Original Article

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Breastfeeding and *Helicobacter pylori* Infection in Early Childhood: a Continuing Dilemma

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Abstract

Objective: *Helicobacter pylori (H. pylori)* is the most common chronic bacterial infection in humans. Chronic colonization increases the risk of duodenal ulcer and gastric cancer. The risk factors for acquiring the infection have been extensively studied. However, there are conflicting results on the role of breastfeeding in the prevention of *H. pylori* infection. We conducted a study to evaluate the effects of breastfeeding on the *H. Pylori* infection in Kurdish children in Sanandaj, IR Iran.

Methods: A historical cohort study was carried out from January 2011 through December 2012. Totally 221 children who were going to attain 2 years old during the study period were randomly enrolled. They were divided into two groups, i.e. breastfed and non-breastfed. We used *H. pylori* stool antigen test to detect infection in the selected group of children after age of 2 years and cessation of breastfeeding. Each group was subdivided into two subgroups, infected and non-infected. The associations of breastfeeding with *H. pylori* infection was assessed using statistical software.

Findings: We found no difference in the odds of infection between breastfed and non-breastfed groups (OR=0.809, 95% CI [0.453-1.444]). An association between age and the prevalence of infection was found (*P*=0.008). There was an increase in the odds of infection as the family size grew (OR=1.93, 95% CI [1.04-3.6]) as well as increasing housing density (OR=2.12, 95% CI [1.10-4.10]).

Conclusion: The data suggests that breastfeeding in infancy does not protect against *H. pylori* infection for long duration among studied children in Iran. The protective effects of breastfeeding, if any, are at most transient.

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Key Words: Helicobacter Pylori; Human Milk; Risk Factors; Children; Iran

Introduction

Helicobacter pylori is the most common chronic bacterial infection in humans, affecting 50% of the world population[1]. Most of infected children have histologic evidence of chronic gastritis but are clinically asymptomatic. However, they may present with abdominal pain, nausea, and iron deficiency. Colonization, in children, leads to clinical disease in adulthood. Chronic colonization increases the risk of duodenal ulcer and gastric cancer, including adenocarcinoma and mucosa associated lymphoid tissue (MALT) lymphoma. The relative risk of gastric carcinoma in infected persons is 2.3 to 8.3 times greater than that in normal population [2]. According to the World

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Health Organization (WHO) classification, *H. pylori* has been classified as group I carcinogens[3].

Although family members are the most likely source of infection, the factors that make some people more susceptible to infection have not been determined[4]. It has been suggested that infection with this organism starts in infancy, and most cases occur before the age of three[4-6]. The mechanism of transmission of this organism is unknown, but the most likely route of transmission is fecal-oral or oral-oral. The prevalence of *H. pylori* infection is much higher in developing countries. In children, the prevalence of infection ranges from less than 10% to over 90%[7]. The risk factors for *H. pylori* infection include socioeconomic status, number of siblings, race/ethnicity, rural residence, institutional residence, lack of maternal education and infection status of family members[8].

The protective effect of breast milk and its beneficial effects in preventing respiratory infections and diarrhea have long been known. Several studies have suggested that breastfeeding can also prevent bacterial colonization of *H. pylori* during childhood[9-11]. However, other studies had conflicting results and the importance of breastfeeding in the prevention of *H. pylori* is still under question[6,8,12,13]. In fact, there are studies that have reported that breastfeeding may even increase the risk of *H. pylori* infection [13-15]. Breast milk may be infected by *H. pylori* and horizontal infection through breastfeeding may occur[14].

We conducted the present study to evaluate the effects of breastfeeding on *H. pylori* infection in Kurdish children in Sanandaj, IR Iran, by using the *H. pylori* stool antigen test (HpSA). Sanandaj is the center of Kurdistan province, in west Iran, with a population of about 450000. A previous study has detected a high prevalence of *H. pylori* in children in this city[6].

Subjects and Methods

The study had a historical cohort design that was carried out from January 2011 through December 2012 in the Pediatric Department of Be'sat Tertiary Hospital affiliated to Kurdistan University of Medical Sciences. Participants were selected from a list of healthy children in 12 primary healthcare centers across the city. We selected them from children who were going to attain 2 years old during the study period. The rationale for selection of these age groups was that the breastfeeding practice usually continues 2 years or even longer in our community. Simple random sampling was used in breastfed children. However, in non-breastfed children, we had to enroll all of them due to paucity of cases. A computer based program generated random numbers in order to draw a selected group of children from the lists.

The sample size for both breastfed and non-breastfed groups was calculated as at least 90 patients for each group. The sample size calculation was based on at least 20% prevalence in the community, 40% prevalence in studied children, 20% power, an odds ratio (OR) of 2.5, and 95% confidence interval (CI), computed from previous epidemiological studies[5,6]. All procedures and tests were carefully explained to the parents of children and informed consent was obtained. Through interviews with the parents, the data regarding age, sex, parents' education, duration of breastfeeding, family size, previous antibiotic usage, height and weight, were recorded for each child. "Breastfed child" was defined as a child that has had at least 1 month duration of continuous breastfeeding. Status of breastfeeding until the date of stool sampling (at least age of 2 years or older) was questioned and on the base of the breastfeeding status the children were divided into two groups, i.e. breastfed and non-breastfed. These two groups were compared in terms of the absolute breastfeeding (no formula exclusively), those with mixed feeding with breast milk and formula for various durations of time and, those with no breastfeeding (no breast milk exclusively). Infants that have received antibiotics or proton pump inhibitors more than a week in the last 2 months were excluded from the study. Other exclusion criteria were children who had known underlying diseases or taking immunosuppressant drugs.

A stool sample was collected from the enrolled children who attained at least 2 years old by parents with varying time after breastfeeding cessation.

We used Premier Platinum HpSA Plus (Meridian Diagnostics, Cincinnati, Ohio, USA) kits to detect

Table 1: Age, Gender and Growth Characteristics of Studied Children

Parameter	breastfed group Mean (±SD)	Non-breastfed group Mean (±SD)	*P. value*
Age (month)	36.17 (±8.67)	38.39 (±7.79)	0.06
Gender (Female/male)	(57/73)*	(48/43)*	0.21
Weight (z score)	-0.51 (±1.27)	-0.24 (±1.33)	0.16
Height (z score)	-0.56 (±2.1)	-0.11 (±1.35)	0.10
Weight/Height (z score)	-0.03 (±1.71)	-0.21 (±1.69)	0.50

*Numbers in each gender group; SD: Standard Deviation

infection in children. The stool assay kit was purchased from a local representative of Meridian Company, Netherlands. It is cleared by the FDA and CE-marked with high sensitivity and specificity in comparison to other stool antigen tests[16-19]. The Maastricht IV/Florence Consensus states that "The diagnostic accuracy of the stool antigen test (SAT) is equivalent to the urea breath test (UBT) if a validated laboratory-based monoclonal test is used[1]." The sensitivity and specificity for the Premier Platinum HpSA Plus test were reported as 92.2% and 94.4%, respectively, in a recent study[20]. The test procedures are described in detail and available online (http://www.meridianbioscience.com/diagnostic-products/h-pylori/premier/premier-platinum-hpsa-plus.aspx). According to the manufacturer's instructions, results are divided into two groups for *H. pylori* infection: negative (the optical density at 450/630 nm <0.100) and positive (the optical density at 450/630 nm ≥0.100).

The data were analyzed with the SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA). By using descriptive statistics, frequency distribution tables and graphs were drawn and crude odds ORs and 95% confidence interval (CI) were estimated. Other statistical differences were evaluated using the chi-square test. Values of $P<0.05$ were considered as statistically significant. We calculated z scores for the indicators of the attained growth standards (weight-for-age, height-for-age, weight-for-height) based on WHO standards, using the respective SPSS macro (syntax) files[21]. We used t test to compare z scores in infected and non-infected groups.

Findings

A total of 221 children were selected and tested for *H. pylori* antigen in stool (Table 1). There was no history of breastfeeding in 91 children. A number of 130 children had a history of variable duration of breastfeeding time (Table 2).

A stool sample was tested for Helicobacter infection after varying times of breast milk cessation. The age range of enrolled children at the time of stool was 2 to 4 years. The calculated prevalence for selected children was 69.7%. We found no difference in the odds of infection between breastfed and non-breastfed groups (OR=0.809, 95% CI [0.453-1.444]). The studied children were divided into five groups as not-breastfed, 1-6, 7-12, 13-18, and 18-24 months of breastfeeding. There was a marginal decrease in the odds of infection as the duration of breastfeeding increased ($P=0.07$). We compared the chance of acquiring infection for the two

Table 2: Prevalence of *H. pylori* infection based on duration of breast milk feeding and gender

Duration of breast milk feeding	Total in each age group	No of patients: Positive test in each age group	Female patients with positive test	No of male patients with positive test
No breastfeeding	91	61 (67%)	31/48 (64.6%)	30/43 (69.8%)
1-6 months	13	12 (92.3%)	4/4(100%)	8/9 (88.9%)
7-12 months	6	3 (50%)	2/2 (100%)	1/4 (25%)
13-18 months	8	8 (100%)	4/4 (100%)	4/4 (100%)
≥19 months	103	70 (68%)	36/47 (76.6%)	34/56 (60.7%)
Total	221	154 (69.7%)	77/105 (73.3%)	77/116 (66.4%)

Table 3: Association of *H. pylori* infection with age, family size, home size, body weight, father education and mother education

Parameter	Variable	No. (%) of children	No. (%) HpSA positive	*P. value*	OR (95% CI)
Age (months)	24-30	131 (59.3)	96 (73.3)		
	31-36	28 (12.7)	21 (75)		
	37-42	19 (8.6)	16 (84.2)		
	43-48	43 (19.5)	21 (48.8)	0.008[a]	
Family size	3	97 (43.9)	60 (61.9)		
	≥4	124 (56.1)	94 (76)	0.02	1.93 (1.04-3.6)
Home Size	<60	29 (13)	21 (72.4)		
	≥60-<100	97 (44.1)	69 (71)		
	≥100	95 (43.2)	64 (67)	0.8[a]	
Housing density	≤20 m^2/person	97 (44)	77 (79)		
	>20m^2/person	124 (56)	80 (65)	0.02	2.12 (1.10-4.10)
Body Weight (Non-Breast-Milk-Fed Group)[b]	Z Score≤-2	6 (6.8)	5 (83.3)		
	-2<Z Score≤-1	16 (18.2)	11 (68.8)		
	-1<Z Score≤0	33 (37.5)	24 (72.7)		
	0<Z Score≤+1	19 (21.6)	14 (73.7)		
	Z Score>+1	14 (15.9)	8 (57.1)	0.8[a,e]	
Body Weight (Breast-Milk-Fed Group)[c]	Z Score≤-2	14 (11.7)	11 (78.6)		
	-2<Z Score≤-1	32 (26.7)	24 (75)		
	-1<Z Score≤0	40 (33.3)	32 (80)		
	0<Z Score≤+1	20 (16.7)	14 (70)		
	Z Score>+1	14 (11.7)	6 (6.9)	0.1[d,e]	
Mother's education	No Education	14 (6.3)	11 (78.6)	-	
	Elementary School	76 (34.4)	57 (75)	-	
	High School	62 (28.1)	42 (67.7)	-	
	University Degree	69 (31)	44 (64)	0.42[a]	
Father's education	No Education	7 (3.2)	5 (71.4)	-	
	Elementary School	71 (32.1)	47 (66.2)	-	
	High School	67 (30.3)	54 (80.5)	-	
	University Degree	76 (34.4)	48 (63.1)	0.11[a]	

[a] Chi square test was used
[b] 7 (3%) values for weight were missed
[c] 4 (2%) values for weight were missed
[d] Fisher's Exact Test was used
[e] *P* values assessing the association of infection with categorized z scores
OR: Odds Ratio; CI: Confidence Interval; HpSA: H. pylori stool antigen test

groups of children that lied at the two ends of the spectrum, the first group fed the longest time with breast milk (18-24 months) and the second group with no breastfeeding. There were no differences in the odds of infection of infection (OR=1.043, 95% CI [0.571-1.904]).

There was no correlation between the prevalence of infection and either the type of feeding in the female gender group (OR=0.44, 95% CI [0.18-1.056]) nor the duration of breastfeeding among girls. (χ^2=5.77, *P*=0.2). Similarly, there was no correlation between prevalence of infection and either the type of feeding in male gender group (OR=1.28, 95%CI [0.569-2.864]), nor the duration of breastfeeding among boys (χ^2=8.16, *P*=0.08).

When the frequency of *H. pylori* infection was calculated in various age groups (Table 3), there was a statistically significant association (χ^2=11.92, *P*=0.008).

The prevalence of infection marginally increased as the family size grew (χ^2=5.08, *P*=0.07). However, when we compared the family size in three member families with four and more

member families, we found significant differences in odds of infection (OR=1.93, 95%CI [1.04-3.6]) (Table 3). We corrected these numbers by dividing family size by home size, i.e. calculated housing density, and observed a significant increase in the odds of infection, as the density increased (OR=2.12, 95% CI [1.10-4.10]).

We calculated the z scores for weight-for-age, height-for-age, and weight-for-height for both non-breastfed and breastfed groups based on WHO standards and the prevalence of infection in respective groups (Table 3). The mean z score for weight-for-age in infected and non-infected children was calculated as -0.07±1.45 and-0.54±1.16, respectively, which was statistically a significant difference (*P*=0.02). The mean z score for height-for-age in infected and non-infected children was calculated as -0.14±1.73 and -0.42± 1.85, respectively, which was not statistically significant (*P*=0.3). The mean z score for weight-for-height in infected and non-infected children was calculated as -0.05±1.61 and -0.22±1.79, respectively which similarly was not statistically significant (*P*=0.5).

Discussion

We were unable to confirm the protective association between breast milk feeding in prevention of *H. pylori* infection. We found higher odds of infection in the selected ages of the two groups, breastfed and non-breastfed. The prevalence of infection was higher in these age groups in comparison to previous studies[5,6,19].

There are multiple research articles that have surveyed the effect of breast milk on infection by *H. pylori*[4,8-11,13]. Some articles deny any protective effect for breastfeeding against *H. pylori* infection[6,8,12,13]. Our study found no protective effect of breast milk against *H. pylori* infection. A systematic review by Chak et al suggested that breastfeeding is protective against *H. pylori* infection[9]. However, they reported that the studies conducted in developed countries did not achieve statistically significant enough OR to conclude that breastfeeding has a protective effect against *H. pylori* infection. Only one study reported statistically significant OR in developed countries[22]. Most of the studies with significant OR were in the low and middle income countries[9].

The prevalence of *H. pylori* infection in each community depends on two factors, the rate of acquisition and the rate of persistence of infection[23]. In developing countries, most of the acquisition of this infection occurs in early childhood and continues as chronic infection to adult life[5,6]. Unhygienic behavior, especially during early childhood, frequent contacts with the sources of infection and frequency of other risk factors lead to persistence of infection. Based on data from United Nations Secretariat, Iran is classified in the upper middle income countries[24]. Consequently, lack of protection by breast milk feeding against *H. pylori* in this country, may be more consistent with the results that have been obtained in developed countries. However, the high prevalence of *H. pylori* in many epidemiologic studies in Iran is more compatible with the epidemiology of *H. pylori* in low income countries[5,6].

The protective effects of breast milk against pneumonia and diarrhea have long been recognized. The components of breast milk, such as antibodies, lactoferrin, lysosomes, and kappa-casein possess antibacterial and antiviral characteristics that protect infants against many infectious agents causing diarrhea and pneumonia. However, these types of immunity are mostly passive and are not long-lasting enough to protect the infant after cessation of breastfeeding. Once breastfeeding has ended, it is likely that it is the better nutritional status and the well development fostered by breastfeeding that protect the child against infections, and not the direct effect of breastfeeding (i.e. the immunity acquired passively by breastmilk). One assumption regarding the different results found in our study is that, in the low and middle income countries, malnutrition is very common. This is not the case in Iran[25]. There is no large variation between nutritional status of infants fed with breast milk and formula fed infants as seen in the low income African countries such as Gambia or Egypt[26]. Unlike low income countries, many types of substitute feedings including various formulas for infants are readily available to even low income people in our country. As there are no significant differences in nutritional status of breastfed and

non-breastfed children in our county, we may be unable to detect differences between the prevalence of Helicobacter infection in these two groups.

One study in Gambian children reported a high prevalence of *H. pylori* infection in children with malnutrition or chronic diarrheal disease[27]. In our study, the mean z scores for body weight in non-breastfed children in non-infected and infected groups was calculated as -0.00 and -0.29, respectively (*P*=0.4). In other words, there was no association between the infection odds and lower z scores for body weight in non-breastfed children. However, in breastfed-children, the mean z scores for body weight in non-infected and infected groups were calculated as -0.12 and -0.69, respectively (*P*=0.02). There was a significant correlation between odds of infection and lower z scores in breast milk fed children. In the absence of correlation of the other malnutrition indicators (height-for-age and weight-for-height) with the rate of infection, it seems that, in Iran, the infection is more associated with a mild type of acute malnutrition. With other words, in the context of *H. pylori* infection, breastfeeding is not protective against acute malnutrition.

Maternal infection with *H. pylori* plays a major role in the acquisition of this infection in the early years of life[28]. Long term and close contact with mother, despite presumptive passive protection by breast milk feeding, involve frequent exposure to infectious sources of *H. pylori*. Non-breastfed infants have less contact with their mothers. In Iran, on the other hand, many mothers avoiding breast milk feeding because of their employment are from high socioeconomic graduated class. Their hygienic behavior poses their infants to less risk for acquiring *H. pylori* infection. These factors may explain the mechanism by which the infection rates increase in breast milk fed infants. Moreover, breast milk feeding causes a decrease of other infectious diseases and less need for antibacterial drugs. Less exposure to antibacterial drugs results in the persistence of *H. pylori* in the stomach, which is usually primarily eradicated by accidental administration of antibacterial drugs for respiratory or diarrheal diseases[29].

High infection odds due to high carriage rate may be a reason for the lack of protective effect of breast milk feeding in our study. There may be a relative protective effect for breastfeeding that is overcasted by the high burden of *H. pylori* infection. In most of the studies that reported a protective effect from breast milk infection, the prevalence of infection was notably below 40-50% of the study population in lower age groups[30,31].

There is a general agreement that the protective role of breastfeeding against *H. pylori* infection is passive, and therefore is not long lasting[32,33]. How long, it is not clear. Reinfection is possible after waning of the protective effect of maternal antibodies occurring with the cessation of breast milk feeding. In our study, the maximal duration of breastfeeding was 2 years. The mean age of the studied children was 39 months and the mean interval between sampling time and the cessation of breastfeeding was calculated as 13 months. Considering the high carriage rate in our community, this interval may be long enough for waning immunity, reinfection and surpassing the suspicious protective effect of breastfeeding.

The prevalence of exclusive breastfeeding and the mean duration of breastfeeding in Iran are above 75% and 23 months duration, respectively[26,34]. The prevalence of continuing breastfeeding for a minimum of 12-15 months is of 84% in Iran[26]. On the other hand, the prevalence of *H. pylori* infection in Iran has consistently been reported as well above 50% in many studies conducted in this country[5,6]. Consideration of these facts challenges largely against the protective effects of breastfeeding in our community.

The calculated OR for time duration of breastfeeding results in a statistically insignificant number. Lengthening breastfeeding duration to multiple months in the early years of age had no effect on the acquisition of infection. This is consistent with data acquired from the majority of the other studies[9,15]. However, this is a contradiction in articles advocating for the protective effects of breastfeeding against *H. pylori* infection. If one assumes any protective effect for breast milk against *H. pylori* infection, it should be a dose-response relationship. Moreover, in many of these articles, we found no case definition for breastfeeding based on duration[9,31,35]. In one study, a prolonged time of breastfeeding was associated with an increased chance of acquiring infection[13].

The majority of the studies regarding the protective effects of breast milk against *H. pylori*

infection have used laboratory tests, other than stool antigen test, as diagnostic tools. Most studies used IgG-based serologic tests to confirm Helicobacter infection[9]. This test has low sensitivity and specificity in comparison to the stool antigen test[36]. Moreover, a positive serology test is not always a reflection of active infection. It may be due to a resolved infection in the past. These variables may cause confusing results in the study of the protective effect of breast milk against infection. The UBT was used in some studies as a tool to confirm infection. It is a worthwhile way with high sensitivity and specificity, comparable to HpSA test. However due to the high cost, limited availability in developing countries and the complexity of the test, it is less applicable on large scale in healthy children[37]. A recent meta-analysis discussed that the use of 13C labeled isotope UBT (13C-UBT) tests are less accurate for the diagnosis of *H. pylori* infection in young children[38]. Our search for the epidemiologic studies that used HpSA as a diagnostic tool to evaluate effects of breast milk feeding have revealed only limited results[4].

One limitation of our study should be considered. We were not able to identify the time of acquisition of infection and its relationship to breastfeeding to unveil any short term protection. Another prospective cohort study may help to solve this problem and clarify the effects of breast milk feeding on *H. pylori* infection.

Conclusion

The presented data suggest that breastfeeding in infancy does not protect against *H. pylori* infection for long duration among studied children in Iran. The protective effect of breastfeeding, if any, is at most a transient one.

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Authors' Contribution

J. Soltani, B. Nikkhoo, J. Khormehr, P. Ataee, F. Gharibi: Substantial contributions to conception and design, acquisition of data, analysis and interpretation of data.
M.S. Hakhamaneshi: Acquisition of data, laboratory performances and interpretation of laboratory data.
All Authors participated in drafting the article and they critically reviewed the manuscript and approved the final manuscript as submitted.

Conflict of Interest: None

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