.** Sixty-five strains were isolated from *H. pylori*-positive patients with dyspeptic symptoms. Endoscopic and histological examination revealed gastritis in 43 patients, PUD in 19 patients (9 with gastric ulcers of the antrum and 10 with duodenal ulcers), and gastric adenocarcinoma in 3 patients. In all patients with gastric ulcers, moderate to severe antral predominant chronic gastritis was diagnosed histologically. Patients with duodenal ulcers presented with duodenitis and, in some cases, with chronic antral gastritis. In six patients, two isolates were obtained from the antrum and the corpus. For each of these patients, both isolates showed identical features by PCR, Southern blotting, and Western blot analysis.

**Determination of *vacA* genotypes and relationship to gastrointestinal disease:** The *vacA* gene was detectable in all *H. pylori* isolates and the predicted PCR products of either 201 or 228 bp were obtained from 64 (98.5%) of 65 isolates. Fifty-four (83.1%) of 65 strains yielded the smaller product, representing genotype s1, and 10 (15.4%) yielded the larger product, representing genotype s2.

## Discussion:

*H. pylori* strains with the *vacA* signal sequence type s1 were predominant in the series of German *H. pylori* isolates, whereas strains with type s2 rarely occurred. Cytotoxic *H. pylori* strains with *vacA* genotype s1 may occur more frequently in such patients. *vacA* genotype s1 was strongly associated with the presence of *cagA* and was more frequent in patients with PUD (24). The difference between *vacA* type s2 and type s1 leader sequences is generated by small inserts totaling 27 bp (20). It has been hypothesized that strains with *vacA* genotype s2 lack effective secretion of VacA through the cytoplasmic membrane of the bacteria (222) because of the use of nonconcentrated broth culture supernatants for vacuolation testing.

Subgroup analysis demonstrated that the *vacA* middle-region type was not independently associated with the clinical outcome of *H. pylori* infection, the presence of *cagA*, or detectable cytotoxin activity. Almost half of the strains contained the combination s1-m2, a finding which is different from the results found in the United States (12) where the genotypes s1-m1 and s2-m2 are more common.

About 74% of all isolates were *cagA*<sup>+</sup> by PCR, which is in agreement with the results of other studies from Europe (60, 270, 275, and 277). The majority of the patients with PUD (84%) were infected with *cagA*<sup>+</sup> strains in contrast to strains isolated from patients with gastritis only, in whom 67% of the *H. pylori* strains were *cagA*<sup>+</sup> (271, 275).

In conclusion, *H. pylori* strains with the *vacA* signal sequence type s1 and middle-region allele m2 were predominant in German *H. pylori* isolates. All combinations of these *vacA* alleles occurred, with the exception of s2-m1. Type s1 strains were associated with peptic ulceration, presence of the *cagA* gene, *VacA* expression, and

cytotoxin activity. Size variations in a distinct region of *cagA* are common, but their significance for clinical outcome needs further evaluation (215).

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# Chapter Eight

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## **Discussions**

## Chapter Eight: Discussions

In 1979 Robin Warren, a pathologist in Perth, Western Australia, began to notice that curved bacteria often were present in gastric biopsy specimens submitted for histological examination. These organisms were not present within the gastric mucosa but were present in the mucus layer overlying the tissue (160, 270). A young trainee in internal medicine, Barry Marshall, became interested in Warren's observations, and together the two sought to isolate the organisms from biopsy specimens. The first isolation of *Helicobacter pylori* in 1982 by Marshall and Warren (161) ushered in a new era in gastric microbiology. Although spiral organisms had been observed in the gastric mucus layer many times in the preceding century, the isolation of *H. pylori*, in conjunction with increased interest in the pathogenesis of gastroduodenal diseases, as well as the relatively frequent availability of clinical specimens via endoscopic biopsy, has led to important breakthroughs in medical care.

Marshall and Warren noted that *H. pylori* infection was associated with duodenal ulceration (161), and this observation too was rapidly confirmed and extended to include gastric ulceration. By 1994, a consensus conference convened by the National Institutes of Health concluded that *H. pylori* was a major cause of peptic ulcer disease (174). A wide body of evidence had indicated that chronic gastritis is linked to the development of adenocarcinoma of the stomach, the most important gastric malignancy in the world (52). In 1994, the International Agency for Cancer Research, an arm of the World Health Organization, reviewed the available evidence and declared that *H. pylori* was a carcinogen of humans (14).

The natural course of infection with *H. pylori* is extremely variable. Most infected individuals remain asymptomatic, and infection with *H. pylori* is not sufficient to induce gastric cancer. Therefore, other factors — known bacterial and host cofactors, and perhaps unknown cofactors — are required. Infection is typically acquired early in life and is followed by a long quiescent phase when there is a

chronic gastritis of variable intensity but with minimal symptoms. Only 10%–15% of individuals infected with *H. pylori* develops peptic ulcer disease, and the risk of gastric cancer is estimated to be approximately 1%–3% (243). The intragastric distribution and severity of this chronic inflammatory process depend on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production. Early investigations of the differential pathogenic properties of *H. pylori* strains indicated that this increased pathogenicity correlated with the ability of more virulent strains to induce morphological changes, vacuolization, and successive degeneration of *in vitro*-cultured cells (150). The 2 genetic loci linked with virulence and inflammatory responses that have been most studied are the *cag* locus (*cag* Pathogenicity Island) and the *vacA* (vacuolating cytotoxin) gene (194). *H. pylori* exhibit enormous genetic variation, resulting mainly from extensive recombination between *H. pylori* strains (97, 238). For many strain comparisons, this has led to complete linkage equilibrium between genes and also (uniquely among bacteria) between loci within genes (98). Sequence analyses of *vacA* alleles from different *H. pylori* strains have shown that there are more pronounced differences between alleles in some regions of the gene than in others (19, 57); for example, a region near the 5' end of the gene is relatively well conserved between alleles. The portion of *vacA* that exhibits maximum diversity in an ~800-bp “mid region”, which encodes part of the 58 kDa domain of VacA (19, 22). In contrast to the region near the 5' end of *vacA*, sequences from the mid region can be phylogenetically divided into two families of alleles, termed m1 and m2. Aside from the *vacA* mid region, various other smaller regions differ markedly between *vacA* alleles. In particular, the “signal region”, encoding part of the amino-terminal signal peptide and the amino terminus of the processed toxin, exhibits striking diversity between strains (19). Two main allelic families of signal regions are recognized, termed s1 and s2, and the s1 type can be further subdivided into s1a, s1b, and s1c (19, 258). As a result of extensive

recombination among *vacA* alleles, all combinations of signal and mid regions can theoretically arise (s1a / m1, s1b / m1, s1c / m1, s1a / m2, etc) (169, 271).

Differences between *vacA* alleles from many *H. pylori* strains can not cause cell vacuolation *in vitro*, despite all strains having a copy of the toxin gene *vacA* (19, 57). Nonsense mutation in *vacA* alleles have been detected, especially in Japan (126), and these account for absence of toxin activity in some *H. pylori* strains. However, most western isolates of *H. pylori* express full-length VacA proteins (25), and for such strains, *vacA* genotype is a major determinant of vacuolating activity. VacA proteins with a type s2 signal sequence undergo amino-terminal signal peptide cleavage at a different site to VacA proteins with a type s1 signal sequence (27). As a consequence, secreted type s2 VacA has a hydrophilic amino terminus, which has been shown to block toxin activity (145). Type s1 / m1 VacA proteins are active on a wide variety of epithelial cell lines, whereas at least some tested type s1 / m2 VacA proteins, although active on primary gastric epithelial cells and RK-13 cells, are minimally active on HeLa cells (185). Further variations among signal and mid regions beyond the s1 / s2 and m1/ m2 divisions have not yet been demonstrated to affect VacA toxicity. Most studies from outside Asia have shown that *vacA* s2 strains are less commonly associated with peptic ulceration or gastric carcinoma than s1 strains (19, 23, 30, 74, 76, 107, and 131). It is still unclear whether *vacA* s2 strains are entirely nonulcerogenic. Functional *cag* PAI encodes a type IV secretion system that translocates CagA (which is also encoded by the *cag* PAI) into gastric epithelial cells. Once internalized, tyrosine residues of CagA are phosphorylated by SRC family kinases, and phosphorylated CagA then activates SRC-homology 2 domain-containing protein tyrosine phosphatase 2 (SHP2) and ERK. This process results in morphological derangement of epithelial cells (111, 195, and 249). On the basis of differences in their tyrosine phosphorylation motifs (TPMs), researchers have suggested that distinct CagA proteins have different biological activities (110). These differences in the TPMs of CagA proteins from strains of *H. pylori* isolated in different parts of the world might explain, in part, the

marked differences in gastric cancer noted in these different geographic regions. Because of the close genetic association between the *vacA* s1 signal type and presence of the *cag* PaI, it is possible that one is merely a marker for the other, although it is equally possible that both are important for disease. A third potential virulence determinant is the blood group antigen binding adhesion, BabA. The *babA* gene, like *vacA*, is polymorphic, and one allelic type, babA2, is disease associated (95). Use of all three markers (*vacA* s1, *cagA*, *babA2*) is a better predictor of pathogenic potential than use of one or two. Two studies have shown an association of type m1 strains with gastric adenocarcinoma (133, 255). Intriguingly, nearly all strains from Japan are *vacA* s1 /m1 (216, 226, 283), the most toxic type, making it interesting to speculate that toxicity may contribute to the high prevalence of gastric adenocarcinoma in Japan. Additionally many adhesions and outer membrane proteins have their putative role in the pathogenesis of *H. pylori* infection. They are BabA (HopS), OipA (HopH), SabA (HopP), *ice A*, *hp0638* gene, AlpA, AlpB, HP-NAP, plasticity region, DupA and lipopolysaccharide (189).

The mechanisms underlying *H. pylori* induced peptic ulceration and gastric adenocarcinoma are unclear. Inflammation, direct toxic activity, or both could be important. The level of inflammation in the gastric mucosa is associated with *vacA* signal type (s1>s2). Gastric epithelial damage is also associated with *vacA* signal type, but one study has shown a further association with *vacA* mid region type (m1>m2) (23, 100).

A variety of tests are now available to diagnose *H. pylori* infection. Histological examination of gastric tissue, bacterial culture, rapid urease testing, fecal occult blood test, esophagogastroduodenoscopy, endoscopic ultrasound, use of DNA probes, and PCR analysis (252).

Based on the molecular pathology, it is possible to improve the quality of histopathological diagnosis by analyzing genetic and epigenetic alterations.

Understanding the structure and function of gastric cancer-associated genes is fundamental for establishing methods of gastric cancer diagnosis and quantification of an individual's risk of the disease. More and more molecular diagnostic markers are being identified to aid in the diagnosis of gastric cancer, e.g., p53, APC, and CD44 have been used as markers for differential diagnosis; epidermal growth factor receptor (EGFR), c-met, and c-erbB2 have been used as markers for the degree of malignancy; and MSI has been used as a marker for the screening of genetic instability (285).

MSI causes accumulation of genetic alterations and has been recognized as one of the earliest changes in gastric carcinogenesis. Researchers have found that inactivation of the *p53* and *APC* genes in early gastric cancer can be used for early detection, whereas the amplification and overexpression of c-met, K-sam, c-erbB2, and cyclin E, and the overexpression of growth factors are indicators of high-grade tumors (285). Recently, an increasing number of technologies, such as laser microdissection, have become available for gastric cancer diagnosis. In addition, the DNA microarray technique is a powerful tool for identifying novel genes that participate in the development and progression of gastric cancer (285). Using cDNA microarray analysis, the expression of hundreds of potential molecular markers can be evaluated in a short period of time (92, 112), in contrast with the current capability to assay only a single or few molecules. For example, Boussioutas and colleagues (40) have recently shown some exciting results on the expression signatures that are characteristic of premalignant gastric mucosa or gastric cancers.

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# Chapter Nine

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**Conclusion**

## Chapter Nine: Conclusion

Gastric cancer is a malignant disease occurs in the stomach. The aim of this exertion is to attain knowledge regarding pathogenesis of *H. pylori* infection, with highlighting on its molecular biological aspects. This review endow with the role of *H. pylori* in gastroduodenal disease. The intragastric distribution and severity of this chronic inflammatory process depend on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production. Early investigations of the differential pathogenic properties of *H. pylori* strains indicated that this increased pathogenicity correlated with the ability of more virulent strains to induce morphological changes, vacuolization, and successive degeneration of *in vitro* cultured cells. Strain-to-strain genetic variability in bacterial virulence factors such as *vacA* and *cagA* not only affects the ability of the organism to colonize and cause disease but also affects inflammation and gastric acid output. In the continuous interactions with the host, the bacteria are able to adapt by mutations and DNA rearrangements, rendering novel genotypes. On the host side, variations in the host immune response to the chronic presence of *H. pylori* directly impact *H. pylori*-associated gastric disease and affect gastric acid output and thereby the density and location of *H. pylori* cells. Additionally many adhesions and outer membrane proteins have their presumed role in the pathogenesis of *H. pylori* infection. They are BabA (HopS), OipA (HopH), SabA (HopP), *ice A*, *hp0638* gene, AlpA, AlpB, HP-NAP, plasticity region, DupA and lipopolysaccaride. Infection with *H. pylori* strains containing *babA2*, *cagA*, and *vacA* S1 confers the highest risk of gastric cancer.

Further the functions of the unknown genes in *H. pylori* will be greatest challenge. It seems likely that these orphan genes encode many of the phenotypic characteristics that allow *H. pylori* to function and thrive in the hostile gastric environment.

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