INFLUENCE OF TYPE OF FEEDING ON LEVELS OF SALIVARY IMMUNOGLOBULIN A (SIgA) IN PRETERM NEONATES

PRESENTED BY: Dr. Priya Subramaniam, Shilpy Dwivedi

BACKGROUND:

There is considerable interest in events occurring during the neonatal period, as it relates to infection susceptibility and establishment of resistance. The effect of environmental factors particularly feeding patterns, on development of mucosal immune response is important. Newborn infants are known to have a higher frequency of microbial infections than older children and adults soon after birth, due to immaturity of the immune system. Preterm delivery is known to cause undermining of development of oral defense mechanism. The mucosal immune system represents the first line of defense in the adaptive immune response to mucosal infection. The induction of mucosal immunity is highly dependent on exogenous stimuli and the neonatal period is critical. Immumoglobulins in saliva consist predominantly of Secretory Immumoglobulin A (SIgA) and may control the oral microbiota by reducing the adherence of bacteria to the oral mucosa and teeth. The total levels of SIgA in saliva have been considered as an indicator of maturation of the mucosal immune system in children. In addition to the reinforcement of mucosal defence provided by maternal SIgA antibodies as a natural (passive) immunization, there is a possible nutritional effect of breast feeding on immune development. Secretory IgA in breast milk provides passsive immunity to new borns. Breast milk stimulates the neonates own secretory IgA production. Breast milk also contains a number of immune cells, cytokines and growth factors that may affect the infant's gut and immunity in an indirect manner. Throughout the first month postpartum, significantly higher concentrations of SIgA was reported to be present in milk, from mothers who delivered preterm infants than that of those giving birth at term. Studies have suggested of an association between neonatal feeding patterns and immunological events. Feeding regimes can modify the pattern of salivary immunoglobulin development. Data on secretory IgA concentrations in breast fed and formula fed infants are conflicting. The presence of SIgA, by virtue of active production by the neonates or retained from the breast milk is controversial. Moreover, minimal data is available on preterm neonates.

AIM:

To assess and compare SIgA levels in saliva of preterm neonates fed on breast milk and infant formula through nasal intubation.
To compare SIgA levels in saliva between preterm and full term breast fed neonates.

DESIGN:

Ethical approval was obtained from institution's ethical committee. Permission was taken from the authorities of Indira Gandhi Hospital for children and written consent from the parents of the new born children. Only healthy neonates were included for the study. Neonates with congenital defects, malformations, perinatal hypoxia, intracranial hemorrhage, weight and length at birth incompatible with gestational age or under antibiotic regimen were excluded from the study.
The study group consisted of 38 preterm neonates, aged 6-10 days, fed through nasogastric intubation. They were considered to be preterm, if the gestational age was less than 37 weeks. Gestational age was estimated from the reported date of last menstruation period and somatic evaluation. Information on gestational age was obtained by interviewing the mother.
The preterm neonates were grouped according to feeding with breast milk or infant formula. They were examined at the hospital by a single examiner.
Another group of 32 full term neonates (gestational age 37-41 weeks) of similar age and fed orally were also selected for comparison with the preterm neonates.
Unstimulated whole saliva was collected from each neonate using sterile polyethylene pipettes, at least 30 minutes after feeding. SIgA was estimated using indirect Enzyme Linked Immuno Sorbent Assay (ELISA). Data obtained was statistically analyzed using ANOVA and level of significance was considered at $p<0.001$

Table 1: Comparison of salivary IgA levels in breast-fed and formula-fed pre-term neonates

Feeding Pattern	Number of neonates (N)	Salivary IgA levels (µg/ml) Mean ± SD	p value
Breast milk	20	4.20 ± 3.94	p< 0.001*
Infant formula	18	12.95± 6.26	

*p<0.001 is significant

RESULTS:

SIgA was detected only in 30 preterm neonates. Among the preterm neonates, breast milk-fed neonates had significantly lower mean SIgA levels (4.20 µg/ml) in their saliva when compared to that of infant formula-fed neonates (12.95 µg/ml) (Table 1)
SIgA levels in saliva of full term neonates was significantly higher than that of preterm neonates.(p<0.001). (Table 2)

Table 2: Comparison of salivary IgA levels in pre-term and full term neonates

Term of Gestation	Number of neonates (N)	Salivary IgA levels (µg/ml) Mean ± SD	p value
Full term	32	30.53±11.97	p< 0.001*
Pre-term	38	8.34±6.75	

*p<0.001 is significant

DISCUSSION:

During the third trimester of human pregnancy, fetal T cells are able to mount antigen-specific responses to environmental and food-derived antigens and antigen-specific T cells are detectable in cord blood in virtually all newborns indicating in utero sensitization. Changes occur at, and soon after, birth in order that the immune system of the neonate becomes competent and functional and that the gut becomes colonized with bacteria. Exposure to bacteria during birth and from the mother's skin and the provision of immunologic factors in breast milk are amongst the key events that promote maturation of the infant's gut and gut-associated and systemic immune systems. The introduction of formula exposes the infant to novel food antigens and also affects the gut flora.

Our results also showed that majority of preterm and all full term infants had detectable salivary levels of SIgA as early as 6-10 days.
The neonatal mucosa is endowed with all major elements of innate and adaptive immunologic repertoire. Rudimentary Peyer's patches and mucosal lymphoid follicles can be observed as early as 10-11 weeks of gestation. CD5+ and IgA+ B cells can be detected in Peyer's patches by 16-18 weeks. CD7+ CD3+ T lymphocytes have been observed in fetal Peyer's patches, epithelial surfaces as well as in the lamina propria.
IgA antibody specificities appear to be influenced by the gestational age. Irrespective of feeding, total salivary IgA levels in full term infants were found to be higher since immune system in full term neonates is more developed than preterm infants. This difference could be attributed to disparity in their chronological and biological age. Compared to full term children, greater numbers of preterm children did not have detectable IgA at birth, suggesting that prematurity of birth delays the appearance of salivary IgA.
The newborn infant leaves a germ-free intrauterine environment to enter a contaminated extrauterine world. Although the intestinal mucosal immune system is fully developed after a full-term birth, the actual protective function of the gut requires the microbial stimulation of initial bacterial colonization. Type of feeding has been shown to influence the immunological development in oral cavity. The predominance of bifidobacteria and lactobacilli (probiotics) in the gut microflora of breast-fed infants is thought to be, at least in part, supported by metabolism of the complex mixture of prebiotic oligosaccharides present in human breast milk, whereas; a more adult-type intestinal microbiota (enterococci and enterobacteria) is found in formula-fed infants. Probiotics, stimulated by prebiotic fermentation, are important to the development and sustainment of intestinal defences. For example, probiotics can stimulate the synthesis and secretion of polymeric IgA, the antibody that coats and protects mucosal surfaces against harmful bacterial invasion.

However, this was not seen in the preterm neonates. Infant formula appeared to be more antigenic as compared to breast milk. As speculated by Gleeson, this increased antigenic challenge can either pose a threat to immature immune system of the preterm neonate or can stimulate faster maturity. Continuous exposure of oral and intestinal mucosa to a multitude of antigens including various microbes could also tend to increase secretory IgA level. Infant formula also has been shown to cause early stimulation of Gut Associated Lymphoid Tissue (GALT).
Besides, the composition of the milk or infant formula, route of feeding could play an important role in stimulating the immune system. The full term neonates were fed orally and the preterm neonates were fed through nasogastric intubation. Moreover, the oral feeding can present greater immunologic challenge both in the oral cavity as well as GALT. Another possible reason could be presence of residual IgA from maternal milk in their oral cavity, even though the samples were collected at least 30 minutes to1 hour after feeding.
Limitations: Birth weight and mode of delivery were not considered as factors that could have influenced the results.
Future studies: Longitudinal comparisons of levels of SIgA in saliva of preterm and full term infants could be helpful to clarify the extent to which the difference is maintained over time.

REFERENCES:

1. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, Frokiaer H et al.Early nutrition and immunity - progress and perspectives Br J Nutr. 2006;96(4):774-90.
2. Ogra PL, Welliver RC Sr. Effects of early environment on mucosal immunologic homeostasis, subsequent immune responses and disease outcome.Nestle Nutr Workshop Ser Pediatr Program. 2008;61:145-81.
3. Forchielli ML, Walker WABr.The role of gut-associated lymphoid tissues and mucosal defence. J Nutr. 2005 Apr;93 Suppl 1:S41-8.
4. Sherman PM, Cabana M, Gibson GR, Koletzko BV, Neu J, Veereman-Wauters G.Potential roles and clinical utility of probiotics in newborns, infants, and children: proceedings from a global probiotic summit meeting, New York City, June 27-28, 2008.J Pediatr. 2009 Nov;155(5):S61-70.
5. Fitzsimmons SP, Evans MK, Pearce CL, Sheridan MJ, Wientzen R, Cole MF.Immunoglobulin A subclasses in infants' saliva and in saliva and milk from their mothers. J Pediatr1994Apr;124(4):566-73.
6. Gleeson M, Cripps AW, Clancy RL, Hensley MJ, Dobson AJ, Firman DW.Breastfeeding conditions a differential developmental pattern of mucosal immunity.Clin Exp Immunol1986Oct;66(1):216-22.