J Prev Epidemiol. 2019;4(2):e27 Review

Journal of Preventive Epidemiology

http jprevepi.com

Mechanisms of *Helicobacter pylori*-induced gastric mucosal injury

Mohammad Bahadoram[1,2], Bijan Keikhaei[2], Mohammad-Reza Mahmoudian-Sani[2], Kosar Alikhani[2], Motahareh Motiee[2], Ammar Helalinasab[2], Maedeh Barahman[1*]

[1]Department of Radiation Oncology, Firoozgar Hospital, Firoozgar Clinical Research Development Center (FCRDC), Iran University of Medical Sciences (IUMS), Tehran, Iran

[2]Thalassemia and Hemoglobinopathy Research Center, Research Institute of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Correspondence to:
Maedeh Barahman, Email: maedeh.barahman@gmail.com, brahman.m@iums.ac.ir

Received: 29 Sep 2019
Accepted: 10 Nov. 2019
ePublished: 18 Dec. 2019

Keywords: Gastric cancer, Helicobacter pylori, Gastric mucosal injury, Atrophic gastritis, MALT lymphoma

Citation: Bahadoram M, Keikhaei B, Mahmoudian-Sani MR, Alikhani K, Motiee M, Helalinasab A, Barahman M. Mechanisms of Helicobacter pylori-induced gastric mucosal injury. J Prev Epidemiol. 2019;4(2):e27.

Abstract

Helicobacter pylori is a common pathogenic microorganism which is able to colonize in harsh acidic environment of the stomach. It also plays an important role in developing gastric cancer (GC) by causing inflammation, epigenetic changes in cancer-related genes, and disturbance in life cycle of cells. Besides, GC is the fourth most common cause of cancer-related mortality in the world. There is still no consensus regarding the eradication of *H. pylori* and its effects in terms of preventing gastric lesion or atrophy progression. The main purpose of this review was to evaluate factors shaping *H. pylori* colonization into gastric epithelial cells and then normal gastric mucosa progression to GC.

Introduction

Helicobacter pylori is a gram-negative spiral type of bacteria that selectively would be colonized in the human stomach epithelium. This bacteria secretes several enzymes called urease, oxidase, and catalase with different roles. Accordingly, the role of urease is converting chemical urea into ammonia, which generates a neutral condition in the stomach and makes it more hospitable for the bacteria concerned. It has been further argued that *H. pylori* affect the microbial environment of gastric tissues, thereby useful bacteria are reduced and harmful ones are multiplied. Moreover, the spiral shape of *H. pylori* prompts it to burrow into the mucus layer, which is less acidic than the inner space or the lumen of the stomach. The superficial proteins of the bacteria also play a leading role in binding to epithelial cells and adapting to long-term colonization. Therefore, long-term infection with *H. pylori* as well as the consequences of chronic inflammation in the mucosa will result in complete loss of the functions of the epithelium. Similarly, there is much evidence that *H. pylori* infection is common due to coexisting of these bacteria with humans for thousands of years. This infection has further spread to more than half of the world's population and a large number of affected patients usually develop coexisting long-term inflammation. It should be noted that *H. pylori* infection is mostly asymptomatic among infected cases. The long-living *H. pylori* notably augment the risk of progressing site-specific diseases. Amongst infected cases, roughly <0.1% of individual's progress mucosa-associated lymphoid tissue (MALT), 1%-3% of them develop gastric adenocarcinoma, and 10% of the patients suffer from peptic ulcer disease (PUD) (4). In 1994, *H. pylori* were classified into carcinogen or cancer-causing agents in humans by an international agency for research and cancer. Afterwards, it has been increasingly accepted that *H. pylori* colonization in the stomach is a significant cause of stomach cancer and MALT or MALT lymphoma (MALToma) (5). For years, the association between gastric tumor and *H. pylori* has been thus a subject of discussions. For example, a study on 1526 patients in Japan, demonstrated that *H. pylori* infection had remarkably raised the risk of gastric cancer (GC) (6).Uemura et al further proved

Key point

Gastric cancer is fatal and *Helicobacter pylori* is considered as a risk factor for this malignancy.

that gastric malignancy had progressed in approximately 3% of *H. pylori* colonized patients, but in no one of the non-colonized ones. Thus, this evidence has confirmed that H. pylori shape initial stages of GC. Therefore, in cases with no premalignant lesions, *H. pylori* elimination could minimize the risk of GC (7). Most of the relevant studies have confirmed that treatments with anti-*H. pylori* agents might be an efficient way to prevent GC. The main purpose of this review was to evaluate several factors affecting *H. pylori* colonization into gastric epithelium and then normal gastric mucosa progression to GC.

Materials and Methods

For this review, a number of databases including PubMed, Embase, Scopus, and Directory of Open Access Journals (DOAJ) were searched to retrieve the relevant articles. The search was accordingly conducted using a combination of the following keywords and/or their equivalents: "gastric cancer, Helicobacter pylori, gastric mucosal injury, intestinal metaplasia, peptic ulcer disease, dysplasia, adenocarcinoma, atrophic gastritis, and MALT (mucosa-associated lymphoid tissue).

Gastric Cancer (GC) and *H. pylori*

GC or stomach cancer is the fourth most common widespread cancer leading to death. Nearly one million people each year are diagnosed with this type of cancer and approximately 700 000 people die from gastric adenocarcinoma (8). Generally, the diagnosis of gastric tumor is postponed by loss of primary special symptoms and majority of patients are detected after the attack of cancer cells to muscular is propria (1). Histologically, two main types of GC have been thus far recognized. First, diffused-type GC that includes infiltrating tumoral cells, which do not configure into glandular organizations, and second, intestinal-type adenocarcinoma that develops by a sequence of well-defined histological stages (9). The second type develops by the modification of normal gastric mucosa to chronic superficial gastritis, to chronic atrophic gastritis (CAG), to intestinal metaplasia (IM), and eventually to dysplasia and adenocarcinoma (5). The second type develops by the modification of normal gastric mucosa into chronic superficial gastritis, to chronic atrophic gastritis (CAG), then intestinal metaplasia (IM), and eventually to dysplasia and adenocarcinoma (5). *H. pylori* initially causes chronic inflammation and gastrointestinal mucosa damage. Altering the proliferation of epithelial cells causes chronic gastric atrophy, in which the mucosal secretory cells would be replaced by connective tissues or intestinal epithelial cells. This complication is assumed as a strong and important precursor of GC, which can significantly mitigate the risk of GC if detected early. In this respect, a study had reported a significant and positive relationship between *H. pylori* infection and CAG, wherein CAG had been spotted in 82.9% of patients with *H. pylori* positive.

Epidemiology of *H. pylori* infection

H. pylori infection proportions vary throughout the world. In this regard, seven types of *H. pylori* have been so far identified based on geographical distributions including hpAfrica1 (hspSAfrica, hspWAfrica, and hspCAfrica), hpAfrica2, hpNEAfrica, hpEurope, hpSahul, hpAsia2, and hpEastAsiafia, divided into hspEAsia and hspAmer (12). Nevertheless, there is a clear link between the areas with elevated levels of *H. pylori* infection and those with higher incidence rate of GC. In less developed countries such as those in Africa and Asia especially India and Bangladesh, the bulk of population infected with *H. pylori* is not diagnosed with GC. In contrast, there is a positive correlation between *H. pylori* infection rates and prevalence of GC in more developed countries including Japan, South Korea, and China (11). These variations can be illuminated by a combination of different factors such as type of *H. pylori* strains and its genotypes, environmental factors, as well as host genetic determinants.

Pathogenesis of gastric malignancy

The pathogenesis of *H. pylori* occurs both indirectly and directly. The indirect way is to create inflammation and the immune system recall as much as possible. In obedience to relevant studies, individuals who were positive for *H. pylori* infection had higher levels of cluster of differentiation 4+ (CD4+) and inflammatory cytokines than those who were not infected with these bacteria. In the direct manner, bacterial factors called *H. pylori* virulence factors can directly target particular molecules in epithelial cells and cause deoxyribonucleic acid (DNA) damage (3). However, in general, a set of factors including environmental risk ones, host conditions, and *H. pylori* virulence factors are all effective in increasing the risk of GC and its progression. In the following, the virulence factors are delineated.

Virulence factors

H. pylori is endowed with some factors, which help it tolerate gastric acidic environment for colonization. These factors are as follows

Cytotoxin-associated gene A (CagA)

H. pylori use a needle-like attachment to inject Cag-A toxin into the junctions of gastric epithelial cells. This toxin (known as CagA) makes changes in the stomach cell structure and prompts the bacteria to attach to them readily. CagA is also considered as an important factor in *H. pylori*-related gastrointestinal complications causing gastritis, epithelial injury, and in due course GC. Injecting this cytotoxin to gastric cells also amplifies oncogene expression and inhibits expression of tumor suppressor genes. This also leads to DNA damage and makes changes in micro-RNA profiles since it releases oxygen (O) and nitrogen (N) free radicals. Eventually, long-term exposure to the toxin induces chronic inflammation. Though not all *H. pylori* strains carry the CagA gene, the ones named

as CagA-positive magnify the risks of acute gastritis, atrophic gastritis, and distal GC (12, 14). In the early 1990s, CagA was firstly detected (15, 17) and its expression was associated with higher rate of PUD. The structure of CagA protein is variable in different species of *H. pylori*. More precisely, they are different in terms of carboxylic terminals of the protein, which are also phosphorylation sites (namely, EPIYA modif). These terminals play a major role in the pathogenesis of *H. pylori* and its ability to interact with epithelial cells (12).

Peptidoglycan and lipopolysaccharide

The Cag secretion system, apart from CagA, is able to hand over *H. pylori* peptidoglycan components into host cells. Peptidoglycan also collaborates with the host intracellular model identification molecule nucleotide-binding oligomerization domain-containing protein 1 (NOD1), emanating from gram-negative bacteria, and acts as a sensor for peptidoglycan components. The collaboration of *H. pylori* peptidoglycan with NOD1 similarly causes stimulation of pro-inflammatory reactions related to nuclear factor-κB (NF-κB) like ß-defensin-2 (BD-2) or interleukin 8 (IL-8) secretion (18, 19). Some former studies have further shown that other host signaling pathways associated with increasing risk of GC can be activated by H. pylori translocated peptidoglycan. For instance, in 2009, Nagy et al proved that the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, leading to decreasing apoptosis and increasing cell migration, could be activated by *H. pylori* translocated peptidoglycan (20). Besides, lipopolysaccharide (LPS) is another important *H. pylori* surface antigen that plays an important role in the stability of *H. pylori* outer membrane and escalates the secretion of cytokines such as tumor necrosis factor-α (TNFα) and IL-1.

Vacuolating cytotoxin A (VacA)

The majority of *H. pylori* strains secrete VacA, which augments the risk of PUD and GC since it targets host cell pathways. This protein is also able to suppress T-cell responses to *H. pylori* that can cause long-lasting infection (21,22). Likewise, VacA increases the expression of the epidermal growth factor receptor and can thus accelerate GC progression. Although significant differences in vacuolating activities are seen between strains, the VacA gene exists in many *H. pylori* strains. This disparity is accordingly ascribed to differences in the structures of VacA gene into the signal (s) region, the middle (m) region, and the intermediate (i) region, situated between the s and m regions. Each region is also separated by two subtypes (s1 [s1a, s1b, and s1c], s2, i1, i2, m1, and m2) with a different role. The region i also plays a significant role in vacuole-creating activity and GC formation. A new region between the regions i and m has been correspondingly identified as region d, which is closely related to the ability of neutrophil infiltration and mucosal atrophy in the antrum and the corpus areas. The distribution of these genotypes varies in different parts of the world. For example, in East Asia, the VacA d1 genotype has the highest frequency. Based on a research on 73 Iranian patients with *H. pylori* infection and colonization with VacA i1 strains were associated with gastric malignancy that should be more potent compared with relations of Cag status, VacA signal, or middle types (12, 23).

Adhesions and outer membrane proteins (OMPs)

Preliminary colonization, infection durability, and surrendering of virulence factors are simplified by the adherence of *H. pylori* to the epithelial cells of the gastric mucosa. In accordance with the arrangement analysis of different sequences of *H. pylori* strains, roughly 4% of the *H. pylori* genomes are composed to encode OMPs that is remarkably above any other recognized bacterial species. OMP representation is also related to gastroduodenal diseases; hence, it may increase the risk of GC (24). Overall, *H. pylori* genomes express about 64 types of OMPs. The most important cases will be described hereinafter. The well-described *H. pylori* OMP is a blood group antigen-binding adhesion (BabA), identified as the first adhesin molecule of *H. pylori*, which has evolved in reaction to host mucosal glycosylation models to allow for adapting *H. pylori* to its host and to retain perdurable colonization (1). It has been suggested that another mechanism of BabA is increasing inflammation in the stomach by escalating CagA transfer to gastric cells. The sialic acid-binding adhesin (SabA) is one other *H. pylori* adhesion, which modulates host cell glycosylation patterns to increase attachment and colonization. SabA is also related to a risk of increased CG but a lower risk of duodenal ulceration. The SabA molecule can further bind specifically to neutrophils and stimulate the release of reactive oxygen species (ROS) as free radicals, which cause oxidative damage to epithelial cells. Moreover, outer inflammatory protein A (OipA) is an inflammation-related OMP. In this regard, *H. pylori* possess non-functional or functional OipA genes and the existence of a functional gene is related to that of enhanced neutrophil infiltration as well as duodenal and stomach cancers. An exact mechanism of action has not been so far established for OipA. While some studies have suggested that increasing IL-8 secretion by epithelial cells is an inflammatory mechanism, others have ruled out its effects on cytokine secretion and have regarded it as an adhesion protein. This molecule seems to cooperate with other pathogenic virulence factors in the pathogenesis of *H. pylori* (25). Moreover, HopQ is another surface antigen of *H. pylori* that binds to carcinoembryonic antigen-related cell adhesion molecule receptors on the epithelial surface of gastric cells, and like BabA, transmits CagA oncoprotein to these cells (12). Similarly, duodenal ulcer promoting A (DupA) gene is one other novel virulence marker located within the plasticity zone of the *H. pylori* genome. In this vein, an initial analysis on 500

H. pylori strains from South Korea, Japan, and Colombia demonstrated an increased duodenal ulcer risk and a decreased GC risk in individuals carrying DupA-positive strains (26). A comparison of different strains from Iraq and Iran also showed that DupA representation was remarkably related to duodenal ulceration in Iraqi isolated strains but not in Iranian ones (27). *H. pylori* also contain 3 to 5 flagella, which are composed of three parts: the basic body, the hook, and the filament. This filament is like a propeller that rotates from the base and is a copolymer of the flagellin (Fla) subunits, i.e., FlaA (as the predominant subunit) and FlaB (as the minor subunit) (28). According to in vivo studies, FlaA and other vital proteins for the assembly of flagella are necessary for perdurable infection in gnotobiotic piglet and rodent models. Therefore, it is evident that motility is essential for effective gastric colonization and pathogenicity (29).

Host factors

As the only determinants of virulence are not the *H. pylori* strain-specific constituents, most individuals infected with *H. pylori* strains remain without symptoms. Therefore, host factors that may affect pathological outcomes have been taken into account. Consequently, host polymorphisms may influence GC development (30). Different host factors are thus described as follows:

Host immune response

The pro-inflammatory cytokine IL-1ß acts as a key mediator of a plenty of pathophysiological events that characterize host-environment interactions. This host effector molecule, which is involved in host response to many antigenic challenges, is accordingly increased within the gastric mucosa of *H. pylori*-infected people. Genetically, IL-1ß has several polymorphisms, each one with a different ability to secrete this IL in the face of infections such as *H. pylori*. Higher IL-1ß secretion also causes more suppression of gastric acid, induces gastric atrophy, and augments risk of GC (30). In 2000, El-Omar et al proved that *H. pylori*-colonized individuals with high expression of IL-1ß polymorphisms, compared with those with limited IL-1ß expression, were facing a notably increased risk of gastric atrophy, hypochlorhydria, and distal gastric adenocarcinoma (31). Apart from IL-1ß, TNFα is also considered as a pro-inflammatory acid-suppressive cytokine, which is enhanced in *H. pylori*-colonized gastric mucosa of humans. As well, TNFα-expressing genes have regulatory regions and are actually changeable, leading to the production of various polymorphisms from this cytokine. In this regard, polymorphisms raising TNFα expression are correlated with GC (31). In this respect, IL-10 is a multifunctional inflammatory cytokine that alleviates immune cell responses and reduces cytotoxic inflammatory ones. Polymorphisms in the promoter region of the IL-10 genes also produce a variety of cytokines that affect IL-10 function and *H. pylori* host sensitivity to GC. With reference to the study by Kim et al. in 2012, the IL-10 concentration in the gastrointestinal mucosa of *H. pylori*-infected patients and GC ones was at a higher level. One of the possible causes of IL-10 carcinogenicity is its two-way function along with being anti-inflammatory. It also has an immunosuppressive function. Therefore, despite its beneficial effects in minimizing tissue damage caused by inflammation, it can lead to inactivation of immune cells in the gastrointestinal mucosa and even reduce phagocytic ability of macrophages, and consequently, their inadequacy in confronting malignant cells at higher concentrations. The combinatorial influence of TNFα, IL-10, and IL-1ß polymorphisms on cancer improvement has been thus far specified, whose risks augment steadily with a rising number of pro-inflammatory polymorphisms (32, 34). The increasing risk of GC has been further linked to genetic polymorphisms, affecting innate immune response genes. IL-8 is also a cytokine secreted in response to *H. pylori* entry into epithelial cells. By augmenting endothelial proliferation and expression of vascular growth factor, this cytokine can induce angiogenesis and thus bring about its carcinogenic effects. Besides, the IL-8 gene has different polymorphisms, each one with a distinctive ability to express these cytokines and their angiogenesis potency and carcinogenicity. High presentation of alleles within the developer region of the chemokine IL-8 gene also raises the risks of severe inflammation and premalignant injuries in Asians and Caucasians although it has not been so far approved in all research studies. Efficient polymorphisms into toll-like receptor 4 (TLR4) has been similarly shown to enhance GC and stomach atrophy risks in white people, which could be connected to a construction shortage of the anti-inflammatory cytokine IL-10 (1). In 2017, Takeda et al had further reported that inflammatory chemokines such as IL-8 and regulated on activation normal T cell expressed and secreted (RANTES) had enhanced in the gastric mucosa by *H. pylori*. They had further investigated the effects of a special strain of *Lactobacillus paracasei* on expression and production induced by *H. pylori* of IL-8 and RANTES, using human gastric epithelial cell lines. This strain was able to release lactic acid, suppressing IL-8 and RANTES production induced by *H. pylori* (35).

Acid secretion

The major stimulant of acid secretion is gastrin, releases from gastrin expressing (G) cell (namely, hormonal), histamine, discharges from enterochromaffin-like (ECL) cells (viz. paracrine), and acetylcholine, liberated from postganglionic enteric neurons (i.e., neurocrine). *H. pylori* can also stimulate or inhibit acid discharge contingent on the state of infection. Harsh infection with *H. pylori* is frequently correlated with hypochlorhydria. In this feature, reduction in acid secretion is supposed to facilitate survival of the organisms and the stomach colonization. The mechanism by which *H. pylori* inhibits acid secretion is complicated and includes direct inhibition of the

parietal cell (and maybe the ECL cell) by a component of the bug (like vacuolating, cytotoxin, LPS, or acid-inhibitory factor) and indirect inhibition of parietal cell task as a result of changes in cytokines as well as paracrine, hormonal, and neural regulatory mechanisms. *H. pylori* itself hinders human gastric hydrogen potassium ATPase, also known as H^+K^+-ATPase α-subunit promoter activity. It also provokes IL-1ß and TNFα secretion that directly prevent parietal cell secretion (36).

Environmental factors

With regard to *H. pylori* strain characteristics and host genetic determinants that influence the risk of gastric carcinoma, environmental factors would be effective. They are described as follows:

Salt intake

High level of salt consumption is a factor that has been regularly related to an increased stomach malignancy risk. This had been identified in one study on a Japanese population, reporting that *H. pylori*-infected individuals with high levels of salt consumption had a higher risk of GC in comparison with *H. pylori*-infected people with lower levels of salt intake (37). The mechanism of increasing the risk of stomach malignancy by salt have been thus far incompletely recognized. However, there are some possibilities like direct effects of salt on gastric mucosa that reduce the malignant transformation threshold. There is one more possibility that gastric mucosa is damaged by salt; thereby carcinogens are allowed to enter into the gastric tissues. Moreover, in response to a high-salt diet, upregulated production of cytokines is synergetic. Eventually, one recent study confirmed that *H. pylori* gene expression could be potentially modulated by high level of salt concentrations and then boost CagA virulence factor (38).

Smoking

Some studies have shown that smoking intensifies the incidence of GC related to *H. pylori*-associated risk. A study on Japanese men in this respect had demonstrated that *H. pylori* and smoking were risk factors for GC. Accordingly, the risk of GC was sharply higher for cases with both *H. pylori* infection and smoking habits (39). Therefore, there is a positive relationship between the higher risk of GC, smoking and *H. pylori* infection. Moreover, the synergistic effect of cigarette smoking on *H. pylori*-related cancer can be due to increased secretion of inflammatory mediators, which enhance gastric inflammation caused by *H. pylori*.

Helminth infection

As concluded in previous studies, the outcomes of *H. pylori* infection may be affected by coinfection with helminths. According to the research on Chinese citizens, serological immunoglobulin G (IgG) responses to *H. pylori* had been altered by simultaneous helminth infections and they were correlated with a lowered risk of gastric atrophy development, as specified by the pepsinogen I/II ratio. Hence, investigations have confirmed that simultaneous helminth infection can decrease *H. pylori*-induced gastritis severity. The reason suggested for this phenomenon is the significant reduction of messenger ribonucleic acid (mRNA) of cytokines in type1 T-cells in inflammatory responses of the gastrointestinal tract (40).

Dietary antioxidant

Reactive oxygen species (ROS) can also cause oxidative damage and various types of cancer by invading the DNA of the cells. Therefore, natural antioxidants mitigate the risk of cancers including gastrointestinal cancers by inhibiting free radicals. The development of GC against the role of antioxidants in foods has been so far investigated. According to a study on individuals with high GC risk, high intake of dietary antioxidant micronutrient supplements like vitamin C and ß-carotene were likely to increase the regression rate of cancer precursor lesions and contribute to preventing GC either alone or in combination with antibiotics (41-43).

Conclusion

GC is fatal and *H. pylori* are risk factors that indicate a developing cancer risk. Though *H. pylori* infection is common, most colonized cases never develop cancer. Thus, infection with this organism is a necessary factor for GC but not a sufficient cause, due to environmental and host-related factors. Finally, the treatment of *H. pylori* infection, parallel with changes in lifestyle and dietary habits, can diminish the incidence of GC.

Authors' contribution

MB, MRMS, KA, MM and AH wrote the manuscript. BK and MB edited the paper. All authors read and signed the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

Funding/Support

None.

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