Original Article

The Comparison of Salivary IgA and IgE Levels in Children with Breast- and Formula- Feeding During Infancy Period

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ABSTRACT

Introduction: Oral local immune factors may play a protective role against oral diseases and defend against microbial agents. Salivary immunoglobulin A (IgA) is a major factor for the local host defence against caries and periodontal disease. The aims of this study were to determine the concentrations of salivary IgA and IgE levels in breast-fed and formula-fed children in infancy period.

Methods and Materials: Totally, 80 healthy 5 years old children were included in the study. According to type of feeding in infancy period, the children divided into two groups: 50 breast-fed and 30 formula-fed. One milliliter of saliva was collected from each participant, centrifuged, and stored at -70 ˚C. The salivary IgA and IgE concentrations were measured, using ELISA technique.

Results: In breast-fed children, the salivary IgA level (39.6 mg/l ± 17.3) was significantly higher than that in formula-fed children (26.9 mg/l ± 14) (P=0.0001). However, the salivary IgE level was significantly lower in breast-fed children, comparing with formula-fed ones (5.01 IU/ml ± 19.70 vs. 11.74 IU/ml ± 39.40) (P=0.047).

Discussion: These results suggest that breast feeding enhances salivary IgA level in the early period of life which may contribute in oral cavity immunity. Higher salivary IgE level observed in formula-fed subjects may have a potential role in development of allergic or inflammatory reactions.

Keywords: Breast Feeding, Formula Feeding, Saliva, IgA, IgE, Children.

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Introduction

The infant immune system matures rapidly during the first 2 years of life, and there is a strong association between type of feeding and development of immune system [1]. Infants who are breast-fed have been shown to have a lower incidence of certain infectious diseases when compared with formula-fed infants [2,3]. Several studies have reported that human milk enhances development of the immune system in breast-fed, compared with formula-fed infants [4,5]. Reports of enhanced humoral response include increased serum antibody titers to *Haemophilus influenzae* type b polysaccharide (Hib), oral polio virus (OPV), and diphtheria toxoid (DIP) in breast-fed infants [6].

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Secretory immunoglobulin A (SIgA) is the dominant immunoglobulin in external secretions that cover mucosal (respiratory and intestinal) surfaces and is often characterized as the first-line of defense against pathogenic microorganisms [7]. Salivary immunoglobulins protect the oral mucosa and teeth surface from invasion of bacteria, viruses, and other antigens and their colonization. Several studies have reported that caries was particularly correlated with IgA level, among the many salivary components [8,9]. In addition, many studies have also demonstrated that the lower incidence of lower caries resulted from a high salivary IgA concentration [10,11]. A lower concentration of IgA in saliva has been presented as a risk factor for upper respiratory tract infections in children and the elderly [12,13]. Furthermore, lower levels of salivary IgA are associated with increased risk for periodontal disease and caries [14,15]. Children who were not breast-fed at all or only for 3 months exhibited significantly higher caries prevalence than those breast-fed for a longer time [16]. In another study in children, aged 3-5, it has been concluded that breastfeeding during infancy may act preventively and inhibit the development of nursing caries in children [17].

Moreover, the results of some studies show that breastfeeding may protect against autoimmune diseases and tumors [18,19]. In addition, exclusive breast-feeding for the first few months has been suggested to be protective against the development of allergic and atopic diseases [20,21]. IgE has an important role in immunopathogenesis of some allergic and inflammatory reactions [22]. This study was conducted to compare the salivary IgA and IgE concentrations in 5 years healthy children who had been breast-fed or bottle-fed in infancy period to clarify the life-long effects of infancy feeding on the mucosal immunity.

Methods and Materials

We conducted this study from October 2005 to March 2006 in the Department of Immunology, Rafsanjan University of Medical Sciences and Health Services, Rafsanjan, Iran.

Totally, 80 healthy children (aged 5 years old) were enrolled in the study. Children with medical history of present or previous recurrent infections, other acute diseases, history of asthma, allergy, atopic diseases, any suspected immunological disorder, and chronic illnesses or syndromes were all excluded from the study. The Informed consent was obtained from the parents before enrolment in the study. Data about medical history of children and type of feeding in infancy were obtained from their parents and public health centers. Complete information about children such as their medical history and type of feeding in infancy were exist in public health centers files. The children divided into two groups according to the type of their feeding during the infancy period. 50 subjects were breast-fed that had been fed with their mothers' breast milk for at least first 18 months of age. 30 children were exclusively formula- fed during infancy.

Collection of the saliva

All saliva samples were collected at morning between 10 and 11 (a.m.). Before collecting the saliva, the subjects had been asked not to eat or drink for at least 1 h. Unstimulated whole saliva samples were collected at one occasion from the mouth, during a period of 5 min. The saliva was collected directly into tubes and placed on ice and all samples were centrifuged for 15 min at 10000 g to remove cells and debris. The supernatants were kept at – 70 ˚C. Samples then were thawed and analysed by enzyme-linked immunosorbent assay (ELISA).

The measurements of IgA in saliva were performed by sandwich enzyme-linked immunosorbent assay (ELISA). In these assay, polystyrene microtitre plates (F96, NUNC, Roskilde, Denmark) were coated overnight at 4 °C with 0.2 µg/well of purified rabbit anti-IgA antibodies (Beta, Iran) in 0.05 m $NaHCO_3$, PH 9.5. Blocking performed using phosphate buffer containing 0.5% bovine serum albumin (BSA) at RT for 90 min. 100 µl of saliva samples (in duplicate) and standard samples (in duplicate) were pipet-

ted into the microtitre wells. The plates were incubated for 90 min at 37 °C. The wells were washed 5 times with washing solution. Then, 100 µl of goat anti-human IgA conjugated with horse radish peroxidase (HRP) was pipetted into each well, and the plates were incubated for 30 min at 37 °C. The wells were washed 5 times with washing solution and tapped dry. A fresh substrate solution, tetramethylbenzidine (100 µl), was added, and the plates were incubated for 15 min at room temperature. The enzyme reaction was stopped with 100 µl of 1 N HCl. Results were quantified using a standard cure whose values are expressed as mg/l. CV% for these ELISA was 3.8%.

Salivary IgE level was quantified in duplicate by ELISA, using commercial kits (Radim, Italy). Salivary IgE concentration expressed as IU/ml.

Statistical analyses

Data were expressed as mean ± SD. Differences of means of two groups were analyzed using t-test and Mann-Whitney U-test. For comparison of the mean salivary IgA levels, t-test (due to homogeneity of variance) was used. For comparison of the mean salivary IgE levels Mann-Whitney U-test (due to skewed variance) was used. The P-values of less than 0.05 were considered significant.

Differences in variables were analyzed using Mann-Whitney U and Kruskal-Wallis tests as appropriate, and P-values of less than 0.05 were considered significant.

Results

Table 1 and Figure 1 show the mean salivary IgA levels in breast- and formula-fed subjects. In breast- and formula-fed children, the mean salivary IgA levels were 39.6 mg/l ± 17.30 and 26.9 mg/l ± 14, respectively. Statistical analysis showed that in the breast-fed children the salivary IgA level was significantly higher than that in formula-fed children (P=0.0001).

Table 1 and Figure 2 show the mean salivary IgE levels in breast- and formula-fed subjects. The mean levels of salivary IgE in breast- and formula-fed children were 5.01 IU/ml ± 19.70 and 11.74 IU/ml ± 39.40, respectively. Statistically, the mean salivary IgE levels were significantly lower in breast-fed, compared with formula-fed group (P=0.047).

Discussion

Secretory IgA antibodies can neutralize viruses, bind toxins, agglutinate bacteria, prevent bacteria from binding to cells, and bind various allergens [7]. The breast-fed infants in the present study showed significantly higher salivary IgA concentration, compared to the formula-fed group. These results are consistent with previous observations of higher IgA levels in different body fluids of breast-fed infants. It has been demonstrated that at early months of life, breast feeding is associated with higher level of IgA in urinary tract which may be cause of reduced incidence of urinary tract infection in breast-fed

Table 1. Comparison of salivary IgA and IgE levels in breast- and formula-fed groups.

Group	Number of subjects	Salivary IgA level (mg/l)*	Salivary IgE level (IU/ml)*	P-value
Breast-fed	50	39.6 ± 17.3	5.01 ± 19.70	† 0.0001
Formula-fed	30	26.9 ± 14	11.74 ± 39.40	‡ 0.047

* Results are expressed as mean ± SD.
† & ‡ Represent the differences of salivary IgA and IgE levels, respectively.

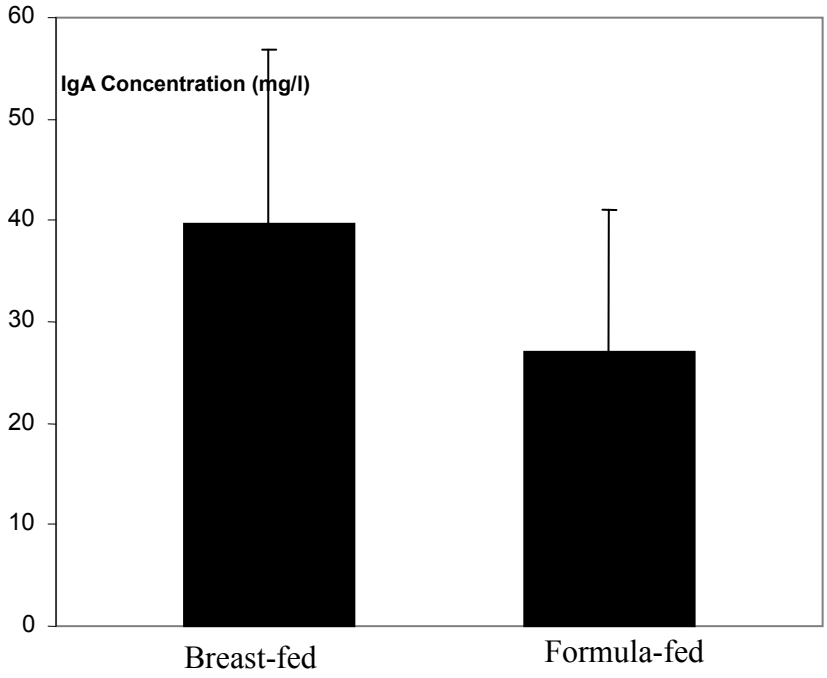

Figure 1. Comparison of salivary IgA levels in breast-fed and formula-fed children.

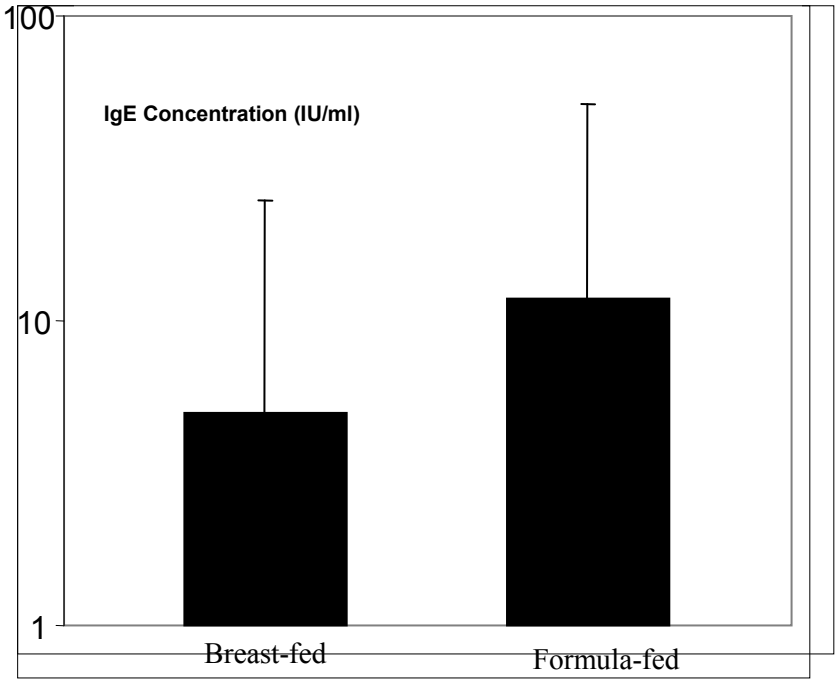

Figure 2. Comparison of salivary IgE levels in breast-fed and formula-fed children.

infants, compared to formula-fed infants [23]. Fitzsimmons et al [24] have reported that the salivary IgA concentration increased more rapidly in breast-fed than in formula-fed infants during the first 6 months after birth. They concluded that although secretory immunity is immature in infants, breast feeding may aid in protection against pathogenic microorganisms by increasing the rate of mucosal IgA maturation. Avanzini et al [25] have demonstrated that the salivary IgA was significantly lower in breast-fed, compared with formula-fed infants at age of one month but salivary IgA increased with age in breast-fed infants and was significantly higher at six months. Köhler et al [26] recently demonstrated that the fecal IgA concentration was significantly higher in breast-fed, than formula-fed infants during the first 3 months. Enhanced fecal IgA in breast-fed infants is not caused solely by the presence of IgA in breast milk; it represents a stimulatory effect of breast milk on the gastrointestinal humoral immunologic development.

The breastfed infants in the present study showed significantly lower salivary IgE concentration, compared to the formula-fed group. IgE has a central role in the development of allergic responses 22. It has been reported that breastfeeding protect from the development of atopic diseases such as asthma, allergic rhinitis, and atopic dermatitis (eczema) 20,21. Moreover, breast feeding is strongly recommended to mothers of infants with family history of atopy, as a possible means of preventing atopic diseases and it has been reported that formula feeding before 3 months of age predisposes the infants to asthma at age 4 years of old 27.

The immunological basis of these differences between breast- and formula-fed subjects could be explained according to the responses of T-helper (Th) cells. These cells can be functionally distinguished based on the profile of cytokine production. Th-1 cells are characterized by secretion of cytokines such as IFN-γ and IL-2. Th-2 cells produce cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13. IL-4 and IL-13 are essential cytokines for IgE production by B cells 28. Moreover, regulatory T cells, also known as Treg cells, are defined by their ability to produce high levels of IL-10 and transform growth factor-β (TGF-β) 29. In vitro studies showed that TGF-ß induces B cell switching to IgA and enhances secretion of this isotype 30. A lack of IgA-committed B cells was seen in TGF-ß-/- mice. It has been showed that IgA levels in both serum and mucosal secretions were significantly reduced in TGF-ß-/- mice. 31. Regarding to the results of this study, lower salivary IgA level in bottle-fed group may be attributed to diminished production of TGF-ß by Treg cells. This observation is consistent with increase in serum IgE level in bottle-fed group. Th-2 cells secretion, especially IL-4 is responsible for IgE production 28. In human and murine models, it has been demonstrate that TGF-ß inhibits Th-2 cells development 31,32. The elevated IL-4 production and the high IgE level detected in TGF-ß-/- mice also indicate a preferred Th-2 responses 31. Recently, it has been demonstrated that TGF- ß supplementation of formula results in a decrease in Th-2 cytokines and down-regulation of allergic reaction. Importantly, this immune profile persisted after weaning when TGF- ß was no longer present in the diet 32. Accordingly, it seems that formula feeding via Treg cell down-regulation and diminished secretion of TGF-ß would generate a Th-2 type of immune response which is responsible for lower IgA and higher IgE production.

In summary, breast-fed children during infancy display higher salivary IgA levels and lower IgE levels compared to formula-fed subjects. These observations represent the effects of infancy feeding on the development of mucosal immunological factors.

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