Role of *Helicobacter pylori* Virulence Factor CagA in Gastric Cancer

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**ABSTRACT**

In 1983, Australian scientists Warren and Marshall identified *Helicobacter pylori* also known as *H. pylori* as the stomach bacteria responsible for peptic ulcer disease. For their discovery, they were awarded the 2005 Nobel Prize in Physiology or Medicine. Other studies conducted in the early 1990s demonstrated that *H. pylori* is also responsible for gastric cancer. As a prominent human infection, *H. pylori* established strong link between ulcers and cancer. It has been proposed that specific analysis of *Helicobacter pylori* virulence factor can be suitable for predicting of *H. pylori* infection disorders like Gastric Cancer (GC). Colonization by *H. pylori* is extremely common worldwide, affecting about half of the world’s population, and most carriers develop neither ulcers nor cancer. Gastric cancer is the third most common cause of cancer-related death in the world. This article is designed to evaluate the association between specific virulence factor of *H. pylori* and Gastric Cancer. One major virulence factor in *H. pylori* is the cytotoxin-associated gene a (CagA). The carriage of CagA+ stain is proinflammatory and associated with increased risk of gastric cancer. The product of the CagA gene CagA enters gastric epithelial cells directly through the type IV secretion system. The overexpression of *H. pylori* CagA antigen results in dysregulation of cellular signalling, which increases the risk of gastric cancer. The review also discusses the epidemiological data linking *H. pylori* strains that are CagA positive to infection and a higher risk of gastric cancer including the eradication.

**Keywords:** *Helicobacter pylori*, CagA, Gastric Cancer, Virulence factor, Type IV.

**INTRODUCTION**

Gastric cancer accounted for almost 1,000,000 new cases and 768,000 fatalities in 2020, ranking it as the fourth most common cause of cancer-related death overall and the sixth most commonly diagnosed disease (Salvatori et al., 2023). Men experience incidence rates twice as high as women do, with East Asia having unusually high rates (Sung et al., 2021). *Helicobacter pylori* (*H. pylori*) is a gram-negative, microaerophilic spiral-shaped bacteria infecting numerous people all over the world (nejati et al., 2018). The human stomach is colonized by this species, a Class I carcinogen that can cause both malignant neoplastic diseases (gastric cancer and mucosa-associated lymphoid tissue) and inflammatory conditions (chronic gastritis and ulceration) (Matsuo et al., 2017). The distinct bacteria have evolved to withstand the severe and acidic conditions found in the stomach (Salvator et al., 2023). Through Outer Membrane Proteins (OMPs), *H. pylori* binds to stomach epithelial cells. *H. pylori*’s outer membrane is essential for the adhesion and colonization of stomach cells. Inflammation is the outcome of *H. pylori* pathogenesis following colonization (Matsuo et al., 2017). So, gastric cancer is a condition that some *H. pylori*-infected patients experience; however, this usually needs a long-term, persistent infection. According to earlier research, the risk of developing Gastric Cancer (GC) was six times higher in those with *H. pylori* infection than in healthy individual (Iran., 2017). All the types of *H. pylori* genetically are located in two major groups, including CagA+ (cytotoxin-associated gene A) and CagA−, such that CagA is the main virulence factor of this bacterium and after that second intensive virulence factor of the bacterium is the vacuolating cytotoxin A, VacA (Kim et al., 2021). These virulence factors contribute to successful *H. pylori* colonization and pathogenicity (Freire et al., 2022).

**Epidemiology of *H. pylori* and Gastric Cancer**

The prevalence of *H. pylori* infection varies worldwide. The incidence of HP infection has been declining in many nations as living conditions have increased. However, this bacterium is still very common, particularly in the Far East (Hooi et al., 2017). Although *H. pylori* is widely distributed, its distribution is not uniform globally as compared to developed countries, developing countries have higher infection prevalence (Azuma et al., 2009).
Nonetheless, there is minimal association seen between regions with high GC prevalence and those with high H. pylori infection rates. While H. pylori infection can affect up to 91% of the population in African nations, GC is extremely rare (Salvatori et al., 2023; Hunt et al., 2011). Reportedly, less developed Asian nations like Bangladesh and India have a similar pattern. Nonetheless, a favourable association between the frequency of GC and H. pylori infection rates has been observed in more industrialized Asian nations including China, Japan, and Korea (Salvatori et al., 2023; Fock et al., 2014).

This variation could be explained by a number of variables, such as the host’s genetic makeup, the kind of H. pylori strains, the age at infection, and environmental factors such as diet and smoking (Wang et al., 2014). Epidemiology studies have showed that people with high salt diets or have iron deficiency are at higher risk, smoking is also a cofactor that increases the risk of gastric cancer (Safaralizadeh et al., 2017). Host factors include inflammatory response which increases the risk. Lastly, we have the bacterial factors that play role (Park et al., 2018).

**CagA: its expression in gastric biology**

CagA was found to be part of a pathogenicity island, which was revealed when investigators sequenced CagA and the genes next to it (Ailloud et al., 2021). A pathogenicity island is a cassette of genes acquired horizontally by some strains of H. pylori but not all strains. (Tahimina et al., 202) Most of the genes in this island resembles components of a molecular microsyringe that is called a type IV secretion system. The type IV secretion system injects CagA into host cells. CagA can have dramatic effect on host epithelial cells (Palrasu et al., 2020).

In 1995, some reported in Cancer Research that carriage of a CagA+ strain was associated with increased risk of gastric cancer (Covacci & Rappuoli., 2002; Blaser et al., 1995). As already described earlier, that adjacent to CagA on the bacterial chromosome was a homolog to a Type IV Secretion System (T4SS) protein (cagE or virB4), and mutating that gene eliminated major CagA effects on epithelial cells but at this stage researchers did not understand all of the steps in the process. After these early studies, a breathtaking expansion of research on CagA occurred (Crowe, 2019). Which revealed that carriage of CagA+ strain is proinflammatory and associated with increased risk of gastric cancer (Leung et al., 2019). The over expression of H. pylori CagA antigen results in dysregulation of cellular signaling, which increases the risk. Cag A protein of H. pylori gets injected into the host cell by exploiting its type IV secretion system (Lettl et al., 2021). Several host surface receptors and H. pylori effectors, BabA and SabA are known to facilitate its translocation in the host cells (Sharndama & Mba., 2022). When five groups independently reported in 2000 that H. pylori may encode a functional membrane-spanning T4SS and that the CagA protein itself is the main substrate that it translocates into host cells, it marked a significant advancement in the study of CagA (Backert & Blaser., 2016). These genes, along with others, are located on a bacterial chromosomal region known as the “Cag Island.” The island was acquired horizontally from an unidentified ancestor and integrated into the H. pylori chromosome (Dampier & Jozeren., 2007). It is a locus of approximately 40 kb that carries up to 32 genes and is normally bordered by 31-bp direct repeats. Later research has revealed that the cag T4SS also translocates additional H. pylori substrates, such as peptidoglycan, proinflammatory response. But H. pylori also has various T4SSs with distinct functions (Venerito et al., 2019).

The production of CagA raises the risk of gastric cancer, and the pathogenicity of the CagA protein causes disruptions in cellular signalling (Ansari & Yamaoka., 2020; Jegtmeyer et al., 2017). Previous research has demonstrated that the expression level of CagA influences the variation in steady-state levels of the bacteria among H. pylori strains (Canzian et al., 2020). The particular motif influences CagA expression. In grown gastric cells, this motif (the +59 motif) also increased the expression of higher levels of IL-8 production, whereas strains lacking this motif showed decreased expression (Loh et al., 2019). The inner surface of the plasma membrane is where translocated CagA is subsequently localized and tyrosine phosphorylated by multiple Src Family Kinases (SFK), including c-Src, Fyn, Lyn, and Yes. Without any external stimulation, SFK phosphorylates CagA, suggesting that SFK are constitutively activated in gastric epithelial cells. In mammalian cells, tyrosine phosphorylation is generally involved in the transmission of intracellular signaling for growth, motility, or differentiation. Thus, the discovery presented the intriguing hypothesis that the bacterial protein disrupts signal transduction upon tyrosine phosphorylation, causing cellular malfunction (Hatakeyama & Higashi., 2005).

**CagA Translocation**

After being synthesized by H. pylori strains, CagA is translocated into the gastric epithelial cells, where it forms T4SS (a syringe-like structure) with the help of at least 15 proteins encoded by the cagPAI (Backert et al., 2017). It has been discovered that more T4SS is expressed by the H. pylori bacteria colonizing the basolateral surfaces than by those colonizing the apical surfaces (Businello et al., 2021). T4SS pili were shown to be present in over 70% of H. pylori strains that colonized the basolateral surfaces, while most strains that colonized the apical surfaces either showed no T4SS pili at all or very few with only one pilus (Akhavasfar et al., 2023). This result implied that H. pylori was more able to express the T4SS system apparatus when it colonized the integrin-rich basolateral surfaces as opposed to the apical surface (Tegtmeyer et al., 2017). Through T4SS, CagA is exposed on the bacterial surface where it interacts with phosphatidylserine (PS) patches on the host cell’s plasma membrane that have abnormally externalized as a result of the H. pylori infection (Sguuras et al., 2015). It has been discovered that the PS patches engage with the
N-terminal region of CagA, causing the bound CagA to flip inside and cause the internalization of the CagA. (Murata et al., 2010). However, a variety of bacterial and host co-factors are required for the translocation of CagA, which is mediated by T4SS across the host cell membrane (Nell et al., 2018). Recent research revealed a strong relationship between CagV and CagA’s surface localization and translocation across the host plasma membrane (Kumar et al., 2017).

**Eradication of H. pylori**

*H. pylori* can be treated with a combination of antibiotics and an acid reducing proton pump inhibitor. Combination therapy with Proton Pump Inhibitors (PPIs) and at least two antibiotics is advised due to the difficulty in treating *H. pylori* infections. However, adverse effects of *H. pylori* therapy may be a factor in patient noncompliance. The most commonly used antibiotics along with their side effects are listed in Table 1.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>side effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>Altered taste, GI upset and diarrhoea.</td>
<td>(Goderska., 2018)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Dyspepsia, a metallic taste, and a disulfiram-like reaction with alcohol consumption.</td>
<td>(Nista et al., 2004)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Diarrhoea or a rash.</td>
<td>(Roesler et al., 2012)</td>
</tr>
<tr>
<td>Proton pump inhibitors</td>
<td>May have a bacteriostatic effect and lengthen the half-life of several antimicrobial medications in plasma.</td>
<td>(Hafuz et al., 2021)</td>
</tr>
</tbody>
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| Table 1: Commonly used Antibiotics used to treat *H. pylori* and their side effects. |

In this study, we address the role of CagA in the development of *H. pylori* mediated gastric cancer, which undergoes important translation and phosphorylation before disrupting cell signalling pathway and protein dysfunction. Gastric cancer is at risk due to *H. pylori* infection. The data exhibit variability due to variations in the control group selection, patient age, and the location and stage of Gastric cancer. Infection with CagA-positive strains of *H. pylori* increases the risk for gastric cancer over the risk associated with *H. pylori* infection alone. Searching for CagA status over *H. pylori* infection may confer additional benefit in identifying populations at greater risk for gastric cancer. *H. pylori* is an important bacterium with a wide variety of virulence factors for thrive in the environment of the stomach. The strong link between *H. pylori* infection and gastric cancer development has led to numerous studies to clarify the role of virulence factors in the establishment of the disease. Lastly, it is indisputable that *H. pylori* has the capacity to develop into a pathogenic bacterium and a carcinogen. This is why it’s so important to clear up any unanswered questions about the subject and keep an eye on the bacterium’s behaviour throughout an infection, as well as the molecular processes and parts like CagA that aid in the identification and management of diseases.

**FUTURE PERSPECTIVE**

I aim to identify the kinases transcriptionally dysregulated by CagA of *H. pylori*, leading to transcriptionally dysregulation. The cellular transcriptome map of AGS cells infected and uninfected will clearly inform the critical kinases that take part in kinase mediated events in post – *H. pylori* infection CDNA synthesized from the isolated RNA infected and control AGS cells will be used in human kinome panel.

The highly distorted kinases from their control expression will be mapped to the related signalling network. Also, functionally relevant of kinases will be identified using cytoscape. This review helps clinicians to better identify those infected individuals who are at high risk of developing gastric cancer and implement the necessary investigations and treatment.

The triple drug therapy has major side effects, urgent need to design therapeutic molecules to inhibit *H. pylori*.

**ACKNOWLEDGEMENT**

I would like to thank the Department of Biochemistry, Jamia Hamdard, Delhi, India for the support and to Dr. Munazzah Tasleem for the help throughout.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.
H. pylori: Helicobacter pylori; CagA: Cytotoxin-associated gene A; vacA: vacuolating cytotoxin A; GC: Gastric cancer; T4SSs: Type IV secretion systems; BabA: Antigen binding adhesin A; SabA: sialic acid-binding adherence A; CagPAI: The Cag pathogenicity island; Sfk: Src family kinase; Ps: phosphatidylinositol; GI: gastrointestinal; PPIs: Proton Pump Inhibitors; AGS: Human gastric adenocarcinoma cell-line.

REFERENCES


