

***Helicobacter pylori* infection in mother and infant pairs in Anatolia**

Anadoluda anne ve bebeklerinde *Helikobakter pilori* enfeksiyonu

Selda HIZEL¹, Ali ÖZDEN², Fatoş TANZER³, Üçler KISA⁴, Emine DİBEK MISIRLIOĞLU¹, Derya BÜYÜKKAYHAN³, Özgül KISA⁴

Departments of ¹Pediatrics, ⁴Biochemistry, Kırıkkale University, School of Medicine, Kırıkkale

Department of ²Gastroenterology, Ankara University, School of Medicine, Ankara

Department of ³Pediatrics, Cumhuriyet University, School of Medicine, Sivas

Department of ⁵Microbiology, Gülhane Military Medical Academy, Ankara

Background/aims: The aim of this prospective study was to determine the seroprevalence rates of *Helicobacter pylori* in mother and infant pairs and to discuss the possible fecal-oral transmission route of *Helicobacter pylori* infection in the early years of life. **Methods:** Forty-eight mother-child pairs were followed for 12 months. *Helicobacter pylori* IgG and hepatitis A virus (HAV) IgG levels were measured in maternal sera, infant sera and breast-milk samples at birth and in breast-milk samples and infant sera at follow-up visits. **Results:** At birth, the rate of *Helicobacter pylori* positivity was 81.25% in breast-milk and 95.8% in maternal and infant sera. Although there was a decrease in seropositivity in both baby sera and breast-milk at the age of nine months, an increase was observed in the 12th month. Hepatitis A virus IgG was measured to show whether *Helicobacter pylori* and hepatitis A virus use the same transmission routes. Hepatitis A was positive in all infants' sera, in 95.8% of mothers' sera, and in 68.75% of breast-milk samples. Seropositivity rates in infants whose mothers were seropositive for *Helicobacter pylori* and hepatitis A virus decreased gradually. There was an increase after the 9th month of life. **Conclusions:** *Helicobacter pylori* seroprevalence rates are high in Anatolia. It is possible that the decrease in breastfeeding with increased introduction of supplemental foods may lead to an increased risk of exposure to *Helicobacter pylori*.

Key words: *Helicobacter pylori*, breast-milk, infant, hepatitis A virus

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is one of the most common infections worldwide, and is usually acquired during childhood. It carries a significant lifetime risk for morbidity (1, 2). It is not only recognized as the main causative agent of se-

Amaç: Bu prospektif çalışmanın amacı anne ve bebek örneklerinde *Helikobakter pilori* seroprevelansını belirlemek ve hayatın erken dönemlerinde olası fekal-oral geçiş yolunun tartışılmasıdır. **Yöntem:** Bu prospektif çalışmada 48 anne-bebek çifti oniki ay süre ile izlendi. Doğumda alınan anne kanı, bebek kanı ve anne sütü örnekleri ile aylık izlemlerde alınan anne sütü ve bebek kanında *Helikobakter pilori* IgG ve Hepatit A IgG değerleri ölçüldü. **Sonuçlar:** Doğumda anne sütündeki *Helikobakter pilori* IgG pozitifliği %81.25, anne ve bebek serumunda %95.8 idi. 9. aya kadar bebeklerin serumunda ve anne sütünde *Helikobakter pilori* seroprevelansında azalma olmasına karşın 12. ayda hem bebek serumu hemde anne sütü *Helikobakter pilori* seroprevelansında artış oldu. *Helikobakter pilori* enfeksiyonunun geçiş yolunun değerlendirilmesi için Hepatit A IgG bakıldı ve tüm bebek serumlarında, anne serumlarının %95.8'inde ve anne sütünün %68.75'inde pozitif saptandı. Annelerinde *Helikobakter pilori* IgG pozitifliği olan bebeklerin serumlarındaki antikor pozitifliği bebeğin yaşı ilerledikçe azalma gösterdi, ancak 9. aydan sonra yeniden artış gözlemlendi. **Tartışma:** Anadolu'da *Helikobakter pilori* seroprevelansı yüksektir. Anne sütü alımının azalması ile ek gıdalara geçiş döneminde *Helikobakter pilori* enfeksiyonu ile karşılaşma riskini artırıyor olabilir.

Anahtar kelimeler: *Helikobakter pilori*, anne sütü, infant, hepatit A virüsü

Address for correspondence: Selda HIZEL
Konrad Adenaur Cad. No: 48/9 Çankaya/Ankara, Turkey
Phone: + 90 312 491 85 32 • Fax: + 90 318 225 28 19
E-mail: seldabulbul@yahoo.co.uk

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ways, such as person-to-person, fecal-oral and oral-oral transmission, play a role in transmission of the infection. Feces, saliva or vomit can potentially transmit the organism (3). Though it was mentioned in a Finnish study that maternal seropositivity is not a straightforward risk factor for acquiring *H. pylori* infection in infancy, infected mothers may play a key role in the transmission of *H. pylori* within the family (4,5). Overcrowded and unsanitary living conditions, sharing a bed and lack of running water have been shown to be major risk factors (5, 6).

The immune system of the newborn is immature at birth and maturation takes time. IgG antibodies transferred transplacentally and secretory IgA antibodies in breast-milk play important roles in the protection of the infant in the first months of life. As *H. pylori* infection is predominantly acquired in early childhood, maternal antibody transfer may be important in the prevention of acquisition of infection (7). *H. pylori* can transfer from mother to baby either during pregnancy or horizontally through breast-milk in the postnatal period. A systematic review that included a birth cohort study enrolling 1066 healthy newborns, using a monoclonal stool antigen test, identified maternal infection as the single significant risk factor for acquisition of infection in childhood (8). However, there are few studies on mother-to-child transmission in the perinatal and postnatal periods.

To our knowledge, there are few follow-up studies in our country describing the specific *H. pylori* antibody profiles and their relation in the mother's serum, infant's serum, and breast-milk during the first year of life.

The purpose of this study was to determine the *H. pylori*-specific immune response in maternal serum and milk, to show the quarterly variations in mother-baby pairs, and to search possible transmission routes of *H. pylori* in the early years of life.

MATERIALS AND METHODS

This prospective study was established in Kırkkale State Hospital and Sivas Cumhuriyet University-Medical School Hospital between November 1999 and November 2000. The study was approved by the local ethics committee. All mothers were informed about the aim of the study, and written permissions were obtained from those who agreed to participate. A total of 72 mother-baby pa-

irs were included in the study. Infants with gestational ages of less than 38 weeks and birth weights less than 2500 grams were excluded, as were infants with respiratory distress and/or sepsis. None of the infants underwent antibiotic treatment during their stay in the hospital and none of them received any kind of transfusion during the study period. Questionnaires comprising 20 questions about demographic characteristics, housing conditions, hygienic behavior, medical history, and gastrointestinal symptoms were administered by the researchers to all mothers. During the one-year follow-up period, 48 of the 72 mother-baby pairs completed the study. Though >48 mother-baby pairs were followed for the first six months, we did not include all these pairs in this study as they did not complete the full follow-up period.

Serum samples of mothers/infants and mothers' breast-milk samples were collected within the first 12 hours after birth. Babies and mothers were asked to come to follow-up appointments 1, 3, 6, 9 and 12 months after birth. During these visits, questionnaires were completed, routine physical examinations of the babies were carried out, and babies' blood samples and mothers' breast-milk samples were collected. Monthly questionnaires consisted of questions on the feeding styles and health status of the babies.

During the follow-up visits, for each infant, anthropometric measurements were taken (i.e. height, weight, head circumference, chest circumference and mid-arm circumference), the infant's nutritional status was assessed, and relevant investigations and treatments were carried out for any significant symptoms in the infant. At the end of each session, blood samples of the infants were obtained by venipuncture and breast-milk samples of the mothers were obtained by expression by the mother herself with a simple tool that was washed carefully after each use.

The collected serum and milk samples were stored at -20°C for one year in deep freezers in the Kırkkale University Medical School Biochemistry Laboratory and in the Cumhuriyet University Pediatric Clinic Laboratory. All samples were first centrifuged to separate the serum. To ensure standardization, they were sent in storage containers with ice packs to Gulhane Medical Academy, Department of Microbiology Laboratory, for serologic analysis.

Anti-*H. pylori* IgG (anti-*Hp* IgG) and anti-hepati-

tis A virus IgG (anti-HAV IgG) analyses in serum and breast-milk samples were performed using the ELISA test. All sera were tested blindly. As our aim was to show the prevalences rather than incidences, and as the test is noninvasive and inexpensive, we preferred to test our patients for *H. pylori* IgG by the ELISA method.

The RADIM Helicobacter pylori IgG EIA WELL kit was used for anti-*Hp* IgG analysis. The cut-off value for anti-*Hp* IgG was 0.474 for maternal and baby serum samples. As the chemical structure of breast-milk is different from the serum, different dilutions were performed and the cut-off value was taken as 0.390 for breast-milk samples. The sampling value was considered positive if it was higher than the cut-off value.

Anti-HAV IgG was analyzed in all serum and breast-milk samples to show the relation between *H. pylori* and HAV seroprevalences. At present, no universal vaccination program against hepatitis A exists in Turkey. Therefore, the epidemicity of HAV is expected to be unchanged.

Anti-HAV IgG was studied in maternal blood, breast-milk and the infant's serum samples with the RADIM anti-HAV EIA WELL kit. The cut-off value was 0.633 for anti-HAV IgG in breast-milk and 0.920 for maternal and baby serum. The sampling value was considered positive if it was less than the cut-off value. The results were accepted as suspicious and re-analyzed if the value was within a range of $\pm 10\%$ of the cut-off value.

The SPSS 10.0 software package was used for statistical analysis. The definitions were provided as number and percentage for discrete variables, and mean and standard deviation for continuous variables.

RESULTS

Of the original 72 mother-baby pairs, 24 pairs were withdrawn from the study as they did not want to continue or did not attend follow-up appointments. Therefore, the study ended with 48 mother-infant pairs who were followed for 12 months. The mean age of the mothers completing the study was 26.3 ± 5.01 years (min: 18; max: 40 years), 72.9% were less than 30 years old, 4.2% were employed, and 18.7% had received more than eight years of schooling while 4.2% were illiterate. Overall, 72.9% of the fathers were qualified workers and 56.3% had received more than eight years of schooling (Table 1).

Table 1. Sociodemographic characteristics of the study group

Mean age of the mothers (years) (Min-max)	26.3 \pm 5.01 (18 - 40)	
	n	%
Age groups of the mothers		
18-22 years	12	25.0
23-29 years	23	47.9
30-40 years	13	27.1
Educational status of the mothers		
Illiterate	2	4.2
<8 years of education	37	77.1
High school graduate	3	6.2
Mother's employment status		
Unemployed	46	95.8
Employed	2	4.2
Educational status of the fathers		
Illiterate	1	2.1
<8 years of education	20	41.6
High school graduate	27	56.3
Father's employment		
Unqualified worker	13	27.1
Qualified	35	72.9
Residential water status		
Constant water supply	42	87.5
Constant hot water	27	56.2
Keeping a pet at home	7	14.5

Though all infants were breastfed at birth, a decrease was observed in exclusively breastfed infants in the first month of life (87.5%). Moreover, the percentage of breastfeeding decreased gradually to 31.2% at the age of 12 months (Table 2).

H. pylori and HAV serology in mother's serum, infant's serum and breast-milk samples was evaluated in 48 mother-baby pairs.

H. pylori Serology

Anti-*Hp* IgG was positive in the serum except in two mothers who lived in Kirikkale (95.8%). The first-day breast-milk samples were positive for anti-*Hp* IgG in 81.25% of the mothers (39/48). First-day serum anti-*Hp* IgG was positive in 95.8% of the infant's sera.

Seropositivity rates decreased until the 9th month in infants' sera and increased by 8.3% between the 9th and 12th months. Four *H. pylori* seronegative infants in the 9th month were found to be positive in the 12th month.

Table 2. Breastfeeding status of the infants

	Breastfed infants		Exclusively breastfed	
	n	%	n	%
Month 1	48	100	42	87.5
Month 3	43	89.5	15	31.2
Month 6	36	75.0	3	6.2
Month 9	27	56.2	0	0
Month 12	15	31.2	0	0

Table 3. Anti-*Hp* IgG and Anti-HAV IgG positivity in infants' sera and breast-milk

	Infants' sera				Breast-milk			
	<i>H. pylori</i>		HAV		<i>H. pylori</i>		HAV	
	n	%	n	%	n	%	n	%
1 st day	46/48	95.8	48/48	100	39/48	81.2	33/48	68.7
1 st month	41/48	85.4	47/48	97.9	40/48	83.3	40/48	83.3
3 rd month	38/48	79.1	45/48	93.7	32/48	66.6	29/48	60.4
6 th month	29/48	60.4	41/48	85.4	23/48	47.9	20/48	41.6
9 th month	19/48	39.6	38/48	79.1	15/48	31.2	13/48	27.1
12 th month	23/48	47.9	31/48	64.5	11/48	22.9	7/48	14.5

The two infants who were seronegative on the first day postpartum remained seronegative at 12 months. The breast-milk of their mothers was positive at first but then became negative. Twelve of the babies who were anti-*Hp* IgG positive on the first day were still positive at 12 months of age. Anti-*Hp* IgG became negative in the 3rd month in 8 babies, in the 6th month in 9 babies, in the 9th month in 10 babies, and in the 12th month in 7 babies.

Although anti-*Hp* IgG positivity rates in breast-milk decreased from the first day to the 9th month, mothers who continued to breast-feed showed a 17.8% increase in anti-*Hp* IgG positivity at the 12th month (Table 3).

Hepatitis A Serology

Except for one mother, all mothers (97.9%) and all infants were seropositive for anti-HAV IgG (100%) on the day of birth. Anti-HAV IgG was positive in all infants' sera, whereas it was negative only in the serum of a single mother. However, this mother's breast-milk was positive for anti-HAV IgG. On the first day, anti-HAV IgG was positive in 68.75% of the breast-milk samples (33/48). Similar to anti-*Hp* IgG, anti-HAV IgG seroprevalence also decreased over time in infants' sera and breast-milk samples (Table 3, Figures 1, 2).

DISCUSSION

H. pylori is responsible for one of the most frequent chronic bacterial infections and seems to occur

predominantly in childhood, especially during the preschool years. The prevalence of *H. pylori* infection is low among children in developed countries. In contrast, it is high in developing countries, where it occurs in early childhood and persists throughout life (9).

Our study was established to investigate the variations in the seroprevalence of *H. pylori* IgG in baby serum and breast-milk in the first year of life. Except for two mothers, all mothers and 46 (95.8%) babies were seropositive for *H. pylori* on the day of birth. In agreement with previous reports, the high degree of correlation between maternal and baby serum on the day of birth could be accepted as indicating the direct placental transfer of IgG antibodies (7). These maternal IgG antibodies may last until the postpartum period and may help to protect the child for the first months of life while its own immune system matures (7). Though it is still under debate in the literature, human milk is shown to have protective effects against *H. pylori* infection by a variety of mechanisms with different studies (8,10-12). These include antibody effects targeted at protection against pathogens in the infant's environment (through milk IgA, IgG, and IgM) and nonspecific inhibition of bacterial adherence to gastric mucosal cells (8, 10). Human milk antibodies are active within the newborn's gut and influence the gut flora (7). In addition, ligand action, which inhibits *H. pylori* adhesion to gastric mucosa by kappa-casein,

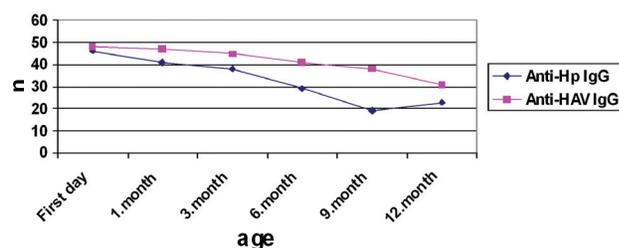


Figure 1. Monthly changes in anti-*Hp* IgG and anti-HAV IgG positivity in infants' sera.

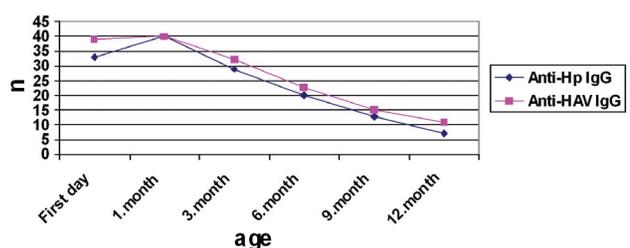


Figure 2. Monthly changes in anti-*Hp* IgG and anti-HAV IgG positivity in breast-milk.

is another protective effect of breast-milk. A prospective study in Gambia showed specific breast-milk IgA antibodies that delay *H. pylori* colonization in the first year of life (13). The study of Tanriverdi et al. (12) from Turkey supported this old Gambian study by showing the *H. pylori* IgA in colostrum. They especially highlighted the existence of *H. pylori* IgA in human milk in developing countries like Turkey where exposure to infections like *H. pylori* is at much earlier ages than in developed countries. In our study, *H. pylori* IgG was positive in breast-milk in 81.25% of the mothers on the day of birth, and seroprevalence rates tended to decline gradually. The same decrease was observed in infants' sera. The gradual decrease in the maternal antibodies transferred prenatally, and in the antibodies in breast-milk, increases the infant's susceptibility to *H. pylori* infection. We were not able to show the serum and breast-milk *H. pylori* IgA levels due to financial limitations. However, as discussed in the study of Lepper et al. (14), interpretation of IgG would also be a very useful tool in seroepidemiological studies. In some studies, ELISA was given as the first-step laboratory test that identifies all truly and potentially *H. pylori*-positive patients with a high sensitivity (14). Diagnostic sensitivity for *H. pylori* IgG antibody testing was 71.9%, and specificity ranged from 72% to 98% (15,16). In addition to several studies published on serology, Leal et al. (17) published a meta-analysis on antibody-based detection tests for the diagnosis of *H. pylori* infection in children. ELISA-IgG assays showed low sensitivity (79.2%) but good specificity (92.4%). Therefore, we believe that *H. pylori* IgG levels could also help us to discuss the situation of the *H. pylori* infection in mother-infant pairs in our study.

In our study group, *H. pylori* seroprevalence began to increase at nine months of age. This can be the result of decreased rates of breastfeeding in these months. It was shown by Weaver (18) that specific breast-milk IgA might play a crucial role in delaying the onset of *H. pylori* infection. Hanson et al. (19) indicated an enteromammary link that is a path for the migration of B lymphocytes from Peyer's patches into the mammary glands that protect the baby while breastfeeding. Chronic *H. pylori* infection has been shown together with chronic active gastritis, peptic ulceration and moreover with gastric malignancies. Infection with this microorganism is acquired mainly in the first and second years of life (11). As shown in Table 2, breastfeeding rates are quite high in our study group,

and there is a parallelism with the seropositivity rates of anti-*Hp* IgG in infants' sera and breast-milk samples by age. Thus, these results could also be an indicator for the protection of the baby from contamination in the first year of life.

Age, socioeconomic level, overcrowding, sharing a bed, contaminated water, hygienic conditions and poor living conditions have been shown to be major risk factors for higher infection rates and earlier age at acquisition (20, 21). Keeping a dog at home also increases risk and indicates the potential zoonotic risk of human infection by *H. pylori* (20). The percentages of our study participants who did not have running water at home and kept a pet at home were 12.5% and 14.5%, respectively. As all mothers and infants in these groups were seropositive for *H. pylori*, it was not possible to show the additional risks of these factors on *H. pylori* infection by assessing the antibody profiles in mothers' and infants' sera in this study.

The sources and routes of transmission of *H. pylori* infection are still a topic of debate (1, 3, 13). The pattern of immunity against this microorganism and the fate of *H. pylori* infection in children are unclear. Detection of antibodies to *H. pylori* denotes past infection, but infection is so common that this information is of limited clinical value (8, 15).

Any mechanism that transfers *H. pylori* organisms from an infected stomach to an uninfected stomach is thought to be a potential mode of transmission. However, little is known on this topic. Understanding the transmission routes of *H. pylori* is essential to prevent children from being infected with this microorganism (1). The majority of evidence supports person-to-person transmission to be via fecal-oral, oral-oral, or gastro-oral routes (1, 5, 6). Several studies have suggested that family members, especially the mother, play an important role in the transmission of the infection within the household (5, 22). Poor hygienic conditions are considered to be a major risk factor both for the acquisition of *H. pylori* and HAV. However, two Turkish studies from Manisa and İzmir did not show a correlation between anti-*Hp* IgG and anti-HAV IgG in children and concluded that they may have the same or different transmission routes (23, 24). Analyses of anti-HAV and anti-*Hp* IgG in the serum of our study groups (infants) and in breast-milk samples showed similar changes. This led us to think that these results could be an evidence for a possible fecal-oral transmission route for *H. pylori*, as with HAV. There are conflicting

results on the transmission route of *H. pylori* infection in the literature that need to be clarified in further investigations.

The results of our study suggest that neonates born from *H. pylori*-infected mothers were possibly protected with anti-*Hp* IgG antibodies transferred transplacentally. Moreover, breastfed neonates are additionally protected with antibodies against *H. pylori* in breast-milk. The gradual decrease of these antibodies in infant serum together with the decreased intake of maternal milk leads to an increased risk of exposure to *H. pylori* when supplemental food is started. Therefore, environ-

mental conditions should be controlled during the weaning period to prevent infants from becoming exposed to *H. pylori* early in life. In conclusion, as mothers are the most important persons affecting the present and future health of the baby, health programs focused on training the mothers in hygienic and healthy behaviors should be started and implemented country-wide.

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