Inducible nitric oxide synthetase genotype and Helicobacter pylori infection affect gastric cancer risk

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AIM: To investigate the association of the inducible nitric oxide synthetase (iNOS) C150T polymorphism with Helicobacter pylori (H. pylori) infection and gastric cancer (GC) risk in Iran.

METHODS: In order to determine whether there was a correlation between iNOS genotype and GC in Iran, we conducted a case-control study using samples from 329 individuals. For each sample, the C150T iNOS polymorphism was genotyped by polymerase chain reaction (PCR) and restriction digestion. Patients were grouped by cancer presence, demographic and behavior characteristics, and H. pylori infection status. Statistical tests were conducted to determine whether any behavioral factors or a particular iNOS genotype was associated with GC in the study population.

RESULTS: In this population, we found that smoking, hot beverage consumption, a familial history of GC and H. pylori infection status were significantly associated with GC development (P = 0.015, P < 0.001, P = 0.0034, and P < 0.015, respectively). The distribution of the C150T iNOS genotypes among the two study groups was not statistically significant alone, but was impacted by H. pylori infection status. When compared to the non-H. pylori infected group, cancer patients who had a heterozygous CT genotype and were also infected with H. pylori were 2.1 times more at risk of developing GC [odds ratio (OR) = 2.1, P = 0.03] while those with a homozygous TT genotype and infected with H. pylori were 5.0 times more at risk of developing GC (OR = 5.0, P = 0.029). In contrast, this association was not seen in patients in the control group.

CONCLUSION: A CT or TT polymorphism at position 150 in the iNOS gene significantly increases the risk of GC and may be a marker for GC susceptibility.

Key words: Inducible nitric oxide synthetase; Gastric cancer; Helicobacter pylori; Heterozygous CT genotype; Homozygous TT genotype

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INTRODUCTION

Gastric cancer (GC) is a major cause of morbidity and mortality throughout the world; it is estimated that GC is responsible for over 700,000 deaths per year\textsuperscript{[1-3]}, which makes this disease one of the leading causes of cancer-related death worldwide\textsuperscript{[4]}. One of the most prominent risk factors for development of GC is infection with the bacterium Helicobacter pylori (H. pylori). Indeed, since its discovery in the early 1980s, H. pylori has been recognized as the causative agent of two types of cancer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma\textsuperscript{[4-8]}. The tight association between infection with this pathogen and the development of GC led to the classification of H. pylori as a class I carcinogen\textsuperscript{[9]}; it is currently the only bacterium so classified.

Although H. pylori infection is relatively common, only a small subset of infected individuals develop GC; some individuals colonized with H. pylori carry the organism their entire lives with no overt disease symptoms, while others develop severe disease. Furthermore, recent evidence has begun to suggest that H. pylori colonization may be beneficial in some individuals; studies indicate that H. pylori protects against the development of esophageal cancer, childhood asthma\textsuperscript{[10-12]} and active tuberculosis\textsuperscript{[13,14]}. These findings have led researchers to try to define risk factors associated with H. pylori-induced disease development. Indeed, years of study have shown that in H. pylori-infected individuals, disease development/carcinogenesis is a complex and multifactorial process that involves an intricate interplay between numerous factors; studies indicate that disease etiology is influenced by a combination of bacterial factors, host genetics, and environmental factors such as diet.

Numerous bacterial factors have been identified as being important for H. pylori-induced disease development. These include the toxins CagA\textsuperscript{[13,14]} and VacA\textsuperscript{[15,16]}, the adhesions BabA\textsuperscript{[17]} and SabA\textsuperscript{[18]}, and the outer membrane proteins HomA, HomB, and OipA\textsuperscript{[19,20]}. These and other bacterial factors act in concert to alter host cell signaling pathways, induce epithelial cell proliferation, and disrupt the delicate balance that exists between the bacterium and the host immune system. However, although these bacterial factors play a key role in the development of gastric disease, they represent only one aspect of the story. Indeed, there is a large body of work that suggests environmental factors also affect the risk of GC development. Some of the more prominent environmental risk factors include smoking\textsuperscript{[21,22]} and high levels of dietary salt intake\textsuperscript{[23-25]}.

Besides these exogenous factors, the host immune system also clearly plays a major role in the development of gastric disease. In response to infection with H. pylori, the localized gastric environment becomes pro-inflammatory via the expression of pro-inflammatory cytokines\textsuperscript{[26-28]}. Because these host factors play an important role in generating and maintaining the pro-inflammatory environment, it is perhaps not surprising that specific mutations in the genes that encode these factors influence gastric disease development. For example, specific interleukin-1 (IL-1), IL-8, IL-10, and tumor necrosis factor-α gene polymorphisms are associated with an increased risk for GC\textsuperscript{[29,30]}.

While these cytokines play an important role in the development and maintenance of the pro-inflammatory environment in the gastric mucosa, there are clearly other host factors involved in the disease process.

Nitric oxide (NO) is a molecule that has multiple functions in the gastrointestinal tract. In addition to having anti-microbial properties and playing an active role in vasodilation\textsuperscript{[31]}, NO is also a component of the pro-inflammatory immune response that has been linked to carcinogenesis\textsuperscript{[32]}. NO is synthesized from L-arginine by three distinct isoforms of the nitric oxide synthetase (NOS)\textsuperscript{[33]}. Whereas 2 of the 3 isoforms (neuronal NOS and epithelial NOS) are constitutively expressed, the inducible isoform (iNOS) is primarily expressed in response to specific cytokines\textsuperscript{[34]}. Furthermore, expression of iNOS is upregulated during H. pylori infection\textsuperscript{[35]}. Several studies have shown a link between increased levels of iNOS and risk of GC development\textsuperscript{[36,37]}. Since one of the end products of NO metabolism is reactive nitrogen species, it is hypothesized that higher levels of iNOS lead to more oxidative DNA damage, thus increasing the risk of cancer development\textsuperscript{[38]}. Interestingly, a single amino acid substitution (Ser 608 Leu) in the iNOS reductase domain has been associated with an increased risk for GC in Asian populations\textsuperscript{[39,40]}. This amino acid change is the result of a single polymorphism (C150T) in exon 1\textsuperscript{[39,40]}, which is believed to affect the activity of the iNOS enzyme\textsuperscript{[39]}.

Even though the number of GC cases has decreased in many countries in recent years, the incidence of GC in Northern Iran remains high\textsuperscript{[41]}. Several provinces, including Manzadaran, Ardabil, Golestan, Semnan, and the metropolitan area of Tehran, have high rates of GC in both men and women\textsuperscript{[42]}. The age-standardized incidence rate for GC in these areas has been reported as high as 49.1 in men, and 25.4 in women\textsuperscript{[43]}. These incidence rates may be at least partially explained by the fact that a significant proportion of this population is colonized by H. pylori. Indeed, the H. pylori infection rate for Iranians aged 40 years and over can be as high as nearly 90%\textsuperscript{[44,45]}. A similar prevalence was reported for the Babol city of Iran where the H. pylori infection rates are 78% for men and 82% for women\textsuperscript{[46]}. In addition to high rates of H.
pylori infection, environmental factors such as consumption of red meat and dairy products, high salt intake, and consuming hot tea have been identified as important risk factors for GC in this region\[^{2}\].

At present, virtually nothing is known concerning which host genetic factors may influence GC risk in this region. Herein, we describe an epidemiologic study in which we investigate the association of the \(\text{iNOS} C150T\) polymorphism with \(H.\ pylori\) infection and GC risk among men and women from the Mazandaran region of Iran. We found that in this population, individuals who are infected with \(H.\ pylori\) are at a significantly higher risk for development of GC if they also harbor a CT or TT \(\text{iNOS}\) genotype. To date, this report is the first study that links a host-genetic factor to GC risk in this population. Furthermore, it is the first study to investigate the importance of the \(\text{iNOS}\) polymorphism in a non-Asian population. \(\text{En masse}\), our data indicated that the \(\text{iNOS} C150\) allele may be a potential marker for susceptibility to \(H.\ pylori\)-mediated GC development in multiple populations.

**MATERIALS AND METHODS**

**Study participants**

This case-control study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Research Ethics Committee at the Mazandaran University of Medical Sciences. Written informed consent was obtained from each individual, and all study participants were given an explanation of the nature of the study prior to enrollment. Patients were enrolled in the study after seeking treatment at Touba Polyclinics or Imam Hospital between April 2008 and March 2011. Patients with GC were diagnosed by gastric endoscopy and cases were defined using the International Classification of Diseases for Oncology and Lauren criteria\[^{44}\]. During the course of the study, 159 individuals with GC were enrolled. This group had a mean age of 61.32 ± 12.5 years and was composed of 75 female (approximately 47\%) and 84 male (approximately 53\%) patients. Patients with inflammatory diseases such as rheumatoid arthritis and/or malignancies other than gastric carcinoma were excluded from the study. Members of the control group were matched by age, sex, geographic location, and ethnic background. This group included 170 individuals with a mean age of 58.86 ± 14.2 years. Ninety-eight were male (approximately 58\%) and 72 were female (approximately 42\%). For this population, healthy controls who were 40 years of age or greater were recruited from the clinics after undergoing an annual medical screening that included a gastric endoscopy. Healthy controls who were less than 40 years of age were recruited after being referred to the clinic for routine laboratory testing. Demographic information, eating habits, and epidemiologic risk factors were provided by study participants during a face-to-face interview. Risk factors that were evaluated included cigarette smoking, drinking hot beverages, and consumption of salted fish and fast food. A person was considered a smoker if they reported smoking at least one cigarette per day for 12 or more months. Consumption of salted fish or fast food was defined as consuming either item one or more times a week for at least 6 mo. Criteria for drinking hot beverages were essentially as described previously\[^{45}\]. Patients were considered to have a family history of GC if either of their parents currently had or had been diagnosed with GC in the past, or if any of their siblings had a history of the disease.

**Inducible nitric oxide synthetase genotyping**

In order to investigate \(\text{iNOS}\) gene polymorphisms, venous blood was collected from study participants and used to isolate genomic DNA by the salting-out method\[^{40}\]. \(\text{iNOS}\) genotyping was performed using an amplicon-restriction fragment length polymorphism (ARFLP) essentially as previously described\[^{46}\]. Briefly, for each individual a polymerase chain reaction (PCR) was performed using 2 pmol each of the \(\text{iNOS}-F\) (5’-TGGATACGCCCCTTTTTGTTG-3’) and \(\text{iNOS}-R\) (5’-174 GTCTCTGGGGTCTGAA-3’) primers, 200 mmol dNTPs, 10 mmol Tris-HCl, pH 8.3, 50 mmol KCl, 1.5 mmol MgCl\(_2\), 0.25 U of Hot start Taq DNA polymerase (Fermentas, Italy) and 50 ng of genomic DNA template in a final reaction volume of 25 \(\mu\)L. Each reaction was denatured at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 58.5 °C for 60 s, and extension at 72 °C for 40 s. A final extension at 72 °C for 5 min was added to ensure complete amplification. Restriction digests were performed by incubating 10 \(\mu\)L of PCR product with 2 U of TSP 509I enzyme (New England Biolabs, Ipswich, MA, United States) in a final volume of 15 \(\mu\)L at 65 °C. Digestion products were separated on a 1.5\% SDS-polyacrylamide gel, and visualized after staining with ethidium bromide. Results of the ARFLP analysis allowed us to differentiate between the 3 possible genotypes: (1) digestion of PCR amplicons from homozygous (CC) individuals generated 2 DNA fragments of 113 bp and 175 bp; (2) digestion of amplicons from heterozygous individuals (CT) yielded 3 DNA fragments of 113 bp, 142 bp, and 175 bp; and (3) digestion of amplicons from homozygous (TT) individuals yielded 2 DNA fragments of 113 bp and 142 bp.

**H. pylori detection**

Infection with \(H.\ pylori\) was detected by culturing gastric tissue collected during endoscopy. Identification of clinically isolated \(H.\ pylori\) strains was confirmed by Gram stain and urease activity. Additionally, all patients were also tested for infection using an \(H.\ pylori\) specific IgG enzyme linked immunosorbent assay (ELISA) (Diagnostic Automation, CA, United States) and by urease breath test (Bahar, Afshan, Iran). Given the difficulties in culturing \(H.\ pylori\), especially from cancerous tissue, individuals were classified as infected with \(H.\ pylori\) if they gave a positive result by ELISA.
Table 1  Patient demographics and risk behavior

|                  | Gastric cancer | Control | P-value |
|------------------|---------------|---------|---------|
| Age (yr)         | 61.3 ± 12.5   | 58.8 ± 12.4 | 0.098   |
| Sex (male/female)| 84/75         | 98/72   | 0.367   |
| Marital status   |               |         |         |
| Single           | 7 (4.4)       | 3 (1.8) |         |
| Married          | 149 (93.7)    | 166 (97.6) | 0.002  |
| Divorced         | 3 (1.9)       | 1 (0.6) |         |
| Occupation       |               |         |         |
| Unemployed       | 4 (2.5)       | 1 (0.6) |         |
| Employee         | 24 (15.1)     | 35 (20.5) | 0.001  |
| Housewife        | 46 (28.9)     | 74 (43.5) |        |
| Others           | 85 (53.5)     | 60 (5.4) |         |
| Smoker           | 33 (20.8)     | 22 (12.9) | 0.015  |
| Hot beverage     | 76 (47.8)     | 38 (22.4) | < 0.0001 |
| Salty fish       | 15 (9.4)      | 14 (8.2) | 0.09    |
| Fast food        | 29 (18.2)     | 39 (22.9) | 0.037  |
| Pickles          | 88 (55.3)     | 97 (57.1) | 0.62   |
| H. pylori positivity | 94 (59.1) | 77 (45.3) | 0.015  |
| Positive family history | 34 (21.4) | 16 (9.4) | 0.0034 |

H. pylori: Helicobacter pylori.

Statistical analysis

Fisher's exact test was used to compare the different iNOS allele types, and the frequency of each iNOS genotype in the patient population(s) was assessed using a $\chi^2$ test. The difference in age between the 2 study groups was evaluated using a two-tailed Student’s $t$-test. Deviation from the Hardy-Weinberg equilibrium was evaluated using a $\chi^2$ test. To evaluate the risk factors in the study populations, unconditional logistic regression models were applied to odds ratios (ORs) and 95% confidence intervals (CIs). For all statistical tests, a $P$ value of less than 0.05 was considered significant.

RESULTS

Demographics and epidemiology of the patient population

Complete epidemiologic and demographic information for the study population is given in Table 1. Across the 329 study participants, ages ranged from 28 years to 86 years. Within the control group the mean patient age was 58.9 ± 14.2 years, whereas within the cancer group the mean patient age was 62.1 ± 12.6 years. There was no significant difference between the ages of patients in the two groups ($P = 0.098$). The control group was comprised of 98 male and 72 female patients while the cancer group was comprised of 84 male and 75 female patients. There was no significant difference between the distribution of male and female study participants in either group ($P = 0.367$). Patients were categorized by marital status as single, married, or divorced. The cancer group consisted of 7 single, 149 married, and 3 divorced individuals. In contrast, the control group consisted of 3 single, 166 married, and a single divorced patient. The breakdown of marital status between the case and control groups was significant ($P = 0.002$). The study participants were also broken down into different groups based on occupation. Patients were considered either unemployed, employed, or worked as a housewife; those participants whose employment status did not fall into one of these 3 categories were grouped into a single category, which was named “other”. In the GC group, 4 of the study members were unemployed, 24 were employed, 46 were housewives, and the remaining 85 were in the other classification. Within the control group, 1 participant was unemployed, 35 were employed, 74 were housewives, and 60 were included in the other category. The distribution of patient occupation among the case group and the control group was significantly different ($P = 0.001$).

Risk factors associated with GC

In addition to gathering demographic information about the study participants, we also analyzed behaviors that are known to be associated with increased GC risk. Of the 159 patients who were diagnosed with GC, 33 were smokers whereas only 22 of the healthy controls reported smoking. Since smoking is an established risk factor for development of GC, perhaps it is not surprising that the increased number of smokers in the cancer group was statistically significant ($P = 0.015$). Another established risk factor for GC is consumption of hot beverages; drinking hot tea is specifically associated with increased risk of esophageal cancer in Iran[23]. Seventy-six participants in the cancer group reported drinking hot beverages on a regular basis. This number was significantly different than the 38 control patients who regularly drank hot beverages ($P < 0.0001$). Although consuming large amounts of dietary salt has been linked to GC[23,24], we found no significant difference in the number of patients who regularly ate salted fish among the two groups. Similarly, we found no significant difference in the number of patients who consumed pickles among the groups. However, more of the healthy controls reported regularly eating fast food than did the participants who developed GC. In contrast to the other 2 food criteria, this difference was significant ($P = 0.037$).

Among the most widely recognized factors that are associated with GC risk are H. pylori infection and a family history of cancer. Among the control group, only 18 patients had a family history of GC. In contrast, 34 participants in the cancer group reported having a family history of GC ($P = 0.003$). As it is the most commonly associated risk factor for GC, we next compared the number of H. pylori-positive individuals in both of our study groups. In the cancer group, nearly 60% of the study participants tested positive for H. pylori. Compared to this relatively high infection rate, the percentage of healthy controls who tested H. pylori-positive (45%) was significantly less ($P = 0.015$).

Frequency and distribution of C150T inducible nitric oxide synthetase polymorphisms

Having established the relative risk factors of our population, we next sought to determine the distribution of the
possible C150T \(iNOS\) genotypes among the two study groups (Table 2). Patients were genotyped essentially as described\(^{37}\), as reported in Japanese populations\(^{36}\), the distribution of these specific \(iNOS\) polymorphisms in the control and cancer patient groups fell within the Hardy-Weinberg Equilibrium (\(P = 0.07\) and \(P = 0.796\), respectively). Among the 159 members of the GC group, 89 were homozygous CC, 59 were heterozygous CT and 11 were homozygous TT. The distribution of allele types within this group was not significantly different when compared to the study control group, which contained 92 homozygous CC, 72 heterozygous CT, and 6 homozygous TT individuals. There was also no difference in the frequency of each individual allele (C or T) found within the two study populations or the frequency of mutant (either CT or TT) genotypes between the GC and control groups.

### CT and TT inducible nitric oxide synthetase polymorphisms contribute to GC risk in \(H. pylori\)-infected individuals

Since there was no significant difference in the frequency or distribution of \(iNOS\) C150T alleles in our two patient populations, we next examined any potential epidemiological interaction between \(H. pylori\) infection and the particular \(iNOS\) C150T genotype within the 159 GC patients. As shown in Table 3, the number of GC patients who were infected with \(H. pylori\) (\(n = 94\)) was slightly higher than the number of patients who were \(H. pylori\)-negative (\(n = 65\)). Among the 94 individuals who were infected with \(H. pylori\), 46.8% were homozygous CC, 42.6% were heterozygous CT, and the remaining 10.6% were homozygous TT. In contrast, the \(H. pylori\)-negative group consisted mostly of the homozygous CC genotype (67.7%), followed by the heterozygous CT genotype (29.2%) and the TT genotype (3.1%). When compared to the non-\(H. pylori\) infected group, GC patients who had a heterozygous CT genotype and were also infected with \(H. pylori\) were 2.1 times more at risk of developing GC (\(OR = 2.1, P = 0.03, \chi^2\) test). Similarly, having a homozygous TT genotype and being infected with \(H. pylori\) also led to an increased risk of developing GC; the risk associated with having the homozygous TT genotype was more than twice the risk associated with having only a single T allele (\(OR = 5.0\)). This association was also significant (\(P = 0.029, \chi^2\) test). Similarly, the presence of either mutant allele (CT or TT) increased the risk of GC nearly 2.5-fold (\(OR = 2.4, P = 0.01, \chi^2\) test). Taken together, these results indicate that for individuals who are infected with \(H. pylori\), carrying either a CT or TT \(iNOS\) polymorphism at position 150 significantly increases the risk of developing GC. Importantly, unlike those patients within the GC group, there was no significant association between \(iNOS\) genotype, \(H. pylori\) infection and risk of developing GC in the control group (Table 3).

### DISCUSSION

The study of \(H. pylori\)-induced disease is complicated by the fact that the etiology of disease appears to result from the intricate interplay between bacterial virulence factors, host genetics and environmental components. Moreover, an increasing body of literature indicates that colonization by \(H. pylori\) may even be beneficial to some hosts\(^{10-12}\). Because of these facts, there is a pressing need to develop new diagnostics that will clearly identify those individuals most at risk for development of the most severe form of \(H. pylori\)-induced disease-GC. To this end, numerous studies have looked at the role of virulence factors, environmental risk factors and host genetic polymorphisms in disease development. While these studies have shed an enormous amount of light on development of GC, it is becoming increasingly more evident that vari-
ous factors play different roles based on the population being studied\[13-21,23-27,30\]. Thus, there is a need for larger population-based studies to define the role of particular factors in the disease process. Despite the high prevalence of GC and *H. pylori* infection in Northern Iran, there have been relatively few studies that have examined specific risk factors associated with disease development in this population. Of those that have been conducted, one of the studies that investigated the high rate of cancer development in this region concluded that the classic *H. pylori* virulence factors were not a good predictor of disease outcome\[45\]. Furthermore, the authors went on to suggest that “...the association between strain virulence markers and disease in Iraq but not Iran suggests that other host and environmental factors may be more important in the disease-prone Iranian population”\[46\]. Given this suggestion and the deficit in our knowledge, we set out to examine the contribution of particular behavioral factors and genetic polymorphism in the *iNOS* gene to GC development in the Iranian population. Using a case-controlled study design we identified a significant association of several factors with an increased risk for GC. Among the behavioral factors that were important, smoking and consumption of hot beverages were both associated with development of GC. Both of these factors have been previously identified as risk factors for cancer in other populations\[16,57\]. However, our study is the first to link consumption of hot beverages with GC development in this particular region.

In addition to behavioral factors and similar to other previously reported risk factors\[46\], we found that a familial history of GC was significantly associated with increased risk of GC development in our study population (Table 1). Also similar to at least one other study in this region\[58\], we found that infection with the gastric pathogen *H. pylori* significantly increased the risk of developing GC. Finally, we found that carrying either a heterozygous (CT) allele or a homozygous (TT) allele at position 150 of exon 16 of the *iNOS* gene combined with *H. pylori* infection resulted in a significant increase in GC development.

In humans, NO is used as an important signaling molecule. However, in the presence of increased and/or sustained levels of this molecule, the beneficial effects of NO can be overcome by deleterious processes such as DNA damage and chronic inflammation. These pathologic events may be catalyzed by increased iNOS activity, which leads to increased NO production and concomitant increases in the amount of downstream free radicals. As the major source of NO in humans, perhaps it is not surprising that even minor amino acid changes in the iNOS enzyme can affect the levels of NO. The (C150T) polymorphism found in exon 16 of the *iNOS* gene was previously suggested to affect activity of this enzyme, leading to increased production of NO\[59\]. Interestingly, in our or other populations\[56,57\], the *iNOS* polymorphism alone was not a risk factor for cancer development. This fact suggests that while the C150T polymorphism may affect NO production, it does not do so to such an extent as to induce cancer development in isolation. Instead, a co-carcinogenic factor is required to synergistically interact in such a way as to promote disease progression. Within the population of Northern Iran, this co-carcinogenic factor appears to be *H. pylori* infection (Table 3). A similar result was also found within the Japanese population\[49\]. Alternatively, the *iNOS* polymorphism was suggested to act as a co-carcinogenic factor with past cigarette smoking and alcohol consumption within the Chinese population\[57\]. Thus, in multiple populations, *iNOS* polymorphism synergistically interacts with various factors to affect GC development.

While the molecular mechanism of synergistic interaction between the *iNOS* polymorphism and *H. pylori* infection is not clear, one plausible hypothesis is that since *H. pylori* infection alone is sufficient to increase NO levels in gastric tissue\[57,61\], the addition of a polymorphism that also increases iNOS enzyme activity could increase the amount of NO in the local tissue to a further degree. In turn, this combined increase in NO could skew the gastric mucosa toward a pro-inflammatory response and increase the likelihood of carcinogenic cellular damage. It is interesting to note that *H. pylori* infection alone creates a chronic pro-inflammatory environment within the gastric mucosa. Furthermore, it has been well documented that the ability of *H. pylori* to establish a persistent infection depends on maintenance of the proper balance between the host immune system and the bacterium (reviewed in\[45\]). In patients with either the CT or TT *iNOS* polymorphisms, the increased levels of NO may upset this delicate balance, thus perpetuating pathological and carcinogenic events.

*H. pylori*-mediated cancer development is a complex and multi-faceted process that involves a combination of bacterial, host, and environmental factors. Moreover, infection with *H. pylori* is inversely correlated with development of esophageal cancer\[63\], and is thought to provide protection against development of autoimmune disorders such as asthma\[11\]. Thus, the apparent benefit(s) of asymptomatically harboring this organism brings to bear the question of whether it is best to treat all patients for *H. pylori* infection; if not, the question becomes-how do we identify individuals most at risk for developing serious disease? One possible way to differentiate patients who have a high risk for disease development from those who are low-risk is to specifically examine bacterial, host-genetic and environmental factors that have been shown to be associated with cancer development in *H. pylori*-infected individuals. By screening individual patients for these three types of markers, it may be possible to specifically identify and treat those individuals who are at most risk for developing severe disease while reducing the risk of losing the apparent protective effects of *H. pylori* infection in low-risk individuals.

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**COMMENTS**

**Background**
As gastric cancer (GC) remains a serious source of morbidity and mortality worldwide, the identification of individuals most at risk for disease development is a major concern.

**Research frontiers**
Recent studies have highlighted the importance of polymorphisms in host genes as a risk factor for GC development. However, as many of these studies have been performed on limited patient populations, it remains unclear whether these polymorphisms are important across diverse ethnic groups and geographic areas. In this study, the authors identified an increase in GC risk in Helicobacter pylori (H. pylori)-infected Iranian patients who harbored a CT or TT polymorphism at position 150 in the inducible nitric oxide synthase (iNOS) gene.

**Innovations and breakthroughs**
To the best of our knowledge, this study describes the first known link between a host-genetic factor and GC risk in an Iranian population. In addition, this study is the first to define the importance of the iNOS C150T polymorphism in a non-Asian population.

**Applications**
The results of this study suggest that the C150T iNOS polymorphism is a potential marker for susceptibility to H. pylori-mediated gastric disease.

**Peer review**
This is a study aimed at investigating the association of the iNOS C150T polymorphism with H. pylori infection and GC risk among patients from a region of Iran. The authors present an interesting study from a region that has not been addressed very often in regard to H. pylori and its related pathologies.

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