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Prevalence and Clinical Relevance of *cagA* and *oipA* Genotypes of *Helicobacter pylori* in Children and Adults with Gastrointestinal Diseases in Tehran, Iran

Abdolreza Esteghamati[1], Shirin Sayyahfar[1],Khadijeh Khanaliha[1], Ahmad Tavakoli[1,2], Mehri Naghdalipour[1], Mehdi Zarean[3], Morteza Haghighi Hasanabad[1]*

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Abstract

Background: *Helicobacter pylori is*a universal pathogen that causes gastric diseases and cancers in humans. In recent years, several virulence genes have been detected in this microorganism. Thus, we aimed to investigate the frequency of *Helicobacter pylori* strains with *cytotoxin-associated gene A*(*cagA*) and *outer membrane inflammatory protein A*(*oipA*) genotypes among children and adult patients in Tehran, Iran, and evaluatetheir relation to themanifestations of different clinical symptoms.

Methods: In this cross-sectional study, biopsy specimens were obtained from patients with gastrointestinal symptomsand evaluated for *Helicobacter pylori infection*and its genotypes (*cagA*/*oipA)* througha polymerase chain reaction PCR assay. Clinical findings and demographic data of patients were documented and analyzed.

Results: A total of 80 patients with *Helicobacter pylori* infection were included in the study (34 children and 46 adults). The *cagA* and *oipA* genotypes of *Helicobacter pylori* were identified in 22 (64.7%) and 24 (70.5%) children and in 31 (67.3%) and 34 (73.9%) adults, respectively. These differences were not statistically significant between the 2 studied groups. In addition, the frequency of *cagA*-positive strains of *Helicobacter pylori* was found more among patients with gastric ulcers rather than other clinical outcomes.

Conclusion: Our findings demonstrate a highfrequency of *Helicobacter pylori strains* with *oipA* and *cagA* genotypes among children and adults in this region. Although we could not find a significant relationship between virulence genes and clinical outcomes in the patients, further studies are suggested to evaluate these factors in patients and assess their potential roles in the presence of antibiotic-resistant strains.

Keywords: *Helicobacter pylori*, Prevalence, Virulence Factors, Children, Adults

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Introduction

Helicobacter pylori (*H.pylori*) is a widespread bacterium that could be found in the stomach of more than 50% of people in the world. It is also known as the main cause of gastritis and peptic ulceration in humans. In developing countries like Iran, over 80% of people carry *H.pylori in* their stomachs (1, 2).

Corresponding author: *Dr Morteza Haghighi Hasanabad, mhaghighi@razi.tums.ac.ir*

1. Pediatrics Infectious Diseases Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran
2. Department of Virology, Iran University of Medical Sciences, Tehran, Iran
3. Department of Parasitology, Mashhad University of Medical Sciences, Mashhad, Iran

↑What is "already known" in this topic:
Helicobacter pylori is a wide spread microorganism that could be isolated from the stomach of more than 50% of individuals worldwide. Studying *Helicobacter pylori* virulence factors and their roles in disease manifestations may promote the health status of patients and inhibit severe conditions.

→What this article adds:
The high frequency of *cagA* and *oipA* genotypes of *Helicobacter pylori* strains among children and adults in this region are notable. Moreover, the frequency of *Helicobacter pylori* strains with the *cagA* and *oipA* genotypeswas found more amongpatients with peptic ulceration.

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In the last decades, many virulence genes of *H.pylori* have been described thatcould alter the severity of clinical outcomes in patients. Therefore, there is an ongoing interest in recognizing their roles in the pathogenesis of *H.pylori* (3). Generally, people achieve *H.pylori* infection during childhood and the microorganism remains in their bodies for lifelong. In other words, the genotypic patterns of *H.pylori* strains in children and adults of each geographical region are similar (4).

One of the important virulence genes in *H.pylori* that has been frequently studied in recent years is *cytotoxin-associated gene A* (*cagA*). It is found in strains harboring *cag* pathogenicity island and typically is recovered from patients with severe diseases (5). In one study, a significant association was reported between active gastritis and infection of patients with the *cagA*+ strains of *H.pylori*, and in another study, it was reported in correlation with ahigher incidence of peptic ulcers and gastric cancer in patients. It is implied that *cagA* could facilitate the injection of bacteria into the human gastric cells and induces the inflammatory response and secretion of chemokines such as interleukin-8 (6).

Another virulence gene in *H.pyloriisouter membrane inflammatory protein A* (*oipA*), which predominantly is found in patients with gastric and peptic ulcers. Several functionshave been reported forthis gene after expression such as IL-8 inducing factor, pH regulation, and acting as adhesion proteins. According to previous studies, the frequency of *oipA*+ genotypes of *H.pylori* has been reported from 30% to 70% in different regions (7).

Currently, the association of these 2 virulence genes of *H.pylori in* the demonstration of different clinical outcomes among patients, especially inchildren with acute infection, is under investigation in several surveys. Thus, in the present study we aimed to evaluate the frequency of *H.pylori* strains with *cagA* and *oipA* genotypes isolated from children and adults with gastrointestinal symptoms in Tehran, Iran, and alsoestimate its relationship with the clinical manifestations in the patients.

Methods

In this cross-sectional study, the target population was defined as patients with gastrointestinal symptoms who were referred to the endoscopy unit of the gastroenterology ward of a university hospital (Hazrate-Rasool) in Tehran, Iran,for1year (2018-2019). This governmental hospital is one of the main medical centers in Tehran, the capital city of Iran, which is located in the central region of the city and provides awide range of facilities and comprehensive care to referrals with lowfees (8).

Participation in this study was voluntary and a consent form was obtained from individuals. Patients weregrouped as children (ages of ≤15 years) and adults (>15 years). Medical and demographic data of patientswere collected using aquestionnaire. Then, biopsy specimens were obtained throughendoscopyand samples weretested viathe rapid urease test(RUT) for *H.pylori* infection, and then evaluated pathologically in positive cases. Patientswith negative results from the RUT assay were excluded from the study.

Samples with positive results of the RUT were transported to the research laboratoryat theinstitute of immunology and infectious diseases (Iran University of Medical Sciences) and prepared for extraction of DNA and *H.pylori* PCR test (9). Isolation of nucleic acids from specimens wasperformedusing a commercial extraction kit (DNP kit) and total DNAs were stored at -20°C until the PCR test (10). An in-house PCR assay with previously published primers was developed toamplify a 294bp fragment of the *glm* gene in *H.pylori*. Additionally, 2 conserved regions in the *cagA* and *oipA* genes of *H.pylori* were amplified by using specific primers and based on described methods by Gregory et al (11). Table 1 illustrates the primer sequences and the size of PCR products.

Statistical Analysis

The prevalence of *H.pylori* genotypes was expressed as percentages and the student *t* test was used to evaluate continuous variables. Additionally, the chi-squared test was used to comparethe frequencies of *H.pylori* genotypes between the 2 studied groups and also estimate its relationship with the clinical manifestations in the patients (12). Statistical analyses of data were performed by using MedCalc statistical software (MedCalc Soft) (13). $P<0.05$ was considered significant.

Results

A total of 96 patients were recruited during 1year of sampling, of which 16 were excluded from the study. Of 80 participants with a mean age of 24.1 years (± SD,13.6 years), 34 cases were at the ages ≤15 years old and classified as children (42.5%), and 46 cases (57.5%) with the ages of >15 years old were grouped as adults. additionally, 35 (43.7%) were males and 45 (56.3%) were females.

Based on the results of the PCR assay, *cagA* and*oipA*-genotypes of *H.pylori* were identified in 53 (66.2%) and 58 (72.5%) cases, respectively. Table 2 demonstrates the frequency of different genotypes of *H.pylori* strains among children and adults. There were no statistical differences between the 2 studied groups for the prevalence of *H.pylori* infection with *cagA* and *oipA* genotypes.

Considering the findings of endoscopic examinations

*Table1.*The sequence of primers used in this study

Primers	Sequences	Size
glm-f	AAGCTTTTAGGGGTGTTAGGGGTTT	294bp
glm-r	AAGCTTACTTTCTAACACTAACGC	
cagA–f	GATCTCGGTGGGTCTTTC	506bp
cagA–r	TCTTTTACGGCATTGTTCA	
oipA-f	GTTTTTGATGCATGGGATTT	400bp
oipA-r	GTGCATCTCTTATGGCTTT	

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Table 2. The frequency of *H.pylori* genotypes among studied groups

H.pylori Genotypes	Children N=34 (%)	Adults N=46 (%)	*P*-Value*
$CagA^+$	22 (64.7%)	31 (67.3%)	0.990
$CagA^-$	12 (25.3%)	15 (22.7%)	
$OipA^+$	24 (70.5%)	34 (73.9%)	0.939
$OipA^-$	10 (29.5%)	12 (26.1%)	

*Chi-squared test

Table 3. Clinical outcomes in studied groups according to the *H.pylori* genotypes

Groups	Genotypes	Peptic Ulceration		Gastritis N=41(%)	*P*-value*
		Duodenal ulcer N=25 (%)	Gastric ulcer N=14 (%)		
Children	$CagA^+$	7 (70.0%)	5 (71.4%)	10 (58.8%)	0.719
	$CagA^-$	3 (30.0%)	2 (28.6%)	7 (41.2%)	
	$OipA^+$	9 (90.0%)	6 (85.7%)	9 (52.9%)	0.059
	$OipA^-$	1 (10.0%)	1 (14.3%)	8 (47.1%)	
Adults	$CagA^+$	10 (66.6%)	6 (85.7%)	15 (62.5%)	0.671
	$CagA^-$	5 (33.4%)	1 (14.3%)	9 (37.5%)	
	$OipA^+$	9 (60.0%)	4 (57.1%)	21 (87.5%)	0.063
	$OipA^-$	6 (40.0%)	3 (42.9%)	3 (12.5%)	
Total	$CagA^+$	17 (68.0%)	11 (78.5%)	25 (60.9%)	0.431
	$CagA^-$	8 (32.0%)	3 (21.5%)	16 (29.1%)	
	$OipA^+$	18 (72.0%)	10 (71.4%)	30 (73.1%)	0.901
	$OipA^-$	7 (28.0%)	4 (28.6%)	11 (26.9%)	

*Chi-squared test (analysis was performed between patients with peptic ulceration and gastritis)

and results of pathological assessments, gastritis and peptic ulceration (duodenal ulcer and gastric ulcer) were found as the main clinical outcomes in the patients. As shown in Table 3, *H.pylori* strains with the *cagA* genotype were mostly isolated from both children (71.4%) and adults (85.7%) with gastric ulcers (5 and 6 of 7 cases, respectively), rather than other clinical outcomes. Additionally, the *oipA*genotype of *H.pylori*was found commonly in 90% (9 of 10) of children with duodenal ulcers and 87.5% (21 of 24) of adults with gastritis. There were no statistical relations between *H.pylori*genotypes and clinical findings in patients of both studied groups (Table 3).

Discussion

More than 50% of people in the world are chronically or acutely infected with *H.pylori*, as noted in the literature. The prevalence of infection varies widely in differentcountries with a higher rate of infection amongpeople with low socioeconomic status and/or living in crowded cities (14). In Iran, *H.pylori*has been found in 60% to 90% of people, and this data is similar to reports from our neighboring countries (Pakistan and Turkey) or other countries in far regions like South America and Japan (15-18). Thus, it is of great importance to identify the genotypic patterns of *H.pylori*strains in different regions and populations. In a cohort study from Iran, for example, *H.pylori* virulence genes were investigated among 222 adult patientswith gastrointestinaldiseases and positive strains for *oipA*and *cagA* genes were reported as 81.1% and 62.2%, respectively (19). However, the molecular epidemiology of *H.pylori* genotypes among Iranian children is not well investigated until now and the present study provides this data for the first time from Tehran, Iran.

Our findings in the current study revealed that the majority of patients in both groups are infected with *cagA*+ strains of *H.pylori* (65.2%). Consistent with our results, similar data have been reported by studies in different regions of the world. Accordingly, the *cagA* genotype of *H.pylori* was commonly isolated from adult patients of Iraq (71%), Turkey (78%), China (90%), and Korea (97%) (20, 21). This finding was also supported by previous studies from Iran in which the *cagA* genotype was reported in the range of 60% to 95% in *H.pylori* isolates (22). Likewise, a high percentage of infection occurrences with *cagA*+ strains of *H.pylori was* observed in American children (about 70%) (23). Moreover, we found that the *cagA*+ genotype of *H.pylori* is more prevalent in patients with gastric ulcer diseases rather than in other clinical outcomes. This finding was supported partially by a study in Poland in which the presence of the *cagA* genotype was reported in 60% of patients with gastric ulcers (24).

Interestingly, it has been proposed by some researchers that the presence and expression of *the cagA* gene in *H.pylori* is associated with the better circulating of blood flow and antibiotics diffusion tothe injured cells, and therefore increasing the chance of successful treatment for the patients (25). However, it seems that the *cagA* gene of *H.pylori* is not a suitable candidate to powerfully predict the clinical outcomes of infection in adultness before the development of diseases during childhood. Consistently, our finding that no difference was observed between the rates of *cagA* genotype among children and adults is confirming this verdict.

In the present study, the *oipA* virulence gene of *H.pylori*was detected in approximately 70% of children and adult groups. This result is following previous reports thatdemonstrated the *oipA*prevalence is between 60% and 90% among Iranian people living in different regions of the country (26). We also found this genotype of *H.pylori* in the majority of children with peptic ulceration (gastric ulcer and duodenal ulcer). Keeping that, the *oipA*+ strain of *H.pylori* was reported as the predominant isolated genotype among Tunisianpatients with gastric ulcers (27).

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Additionally, the *oipA* genotype of *H.pylori* was reported in association with peptic ulcer diseases, according to one Turkish study (28). Although we could not find a significant association between the *oipA* genotype of *H.pylori* and peptic ulceration in children,possibly due to the few numbersof included patients, our results support the above-mentioned data.

Finally, our study had some limitations, including recruiting participants from only one hospital and/or using small sample sizes of the examined patients in each group. These issues should be taken into account when interpreting the results.

Conclusion

Our results disclose a high prevalence of *H.pylori* virulent genotypes among children and adults in Tehran, the capital city of Iran. The presentstudy also revealed that the high frequency of the the*oipA*genotype of *H.pylori* could contribute to the development of peptic ulcer diseases among children. At last, we recommend continuous monitoring of the patients on this issue and also suggest further studies to evaluate the potential roles of these virulence genes in the presence of antibiotic-resistant strains of *H.pylori*.

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Ethical Approval

This study was reviewed by the board members of the ethical committee at Iran University of Medical Sciences and approved (Ethical Code: IR.IUMS.REC 1396.30929).

Authors' Contribution

Esteghamati A. designed and supervised the project. Sayyahfar S., Khanaliha KH., and Tavakoli A. were involved in the different steps of the study. Naghdalipour M. performed laboratory tests. Zarean M. analyzed data. Haghighi H.M. optimized diagnostic assays and prepared the first draft of the manuscript. All of the authors approved the final version of the paper.

Conflict of Interests

The authors declare that they have no competing interests.

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