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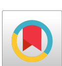

Prevalence of Oral *Helicobacter pylori* and *cagA* Gene in Healthy Young Iranian Children: A Cross-Sectional Study

Nazanin Zargar [1], Neda Shariatinia [2], Bahareh Hajikhani [3], Soodabeh Taheri [3], Payam Paymanpour [1,*], Shivasadat Tabatabaei [2,4]

[1] Department of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] School of Public Health, Boston University, Boston, USA

[*] ***Corresponding Author:*** Department of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: payam_paymanpour@yahoo.com

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Abstract

Background: The oral cavity has been suggested as a potential reservoir for *Helicobacter pylori*, which plays a significant role in various health conditions. The cytotoxin-associated gene A (*cagA* gene) encodes an important virulence factor in *H. pylori* infection.

Objectives: This study aimed to determine the prevalence of oral *H. pylori* and the *cagA* gene in healthy children (< 5 years) in Tehran, Iran, and explore associations with age, sex, family history of gastritis, and dental health status.

Methods: This cross-sectional study included 160 asymptomatic children. Informed consent was obtained from parents or guardians after discussing the study details. Age, sex, and family history of gastritis were recorded using questionnaires. Dental health status was assessed by a trained dentist using the decayed, missing, and filled teeth (dmft) index. Oral samples were collected with sterile cotton swabs from the buccal and sublingual mucosa. Polymerase chain reaction (PCR) was used to detect *H. pylori* and the *cagA* gene. Statistical analysis was performed using Fisher's exact test ($\alpha = 0.05$).

Results: *Helicobacter pylori* was detected in six participants (3.75%), and the *cagA* gene was present in all *H. pylori*-positive samples. No significant associations were found with age, sex, family history of gastritis, or the dmft score.

Conclusions: A small percentage of participants had oral *H. pylori*, with no significant link to demographic factors, family history of gastritis, or dental health. Larger studies are needed to better understand the epidemiology and long-term risks of oral *H. pylori* colonization in Iranian children.

Keywords: Genes, *Helicobacter pylori*, Oral, Child, Polymerase Chain Reaction (PCR)

1. Background

Helicobacter pylori is a Gram-negative, microaerophilic bacterium that infects the gastrointestinal epithelium and affects nearly half of the global population (1). It is a common cause of chronic gastritis, which often remains asymptomatic (2). Additionally, it has been linked to various health conditions, including the aggravation of gastritis, duodenal ulcers, iron deficiency anemia, lymphoma, and reactive adenocarcinoma (3-5). As the only bacterium classified as a class 1 carcinogen (6), *H. pylori* is the strongest known risk factor for gastric adenocarcinoma, which accounts for nearly two-thirds of gastric cancer cases worldwide (7). Moreover, the expression of the cytotoxin-associated gene A (*cagA*) in *H. pylori* has been considered a risk marker for mucosal atrophy and carcinogenesis. The encoded protein induces morphological and malignant transformations in gastric epithelial cells by disrupting intracellular signaling pathways (5, 8, 9). The bacterium can evade the host's immune system by entering the cytoplasm of gastric epithelial cells, where neoplastic transformation occurs through the oncogenic protein encoded by the *cagA* gene (5, 9). Notably, gastric inflammation works in tandem with compensatory genetic and epigenetic

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alterations, leading to self-sustaining carcinogenesis (5, 10).

Helicobacter pylori infection commonly occurs within the first decade of life (11). In children, it is often transient and self-limiting (12), with symptoms typically manifesting later in life (13). The presence of the bacterium in the oral cavity supports both fecal-oral and oral-oral transmission routes (14-17). Additionally, poor personal and food hygiene practices in densely populated areas significantly contribute to intrafamilial transmission (18). Interestingly, the eradication of *H. pylori* from the oral cavity has been shown to improve the success rate of gastric infection treatment (19). Consequently, several previous studies have suggested that oral *H. pylori* may indicate actual infection rather than mere transient colonization (16, 20, 21).

The established relationship between *H. pylori* infection and gastric cancer (7), along with its poor prognosis as a leading cause of cancer-related mortality (22, 23), underscores the importance of early detection (24). This is particularly relevant since the prevalence of *H. pylori* in adulthood has been suggested to depend on childhood infection (25). Previous studies on the Iranian population have reported *H. pylori* infection prevalence ranging from 30.6% to 82% (26). However, given the close relationship between oral *H. pylori* and actual infection (27), and the fact that *H. pylori* infection typically occurs before the age of five (28), the prevalence of the bacterium in young Iranian children has yet to be investigated.

2. Objectives

The present study aimed to detect *H. pylori* and the *cagA* gene using polymerase chain reaction (PCR) in oral samples from asymptomatic children under the age of five residing in Tehran, Iran. Additionally, the study explored associations with age, sex, family history of gastritis, and the decayed, missing, and filled teeth (dmft) score.

3. Methods

3.1. Study Design and Participants

Ten kindergartens across districts #1 and #2 of Tehran were selected for this cross-sectional study. A total of 160 asymptomatic, healthy children under five years of age participated. Participants were chosen using simple random sampling from a list of enrolled children provided by the kindergartens. The sample size was determined based on feasibility considerations and a previous study by Castro-Muñoz et al. on 162 asymptomatic young Mexican children, which reported a prevalence rate of 13% for oral *H. pylori*, with an expected precision of 5.21% (29). Only asymptomatic children whose parents provided written informed consent were included in the study. Confidentiality and anonymity of the participants were ensured throughout the research process. Children or parents/guardians unwilling to participate, as well as those with systemic diseases or gastrointestinal symptoms, were excluded from the study.

3.2. Data Collection

Data collection took place between January and March 2020. For each participant, information on age, sex, and family history of gastritis was obtained from parents or legal guardians using questionnaires specifically designed for this study. A trained dentist then conducted an individual oral examination for each participant through visual inspection, using a dental explorer and mirror under adequate lighting conditions, to record the dmft score (30).

3.3. Sample Collection

Oral samples were collected by swabbing the buccal mucosa and sublingual areas of each participant using sterile cotton swabs (Arta Teb, Iran) (29). The swabs were immediately placed in sterile test tubes containing Luria-Bertani (LB) broth (Thermo Fisher Scientific, USA) and transferred to an independent laboratory for further analysis. To minimize potential detection bias, PCR analysis was conducted by trained personnel who were blinded to the participants' information (31).

3.4. DNA Extraction

Bacterial genomic DNA was extracted from the collected samples using the HiPurA™ Bacterial Genomic DNA Purification Kit (HiMedia, India) following the manufacturer's instructions. The quantity and purity of the extracted DNA were evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA).

3.5. Polymerase Chain Reaction Analysis

The presence of *H. pylori* and the *cagA* gene in oral samples was evaluated using PCR with specific primers (Table 1). Each PCR reaction was carried out in a total volume of 25 µL, consisting of 12.5 µL of master mix (Amplicon, Denmark), 1 µL of each primer (10 pmol/µL), 2 µL of template DNA, and 8.5 µL of nuclease-free water. The thermal cycling conditions were as follows: Initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds (35 cycles); annealing at 55°C for 30

Table 1. Primer Sequences Used for Polymerase Chain Reaction

Gene	Primer Sequence (5' - 3')	Product Size (bp)	Reference
16S rDNA	F: AGAGTTTGATCCTGGCTCAG	1500	(36)
	R: AAGGAGGTGATCCAGCCGCA		
cagA	F: AATACACCAACGCCTCCAAG	400	(33)
	R: TTGTTGCCGCTTTTGCTCTC		

Abbreviations: bp, base pair; *cagA*, cytotoxin-associated geneA; F, forward; R, reverse.

seconds; extension at 72°C for 1 minute; and a final extension at 72°C for 7 minutes (32). Genomic DNA from the standard *H. pylori* strain (ATCC 49503) was used as a positive control (33-35).

3.6. Gel Electrophoresis

The PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The gel was run at 100 volts for 45 minutes in 1X TAE buffer and visualized under ultraviolet light using a gel documentation system (Bio-Rad, USA). The presence of specific bands corresponding to *H. pylori* and the *cagA* gene indicated positive samples (32).

3.7. Statistical Analysis

Statistical analysis was performed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Fisher's exact test was used to examine associations between *H. pylori* presence and variables such as age, sex, family history of gastritis, and dmft scores. A P-value of less than 0.05 was considered statistically significant.

4. Results

4.1. Participants' Demographics and Dental Health Status

The mean age of the children was 3.15 ± 1.30 years. No pathological soft-tissue oral lesions were observed during intraoral visual examinations. The dmft scores ranged from 0 to 3, with a mean of 1.56 ± 0.49. A positive family history of gastritis was reported in 25 children (15.6%).

4.2. Prevalence of Helicobacter pylori

The PCR identified bacterial genomic DNA in 6 children (3.75%), all of whom were also positive for the *cagA* gene. Statistical analysis did not reveal any significant association with the participants' age, sex, family history of gastritis, or dental health ($P > 0.05$). Table 2 provides a breakdown of participants by age group, sex, dmft score, and family history of gastritis.

5. Discussion

To the best of the authors' knowledge, no previous study has investigated the prevalence of *H. pylori* in young children in Tehran. Therefore, this study aimed to explore the oral prevalence of *H. pylori* and the *cagA* gene in symptom-free young children in this city. The PCR was used in this study due to its ability to exclude other phylogenetically close bacteria, such as *Campylobacter* species (37), as well as its high reliability and efficiency in detecting *H. pylori* in children (38). The results showed a detection rate of 3.75% among children, which is considerably lower than that reported for Iranians under 15 years old (42% prevalence, 95% CI: 41 - 44%) or for the general healthy Iranian population (30.6% to 82% overall prevalence, with an age range of four months to 83 years) (26, 39).

This disparity may stem from differences in detection methods—such as enzyme-linked immunosorbent assay or stool antigen—, age-related inclusion criteria, or socioeconomic factors. In fact, social class, parental educational level, living conditions, household size, and drinking water sources have all been shown to influence the prevalence of *H. pylori*, even within different regions of the same country (17, 18). Moreover, the low prevalence in this study can be explained by our sampling method, which did not include dental plaque. Indeed, dental plaque has the highest detection rate of H. pylori in the oral cavity owing to its biofilm characteristics (21, 40). The lower prevalence of *H. pylori* in younger children, compared to older children or adults, in other countries also help explain the low detection rate in this study (15, 26, 41).

In this study, the virulence gene *cagA* was detected in all *H. pylori*-positive oral samples. This finding is consistent with a previous study that reported similar results using saliva samples from Iranian adults with gastroduodenal disease, who were referred to an endoscopy center in a different city (34). Additionally, another study investigating *H. pylori* genotyping and host antibody response identified the *cagA* gene in 91% of Iranian *H. pylori* strains isolated from gastric biopsies

Table 2. Demographic Information, Dental Health Status, and Family History of Gastritis [a]

Variables	Value	Positive for *Helicobacter pylori* (%)	P-Value [b]
Gender			0.109
Male	82 (51.25)	5 (83.3)	
Female	78 (48.75)	1 (16.7)	
dmft Score			0.173
0	94 (58.8)	1 (16.7)	
1	17 (10.6)	1 (16.7)	
2	33 (20.6)	3 (49.9)	
3	16 (10)	1 (16.7)	
Age			0.567
< 1	20 (12.5)	0	
1 - 2	33 (20.6)	2 (33.3)	
2 - 3	42 (26.25)	3 (50)	
3 - 4	34 (21.25)	1 (16.7)	
4 - 5	31 (19.4)	0	
Family history of gastritis			0.913
Present	25 (15.6)	1 (16.7)	
Absent	135 (84.4)	5 (83.3)	

Abbreviations: dmft, decayed, missing, and filled teeth; y, year (s).

[a] Values are expressed as Number (%).

[b] $P \leq 0.05$ was considered as statistically significant.

(35). These findings suggest that the *cagA* gene may exhibit low regional and possibly age-related variability within Iran. However, further studies are needed to validate this hypothesis.

In the present study, none of the *H. pylori*-positive subjects reported experiencing gastrointestinal symptoms at the time of investigation. This lack of symptoms may be attributed to a diminished immune response and reduced cell infiltration in children following bacterial colonization, leading to less gastric inflammation compared to adults (42, 43). However, even in the absence of symptoms, when *H. pylori* infection is suspected, it is important to consider the increased risk of gastrointestinal ulceration, particularly from nonsteroidal anti-inflammatory drugs and chronic immune thrombocytopenic purpura (44).

Although *H. pylori*-positive participants in this study were predominantly male, the difference between the two sexes did not reach statistical significance. This finding aligns with a previous study evaluating anti-*H. pylori* IgG in the serum of 2,561 healthy adults living in rural and urban areas of Tehran province (45). A meta-analysis also confirmed the absence of a clear gender-based predisposition for *H. pylori* infection in children, possibly due to differential antibiotic exposure or gender-specific protective immunity rather than solely differential exposure (46). Interestingly, while sex was found to be of no significance in *H. pylori* prevalence among children in South Korea, being male was reported as a risk factor in adults (41). Thus, although no significant association was found in the present study, a higher bacterial detection rate in males may suggest a possible trend in young Iranian children that warrants further investigation.

The present study did not show any association between *H. pylori* detection and the dmft score, which aligns with a previous report by Mehdipour et al. (mean age: 7.97 ± 1.83 years) (30). In contrast, Sruthi et al. reported significantly higher mean scores in Indian *H. pylori*-positive children aged 3 - 6 years, demonstrating that higher caries status and caries severity were associated with a greater prevalence of *H. pylori* (47). This discrepancy may be attributed to their small sample size (n = 20), as well as differences in geographical location, age range, and sampling method.

The present study had several limitations. First, a larger sample size was required to detect small effect sizes. Second, the cross-sectional nature of the study was unable to provide data on incidence rates, sources of infection, or lifelong trends, all of which are essential for devising effective future prevention strategies. Third, while PCR is a highly specific method, it does not differentiate between transient colonization and persistent infection. Fourth, participants were recruited

from only two districts, which may limit the generalizability of the findings. Consequently, future studies should include larger, more diverse samples and incorporate additional diagnostic techniques.

5.1. Conclusions

The prevalence of oral *H. pylori* was low in the study population. However, the *cagA* gene was detected in all *H. pylori*-positive participants. No associations were found with age, sex, family history, or dental health status.

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Footnotes

Authors' Contribution: Study concept and design: N. Z. and B. H; Acquisition of data: N. S. and B. H.; Analysis and interpretation of data: N. S., N. Z., B. H. and S. T.; Drafting of the manuscript: N. S., P. P., N. Z., B. H. and S. T.; Critical revision of the manuscript for important intellectual content: P. P., N. Z. and B. H.; Statistical analysis: N. S.; Administrative, technical, and material support: N. S., B. H., and S. T.; Study supervision: N. Z., B. H. and S. T.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1396.506).

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