

## Original Article

# The Comparison of Haemophilus Influenza in the Throat of Healthy Infants with Different Feeding Methods

A. Kazemi MD\*, D. Torabinia MD\*\*, R. Iranpour MD\*

### Abstract

**Background:** Haemophilus influenza (HI) is the most commonly found pathogenic bacteria in pediatric otitis media and lower respiratory tract infections. Bacterial attachment to pharyngeal cells and proliferation may be necessary for infection. In the presence of human milk, attachment of HI to pharyngeal cells and colonization may be inhibited. To evaluate the protecting role of breast milk, we investigated the incidence of HI isolated from the throat of healthy infants with different feeding methods.

**Methods:** Between August 2002 and March 2003, 210 healthy infants (70 purely breast-fed, 70 purely formula-fed, 70 mixed-fed), aged 1-6 months were enrolled into the study and a throat culture was taken in all of them. The incidence of HI was evaluated using Haemophilus Test Agar Bose (HTAB) plates.

**Results:** The incidence of HI in purely breast-fed, mixed-fed and purely formula-fed infants was 2.9%, 42.9% and 75.7% respectively ( $P = 0.000$ ). The mean age and weight of cases in the three groups were not statistically different.

**Conclusion:** These data suggest that human milk protects the throat of healthy infants from HI colonization especially in purely breast-fed cases.

**Key words:** Breast milk, Haemophilus influenza, Throat culture

Haemophilus influenza is one of the respiratory bacterial pathogens and the major cause of otitis media and lower respiratory tract infection in childhood. Bacterial attachment to pharyngeal cells and proliferation may be necessary to infect the lower respiratory tract or middle ear<sup>1,2</sup>. The relationship between nasopharyngeal colonization with nontypeable Haemophilus influenza and recurrent otitis media have been reported by Harabuchi et al<sup>3</sup>. The Reduction or elimination of the organism was associated with mucosal immune response.<sup>5</sup> Human milk contains numerous host defense factors such as lactoferrin, lysozyme, receptor analogs and especially secretory IgA antibody to P6, a highly conserved outer membrane protein of nontypeable Haemophilus influenza<sup>6,7</sup>. Thus, in the presence of human milk, Haemophilus influenza may be inhibited from attaching to and colonizing pharyngeal cells.

However, the protective effect of breast milk in preventing the gastrointestinal infection was proved in previous studies but little attention has been focused on the preventive role of human milk in respiratory tract infections<sup>8,9</sup>. In order to confirm the protecting role of breast milk, we investigated the incidence of Haemophilus influenza in the throats of infants with different feeding methods.

### Materials and Methods

Between August 2002 and March 2003, we studied the incidence of Haemophilus influenza in the throat of full term infants aged 1-6 months, cared for at home, who referred to the well-baby clinic with one of the following feeding methods: 70 purely breast-fed, 70 mixed fed (breast-fed and formula) and 70 purely formula-fed infants. Low-birth weight infants, those with symptoms of respiratory tract disease or currently on antibiotics were excluded from the study.

\*Assistant Professor, Department of Pediatrics, Isfahan University of Medical Sciences, Isfahan, Iran.

\*\*Resident of Pediatrics, Isfahan University of Medical Sciences, Isfahan, Iran.

Correspondence to: Dr Akbar kazemi, Al-Zahra hospital, Isfahan, Iran.

Cultures were obtained with a sterile cotton-tipped applicator stick pressed around the Waldeyer's ring in throat. All patients referred to the same laboratory and samples were analyzed immediately. We used a standard culture media, Haemophilus Test Agar Bose (HTAB, Biomark, Germany). We dissolved 43 grams HTAB powder in 1 liter distilled water and autoclaved it for 15 minutes in 120°C. This solution defibrinated with 50 milliliters of rabbit blood in 45-50°C and then bacitracin (5 IU) was added to it. This media composition is one of the best standard microbiological procedures for isolation of Haemophilus influenza<sup>10</sup>. Encapsulation was not determined but as mentioned before even colonization with non-capsulated organisms is also important for recurrent upper respiratory tract infection in infancy. The same microbiologist evaluated the cultures and if it was positive for Haemophilus influenza, a second passage culture was performed. A colony count of at least 10<sup>3</sup> colony forming unit/ml (CFU/ml) was considered positive.

Data were analyzed with Statistical Package for Social Sciences (SPSS version 10.05). Chi-square test was used to compare the incidence of positive cultures among breast-fed, mixed-fed and formula-fed infants. Mean values of age and weight were compared among groups using Kruskal-Wallis and ANOVA tests respectively. A P value < 0.05 was considered as statistically significant.

## Result

The incidence of Haemophilus influenza differed significantly among different feeding methods with the lowest value in purely breast-fed, followed by mixed-fed and purely formula-fed infants (P = 0.000, table 1).

The minimum and maximum ages of infants were respectively 1 and 5 months in purely breast-fed infants and 1.5 and 6 months in the other two groups. There was no significant difference for mean age among the three groups (table 1).

Minimum weights in breast-fed, formula-fed and mixed-fed groups were 3000, 3200 and 3300 grams and maximum weights were 7900, 8500 and 8500 grams respectively. There was also no significant difference among these groups (table 1).

Sex distributions in all groups were comparable (P= 0.1).

## Discussion

Colonization of various Haemophilus species in upper respiratory tract is common. Among those species, Haemophilus influenza is the most commonly found pathogenic bacteria in pediatric otitis media and lower respiratory tract infections. Haemophilus influenza type b is responsible for a life threatening and invasive infection in children. However, when local defenses are compromised, such as during viral infection, the non-encapsulated Haemophilus influenza, which is regarded as opportunistic, can also produce the disease<sup>5</sup>. So viral infections could be a predisposing factor for bacterial invasion of the middle ear, respiratory sinuses and bronchi in children.

The prevalence of various Haemophilus species, Haemophilus influenza and Haemophilus influenza type b in healthy Japanese infants were reported to be 7.9%, 2.3-16.7%, and 0.5-0.8% respectively<sup>9,11</sup>. In a study on 162 healthy Japanese infants, the incidence of Haemophilus influenza in breast-fed infants, mixed-fed infants and formula infants was 0, 0 and 7% respectively<sup>5</sup>. In our study, the incidences of Haemophilus influenza were significantly higher in all three groups especially in formula and mixed-fed infants. Pharyngotonsillar flora is affected by many demographic factors like climate, age and the extent of exposure to infectious disease or vaccines<sup>12</sup>. Immunization against Haemophilus influenza type b has decreased but the carrier state has not been eliminated<sup>13</sup>. None of the infants in our study were immunized against Haemophilus influenza type b. Leach et al<sup>14</sup> showed a high incidence of nasopharyngeal colonization with multiple strain of nontypeable Haemophilus influenza in rural aboriginal infants. The higher prevalence of Haemophilus influenza in our study compared with Japanese infants maybe due to poor hygiene which has been suggested as a contributing factor to the high rate of bacterial colonization<sup>14</sup>. More studies are needed to determine the causes of these differences. In this study we did not match families of infants in the three groups with respect to hygiene, economic status and climate because all the cases were randomly selected from a baby clinic in the center of Isfahan. However we suggest considering these factors in future studies.

Long et al<sup>15</sup> found that in patients with nontypeable Haemophilus influenza otitis media,

Haemophilus influenza accounted for over 50% of bacteria colonizing the nasopharynx. In another study from the New York School of medicine, 68 breast-fed infants were followed prospectively from birth to 12 months of age to assess the effect of human milk secretory IgA antibody on nasopharyngeal colonization of nontypeable Haemophilus influenza<sup>3</sup>. Nasopharyngeal colonization was found in 22 infants (32%), and 39 infants (57%) had otitis media. In this trial, frequency of isolation of nontypeable Haemophilus influenza was directly related to episode of otitis media ( $r = 0.35$ ;  $p = 0.001$ ) and the level of human milk anti-P6 secretory IgA antibody was inversely related to the frequency of isolation of organism ( $r = -0.27$ ;  $P = 0.026$ ). However in our study we did not assay the level of secretory IgA, the lower incidence of Haemophilus influenza in purely breast-fed infants in comparison with mixed-fed infants is probably due to lower secretory IgA in the latter group. The assessment of secretory

IgA levels will be necessary for future studies to prove this relationship.

Although we did not identify the nontypeable and typeable Haemophilus influenza, but non-invasive Haemophilus influenza colonization could also be a cause of bacterial infection in upper respiratory tract especially during viral infection. Therefore, breast-milk feeding and especially purely breast-milk feeding could be a protective factor for prevention of Haemophilus influenza colonization and recurrent upper respiratory tract infection. In addition in formula-fed infants with recurrent upper respiratory tract infection Haemophilus influenza colonization should be considered.

### Acknowledgements:

We are grateful to Dr fazeli for examining the throat cultures and also Mrs Jafari, for her co operation.

**Table 1.** Comparison of age, weight and incidence of positive Haemophilus influenza cultures among infants with different feeding methods. Data are mean  $\pm$  SD or n (%)

	Breast-fed (n=70)	Formula-fed (n=70)	Mixed-fed (n=70)	P value
Age (months)	3.84 $\pm$ 0.89	3.53 $\pm$ 1.04	3.81 $\pm$ 1.14	0.12
Weight (grams)	5475 $\pm$ 1285.70	5435 $\pm$ 1073.87	5824 $\pm$ 1187.30	0.10
Sex				
Male	37 (52.9)	42 (60)	37 (52.9)	
Female	33 (47.1)	28 (40)	33 (47.1)	0.1
Positive throat culture	2 (2.9)	53 (75.5)	30 (42.9)	0.000

### References

- 1- Hokama T, Yara A, Hirayama K, Takamine F. Isolation of respiratory bacterial pathogens from the throats of healthy infants fed by different methods. *J Trop Pediatr* 1999 Jun; 45 (3): 173 – 6.
- 2-Faden H, Duffy L, Hong JJ. Adherence of nontypable Haemophilus influenzae to respiratory epithelium of otitis prone and normal children. *Acta Otolaryngol Suppl* 1996; 523: 142-4
- 3- Harabuchi Y, Faden H, Yamanaka N, Duffy L, Wolf J, Knjstofik D. Human milk secretory IgA antibody to non-typable Haemophilus influenzae: possible protective effects against nasopharyngeal colonization. *J Pediatr* 1994; 124: 193 – 8.
- 4- Hokama T, Imamura T. Members of the throat micro flora among infants with different feeding methods. *J Trop Pediatr* 1998 Apr; 44(2): 84-6
- 5- Hokama T, Sakamoto R, Yara A, Asato Y, Takamine F, Itokazu K. Incidence of Haemophilus influenzae in the throats of healthy infants fed by different methods. *Pediatr Int* 1999; 41(3): 277-80
- 6- Andersson B, Porrás D, Hansan LA, Lagergard T, Svanborg-Eden C. Inhibition of attachment of streptococcus pneumonia and Haemophilus influenzae by human milk and receptor oligosaccharides. *J infect Dis* 1986; 153: 232 – 7.

- 7- Kalieda PH, Nativio DG, Chao HP, Cowden SN. Prevalence of bacterial respiratory pathogens in the nasopharynx in breast-fed versus formula-fed infants. *J Clin Microbiol* 1993; 31: 2674-8
- 8- Hokama T, Hamamoto I, Takenaka S, Hirayama K, Yara A, Adjei A. Throat microflora in breast-fed and formula-fed infants. *J Trop Pediatr* 1996; 42(6): 324-6
- 9- Kuroki H, Ishikawa N, Uehara S, Himi K, Sonobe T, Niimi H. Nasopharyngeal colonization with *Haemophilus influenzae* among infant and children in Japan. *Acta Paediatr Jpn* 1997; 39: 541-5
- 10- Sinegold Sydney N, Martinwilliam G. Gram negative coccobacillary facultative bacteria. In: Sinegold Sydney N, Martinwilliam G. *Diagnostic microbiology*. London, C.V.Mosbey, 1982: 273-279
- 11- Terasima S. A study on the pharyngeal flora of children. *J Jpn Pediatr Soc.* 1977; 81: 692-704
- 12- Berkovitch M, Bulkowstein M, Zhovits D, Greenberg R, Nitzan Y, Burzilary B, et al. Colonization rate of bacteria in the throat of healthy infants. *Int J Pediatr Otorhinolaryngol* 2002; 63: 19-24
- 13- Murphy TV, Pastor P, Medley F, Osterholm MT, Granoff DM. Decreased *Haemophilus* colonization in children vaccinated with *Haemophilus influenzae* type b conjugate vaccine. *J pediatr* 1993; 122: 517-523
- 14- Leach AJ, Boswell JB, Asche V, Nienhuys TG, Mathews JD. Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian aboriginal infants. *Pediatr Infect Dis J* 1994; 13: 983-989
- 15- Long SS, Henretig FM, Teter MJ, McGowan KL. Nasopharyngeal flora and acute otitis media. *Infect Immun* 1983; 41: 987-91