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Research Article



# Comparison of the Prevalence of *Helicobacter pylori* Infection Between the Exclusively Breastfed and Formula- or Mixed-Fed Infants in the First Six Months of Life

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#### **Abstract**

**Background:** Helicobacter pylori is a common gastrointestinal pathogen in human. The mechanism of its acquisition and transmission is unclear, although the most likely mode of transmission is fecal-oral or oral-oral. Most of infections occur in childhood. The current cohort assessed the prevalence of infection in infants with different feeding strategies (exclusive breastfeeding, formula, or mixed feeding).

**Methods:** The current study was carried out in Vali-Asr hospital of Imam Khomeini hospital complex, Tehran, Iran, from 2012 to 2015. The study population included infants born in this hospital and registered in breastfeeding research center during the first 6 months of life. They were compared in 2 groups; exclusively breastfed, and formula or mixed fed. Mothers' *H. pylori* infection was checked serologically using the enzyme-linked immunosorbent assay (ELISA) technique and those of infants by monoclonal antibody test using ELISA technique on stool samples at 1st and 6th month of life.

**Results:** A total of 102 infants in 2 groups (54 exclusively breastfed and 48 formula or mixed fed) completed the study. Mothers' serological assessments were positive in 68% of exclusively breast-fed subjects and 60% of formula or mixed fed subjects. All 1-monthold infants were negative for the infection and in the 6-month-old infants 10 cases (18.5%) were positive, 1 case was at borderline in the exclusively breastfed, and 13 cases (27%) were positive in formula- or mixed-fed subjects. This difference was not statistically significant (P = 0.302). The Mantel-Hanzel test was used to find a relationship in *H. pylori* positivity in mothers and infants during the first 6-month of life in each of the 2 groups. It was found that out of 37 serologically positive mothers in the breastfed group, 8 infants (22%) were *H. pylori* positive and in the formula and mixed-fed group, of 29 serologically positive mothers 10 infants (34%) were also *H. pylori* positive, but the difference between the groups was not statistically significant (P = 0.727). It meant that the prevalence of infantile *H. pylori* infection was similar in the 2 groups.

**Conclusions:** The current study findings showed that exclusive breastfeeding had no role in protection or facilitation of *H. pylori* infection.

Keywords: Breastfeeding, Formula Feeding, Helicobacter pylori

### 1. Background

Helicobacter pylori are among the most common human gastrointestinal pathogens with worldwide spread in both children and adults (1-3). It is a Gram-negative S-shaped bacillus producing urease, catalase, and oxidase and these enzymes may play an important role in the pathogenesis of the microorganism in peptic ulcer disease (4).

The mechanism of H. pylori acquisition and transmis-

sion is unclear, although the most common way of transmission is fecal-oral or oral-oral. The bacteria can be isolated from the stool or vomitus of the infected people. The risk factors such as low socioeconomic status during the childhood or infection of other family members may influence the incidence (4).

Maternal infection is an independent risk factor for childhood infection. Acquisition of infection occurs in early childhood years (1).

Helicobacter pylori cause chronic active gastritis in all

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infected children, although they are often asymptomatic. The infection may present with abdominal pain or vomiting and less frequently refractory form of iron deficiency anemia or growth retardation. Rarely, it may be associated with chronic autoimmune thrombocytopenia. Chronic colonization with *H. pylori* can be associated with significantly increased risk of duodenal ulcer, gastric cancer (CA) such as adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma. The relative risk of gastric CA in the infected adults is 2.3 - 8.7 times higher than that of the non-infected adults and the world health organization (WHO) classified it in the 1st group of carcinogens (4).

Anemia, idiopathic thrombocytopenic purpura (ITP), short stature, and sudden infant death syndrome (SIDS) are reported as extra gastrointestinal (GI) findings of *H. pylori* infection. In a study, *H. pylori* infection was associated with SIDS, but there was no evidence of *H. pylori* effect on its pathogenesis (4).

The diagnosis is made according to histology and finding the bacteria in biopsy specimens. Urea breathing test with C13 and stool antigen tests are the non-invasive methods to diagnose *H. pylori* infection (4).

According to the literature, *H. pylori* infection is an important risk factor for developing chronic active gastritis, peptic ulcer disease, and even gastric CA (1, 2, 5). Most of the infected people get it during childhood (6-8). Recent epidemiologic evidence suggests that chronic *H. pylori* infection acquired during very early childhood can result in gastric CA and primary gastric lymphoma in adults and children (8-10). The risk factors for *H. pylori* acquisition are extensively studied, but there is confusing data about the role of breastfeeding in preventing the infection (11). The current study mainly aimed at comparing the incidence of *H. pylori* infection in 2 groups of exclusively breastfed infants and formula- or mixed-fed infants during the first 6 months of life.

Some references suggest that breastfeeding for a longer period can decrease the risk of H. pylori infection (12). On the other hand, maternal *H. pylori* infection is an important risk factor to acquire H. pylori infection in infants and in a study in Brazil there was no difference between H. pylori infection in breastfed and never breast-fed infants (13), while in a study in Japan transmission of H. pylori infection from mother to the child was shown as the most probable cause of intrafamilial spread of the microorganism (14). In some countries such as Iran, with high prevalence of *H. pylori* infection, it is possible that breastfeeding increase the risk of infection because of longer and closer contact with mother. In a study in Germany the prevalence of H. pylori infection was higher in breastfed infants than the formula-fed ones (15). In the current study, the enrolled infants were divided into 2 groups based on

feeding strategy and variables such as gender, GA, maternal serology for *H. pylori*, keeping place of the infant, delivery method, and the incidence of *H. pylori* infection were compared.

#### 2. Methods

The current cohort was conducted on infants registered by Breast Feeding Research Center of Imam Khomeini Hospital Complex from 2012 to 2015. They were randomly included in the study and followed up for 6 months in 2 groups; exclusively breastfed, and formula- or mixedfed. A datasheet was filled for each subject. The infants whose parents did not want to continue the study or did not refer at 6 months of age or did not provide the stool samples were excluded from the study. Totally, 102 of 180 enrolled cases completed the study. Mothers serologically were assessed by the enzyme-linked immunosorbent assay (ELISA) kits. Infants' infection status was also checked by the stool test of monoclonal antibody (Ab) against H. pylori using ELISA (Astra Formedic). The 1st infant's stool specimen was received in 3-5 weeks of age and the 2nd one during 5 to 7 months of age. By comparing the 2 groups for the acquisition of infection, it was investigated whether exclusively breastfeeding can protect or facilitate H. pylori infec-

Data were transferred into SPSS version16 and analyzed with chi-square, T test, ANOVA, and logistic regression; P value < 0.05 was considered statistically significant and the power of study was 80%. The Mantel-Hanzel test was also performed to find a relationship between maternal serology of *H. pylori* and infant's *H. pylori* infection at the first 6 months of age in each of the 2 groups. The study was ethically and scientifically approved by Tehran University of Medical Sciences (registration code: 91-01-105-16660).

## 3. Results

Totally, 2 groups of 54 and 48 breastfed, and formula-or mixed-fed infants completed the study. The demographic features were matched by the 2 groups (Table 1).

In the breastfed group, 37 mothers (68%) were positive for *H. pylori* infection and in the formula- or mixed- fed group it was 29 cases (60%).

The mean age of mothers in the breastfed group was 29.9 years, ranged 17 to 44, and in the mixed-fed group was 29.8 years, ranged 17 to 40.

The mean duration of exclusive breastfeeding in the breastfed group was 5 months and 10 days (ranged 4.5 to 6 months) and 13 days in the mixed-fed group (ranged 0 to 45 days).

Table 1. Demographic Information of the Study Groups

Feeding Strategy	Exclusively Breastfed (N = 54)	Formula- or Mixed-Fed (N = 48)	P Value
Gender			0.560
Male	25	25	
Female	29	23	
Gestational age, w (mean)	$38 \pm 3d$	35	0.0001
Delivery method			0.728
C/S	41	35	
NVD	13	13	
Mother's age, (mean)	29 y $\pm$ 9 m	$29\mathrm{y}\pm8\mathrm{m}$	0.606
Mother's H. pylori status			0.393
Positive	37	29	
Negative	17	19	
GI problems			0.023
GERD	10	12	
Colic	8	11	
Both	0	3	

Abbreviations: C/S, caesarian section; GEDR, gastro esophageal reflux disease; NVD, normal vaginal delivery.

The mean age of infants when starting formula in the mixed-fed group was 9 days (ranged 1 to 30 days).

The keeping place of infants in the breastfed group was relatives' house in 1 case and in mixed-fed group was the day care center in 1 case; all other infants were looked after at home.

About *H. pylori* infection in 1 month of age, all the cases were negative and in 6 months of age in the breastfed group 10 cases(18.5%) were positive and 1 case (1.8%) was reported borderline, while in the mixed-fed group 13 cases (27%) were positive. In 2 positive cases of breastfed and 3 of the mixed-fed groups, mothers were serologically negative for *H. pylori* infection and the result was positive for other cases.

Demographic information of *H. pylori* positive infants is shown in Table 2.

The mean duration of exclusive breastfeeding in positive cases of the breastfed group was 5 months and 7 days. In the mixed-fed group, in 10 positive cases (77%) formula was first used during neonatal period and in 3 cases (23%) formula feeding was started from 1 month of age.

The incidence of *H. pylori* infection in infants with infected mothers was 22% (8 infants of 37 infected mothers)

Table 2. Demographic Information of Helicobacter pylori Positive Cases

H. pylori Positive 6-Month-Old Infants Based on Stool Specimen Assessments	Exclusively Breastfed (N = 10) (18.5%)	Formula- or Mixed-Fed (N = 13) (27%)	P Value
Gender			0.012
Male	3+1(borderline)	3	
Female	7	10	
Delivery method			0.941
C/S	9	8	
NVD	1+1(borderline)	5	
GA (mean)	$38\text{w}\pm2\text{d}$	36	0.306
Infant's keeping place			0.154
Home	10	12	
Day care center	0	1	
GI symptoms			0.539
GERD	0	2	
Colic	3	2	
Both	0	1	

Abbreviations: C/S, caesarian section; GEDR, gastro esophageal reflux disease; NVD, normal vaginal delivery; GA: gestational age.

in the exclusively breastfed group and 34% (10 infants of 29 infected mothers) in the mixed- and formula-fed group (P = 0.727).

#### 4. Discussion

In the current study, 2 groups of breastfed and formulaor mixed-fed infants were compared for H. pylori infection during the first 6 months of their lives. At the age of 1 month, there was no positive case in the 2 groups, and at the age of 6 months, there was no statistically significant difference in the incidence of H. pylori infection between the 2 groups (P = 0.302).

In a study by Okuda et al., in Japan, it was shown that longer duration of breastfeeding can decrease the incidence of *H. pylori* infection in early childhood (12). Also, in a study in Shiraz, Iran, it was concluded that the incidence of *H. pylori* infection in exclusively breastfed infants was significantly lower than that of infants with other feeding regimens (16). In a case-control study by Children's medical center, it was shown that breastfeeding during the first 6 months of life resulted in lower *H. pylori* colonization, decrease in duration of symptoms, and less severe gastritis (17). In a Turkish-American study, breastfeeding was dis-

cussed as an important protective factor for H. pylori infection (18, 19). In a systematic review by Klein et al. it was suggested that breast milk protects against H. pylori infection (20). In a study by Soltani et al. in Sanandaj, Iran, there was no difference in H. pylori infection between the breastfed and non-breastfed infants (11). Also, in a study in Brazil, there was no significant difference in the incidence of H. pylori infection between the breastfed and never breastfed infants (13). In a study in Germany, the incidence of *H*. pylori infection was higher in breastfed infants than nonbreastfed ones (15). In the current study, similar to the studies in Brazil and Sanandaj, there was no statistically significant difference between the incidence of H. pylori infection in breastfed and formula-fed infants, which may be due to the role of other factors in the acquisition of H. pylori infection in infants.

A cross sectional study by Klein et al. in Peru showed a high prevalence of *H. pylori* infection in infants (71.4%) and lower prevalence in 1-4-year-old children (49.4%) (21). A retrospective serological study by Blecker et al. in a developed country showed that a small portion of the population acquired H. pylori infection (< 1%) during the 1st year of life (22). Acquisition of H. pylori infection usually occurs during the first 5 years of life (23). In Sanandaj study by Soltani et al. there was a statistically significant correlation between age and H. pylori infection (11). In Brazil and Turkey studies, the prevalence of H. pylori infection showed an increase with age increase (13, 18). In a study by Monajemzadeh et al. in Children's medical center, there was also a statistically significant correlation between breastfeeding and age among children infected with H. pylori (17). In a study by Konno et al. the peak age for acquisition of *H*. pylori infection was 1-5 years and transmission of H. pylori from mother to the child was suggested as the most probable cause of intrafamilial spread (14). In the current study, there was no significant correlation between gestational age and H. pylori positivity (P = 0.306).

In a study in Children's medical center, there was no statistically significant difference in gender between H. py-lori positive and H. pylori negative cases (17) that was in agreement with the findings of similar studies (11, 14), but in the current study, the prevalence of H. pylori infection was significantly higher in female infants(74% vs. 26%) (P = 0.012); although the number of female and male infants in the study was almost equal (51% vs. 49%).

About relationship between H. pylori infection and type of delivery, previous studies reported no evidence, but in the current study, although in 74% of H. pylori positive cases of the 2 groups the delivery method was cesarean section (C/S), the association was not statistically significant (P = 0.941) and it can be attributed to the higher rate of C/S than normal vaginal delivery (NVD) in both groups (74.5% vs.

25.5%).

About the relationship between the place of keeping infants and mothers' H. pylori infection, there was no statistically significant difference (P = 0.154) (P = 0.122).

There was no significant correlation between *H. pylori* infection and infantile colic in the current study (P= 0.539). A study in Egypt showed significant correlation between *H. pylori* infection and symptoms of infantile colic (24).

No significant correlation was observed between H. py-lori infection and gastroesophageal reflux disease (GERD) among the studied infants (P = 0.655), which was according to the outcomes of the study by Emiroglu et al. (25). In a study by Abdollahi et al. the possibility of protective effects of H. pylori against GERD in children was discussed (26).

Data were also analyzed using the logistic regression model, but no statistically significant correlation was observed.

The Mantel-Hanzel test was performed to find a relationship between mother's serologically H. pylori positive and infants H. pylori infection at the age of 6 months in each of the 2 groups. It was observed that out of 37 mothers with serologically positive H. pylori in the breastfed group, 8 infants (22%) were also H. pylori positive at the age of 6 months; in the mixed-fed group, out of 29 mothers who were serologically H. pylori positive, 10 infants (34%) were H. pylori positive at the age of 6 months and the difference between the groups was not statistically significant (P = 0.727). It meant that the incidence of infantile H. pylori infection at the age of 6 months was similar in the 2 groups.

#### 4.1. Conclusions

According to the results of the current study, breast-feeding does not protect against *H. pylori* infection and also does not increase the risk of acquisition.

## 4.2. Limitations

As Breastfeeding research center and Imam Khomeini hospital complex are the national referral centers for high risk pregnancies, a lot of cases were excluded from the current study and the study was continued with 2 groups of 54 and 48 infants.

It was a cohort of a limited number of infants and the results may not be generalized to the total population; more studies are needed to evaluate the effect of breast-feeding on the incidence of *H. pylori* infection.

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