Original Article

Prevalence of *Helicobacter pylori* and its *cagA* gene in patients with gastric cancer or peptic ulcer at an Iranian medical center

Maryam Kianmehr[1], Mohsen Zargar[1*], Ahmad Hormati[2*], Roohollah Fateh[3], Razieh Nazari[1]

[1]Department of Microbiology, Faculty of Basic Science, Qom Branch, Islamic Azad University, Qom, Iran
[2]Gastroenterology and Hepatology Disease Research Center Qom University of Medical Science, Qom, Iran
[3]Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran

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Abstract

Background: Iran has a high incidence rate for gastric cancer among the Middle East countries. In addition to gastric cancer, peptic ulcer is also life-threatening; thus, investigating the prevalence of *Helicobacter pylori* infection and other risk factors are essential. The present study was aimed to assess the frequency of *H. pylori* and the *cagA*-positive strains in patients with gastric cancer or peptic ulcer at a teaching hospital in Qom, one of the most populated cities of Iran.

Materials and Methods: The presence of *H. pylori* was investigated in gastric cancer and peptic ulcer biopsy specimens using the standard culture method. PCR analysis was performed to detect the presence of the *cagA* gene.

Results: The frequency of *H. pylori* isolates among 86 investigated biopsies was 20 (23.2%). Likewise, the rate of *H. pylori* was the highest when samples were examined from patients with gastric cancer (25.8%), while it was 21.8% when obtained from peptic ulcer patients. The frequency of the *cagA* gene in *H. pylori* isolates was 9 (56.2%), as confirmed by PCR.

Conclusion: Our results indicated that *H. Pylori* infection and its virulent strains are frequent and widely spread in Qom city. The *cagA* gene was present in almost half of *H. pylori* isolates from peptic ulcer or gastric cancer patients. Therefore, it is necessary to screen it in all cases with *H. pylori* infection for early detection of gastric cancer.

Keywords: *Helicobacter pylori*, *cagA*, Gastric Cancer, Peptic ulcer, Qom

***Co-corresponding Authors:** Mohsen Zargar, PhD, Department of Microbiology, Faculty of Basic Science, Qom Branch, Islamic Azad University, Qom, Iran. Email: zmohsen2002@yahoo.com. Ahmad Hormati, MD, Gastroenterology and Hepatology Disease Research Center Qom University of Medical Science, Qom, Iran. Email: hormatia@yahoo.com; https://orcid.org/0000-0002-3108-5655.

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Introduction

Helicobacter pylori is a global issue with increasing rates of infection in the world, especially in developing countries [1, 2]. Infection caused by *H. pylori* leads to various gastric diseases including chronic gastritis, peptic ulcer, and gastric cancer[3-5]. Recently, the World Health Organization (WHO) has classified *H. pylori* as Class I carcinogen and a risk factor for gastric adenocarcinoma[6]. According to the epidemiological studies, more than 50% of the human population is infected with *H. pylori*, although there are large geographical variations[7]. Generally, the prevalence of *H. pylori* infection is higher in developing countries in comparison of developed countries[7,8]. Although infection caused by *H. pylori* is common

among people, only a small group of the infected population develops peptic ulcer and gastric cancer[9]. Thus, factors such as host genetic, environmental, and bacterial virulence factors, such as the *cagA* gene, are considered to be responsible for neoplastic development [9-12]. Poor hygiene standards and crowded households are among the important environmental factors that are associated with an increased risk of *H. pylori* infection, and these factors are usually found among those living in developing countries[13-15]. The cytotoxin-associated gene A (CagA) protein is the most extensively studied virulence factor of *H. pylori*. Several studies showed that CagA positive strains of *H. pylori* infection developed gastric cancer[16-19].

In Iran, a high prevalence of *H. pylori* infections has been reported, more than 80% in the general population[20]. To genotypes, *cagA* positive *H. pylori* strains in Iranian isolates were associated with gastric cancer[20-22]. Although the prevalence of *H. pylori* and its CagA phenotype has been reported in several regions of Iran, but our knowledge is incomplete about Qom, one of the central cites of Iran. Thus, the aim of this study was to assess the frequency of *H. pylori* and *cagA*-positive strains in patients with gastric cancer or peptic ulcer at a teaching hospital in Qom, one of the most populated cities of Iran.

Methods

This study was conducted at a teaching hospital in Qom (2018-2019), Iran, from July 1 to April 20. The study protocol was approved by the internal review board of Azad University of Medical Sciences.

Study population

Patients who were diagnosed with gastric cancer or peptic ulcer, based on clinical examination, were consecutively included in the study. Experienced gastroenterologist collected three biopsy specimens during each endoscopy session: one sample from the greater curvature of the corpus and two samples from the antrum. Biopsy specimens for bacterial culture were immediately placed in transport media and brought to the laboratory on the day of endoscopy.

Isolation and identification of *H. pylori*

We followed the protocol of Dabiri and colleagues for the isolation of *H. pylori*, using Columbia blood agar supplemented with 10% horse blood, and selective supplement of *H. pylori*[21]. Then, we incubated the inoculated plates for 4 to 7 days at 37°C under microaerophilic conditions. The *H. pylori* were identified by its biochemical profile, such as oxidase, catalase, and urease reactions[21].

DNA extraction and PCR amplification

The extraction of DNA from *H. pylori* isolates was done from freshly harvested bacterial cells. The DNA was extracted using the DNA Mini Kit (Bio Basic, Canada) according to manufacturer specifications. For confirmation of *H. pylori* isolates, PCR for *16SrRNA* and *glmM* genes using specific primers was performed based on published papers[23].

Screening of the *cagA* gene was performed by a reaction mixture[22]. PCR amplification was carried out in a PCR system[21]. Sterile distilled and DNA from colonies of *H. pylori* ATCC 26695 were used as negative and positive controls, respectively. PCR product was detected by 1.5% gel electrophoresis.

Statistical analysis

Data were analyzed using statistical program for social science (SPSS) version 23.0. Qualitative data were expressed as frequency and percentage.

Results

A total of 86 biopsies were collected from patients (age range: 40 to 65 years) with either peptic ulcer (n=55) or gastric cancer (n=31). Based on the culture, the frequency of *H. pylori* among all investigated biopsies were 20 (23.2%). Likewise, the rate of *H. pylori* was the highest when samples were examined from patients with gastric cancer (25.8%), while it was 21.8% when obtained from peptic ulcer patients.

In the 16 *H. pylori* isolates which were used for molecular analysis, the frequency of the *cagA* gene in all *H. pylori* isolates was 9 (56.2%), as confirmed by PCR. Patients with gastric cancer had a greater proportion of *cagA*-positive *H. pylori* strains than those with peptic ulcers (62% vs. 50%).

Discussion

Iran is known for the high mortality rate from gastric cancer in the world and digestive tract diseases are common[20]. *H. pylori* is one of the most important risk factors for gastric cancer. Therefore, we investigated the frequency of *H. pylori* and its *cagA* gene among patients with gastric diseases.

In the current study, the total frequency of *H. pylori* in patients with either peptic ulcer or gastric cancer was comparable with the reported prevalence in developing countries and similar to gastric cancer samples[24,25]. In this study, we investigated the prevalence of the *cagA* gene, from *H. pylori*-positive gastric cancer and peptic ulcer samples using PCR. In Iran, the majority of *H. pylori*-infected individuals are *cagA* positive strains. The prevalence lies between 66% and 90% in different ages and geographic regions[20]. The frequency of *cagA*-positive *H. pylori* is 90% to 95% in Asian countries, whereas it is 50% to 60% in western developed countries[24]. In the current study, we found that 56.2% of isolates from gastric cancer or peptic ulcer patients were positive for *cagA*. This was relatively higher than the prevalence of *cagA*, as reported by other studies, such as 46% in Egyptian isolates of *H. pylori*[26]. While in other countries in the Middle East, the *cagA* gene was even lower in *H. pylori* isolates from Jordan (26%)[27].

The correlation of *cagA*-positive *H. pylori* strains with gastric cancer was established in some previous studies [28-32]. However, other studies reported no association between *cagA* genotype and gastric cancer patients[33,34]. The results of different studies across several geographical regions support the unpredictability of the expression of the *cagA* gene of *H. Pylori* across various populations [35]. The variability of *cagA* gene roles in the development of gastric cancer in different ethnic groups shows the effect of host genetics or other environmental factors that moderate its expression. Thus, further studies are still needed to explore the moderating correlation of the *cagA* gene in gastric cancer patients.

This study had some limitations. First, we only obtained samples from one city in Iran. Therefore, our results might not be generalizable across the country. Second, the study population in this study came from peptic ulcer or gastric cancer patients; therefore, the specifically was not the general population. Third, an additional study using a large number of participants will be necessary to confirm our current data. Finally, further investigation is necessary to obtain samples from other regions throughout the country.

Conclusion

Our results indicated that *H. Pylori* infection and its virulent strains are frequent and widely spread in the Qom. The *cagA* gene was present in almost half of *H. pylori* isolates from peptic ulcer or gastric cancer patients. Therefore, it is necessary to screen it in all cases with *H. pylori* infection for early detection of gastric cancer.

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