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The Difference Secretory Immunoglobulin A between Faeces Sample of Full Breastfeeding and Mixed Feeding Infant

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ABSTRACT

Newborn infants immune systems are immature and inadequate at birth. Infants have limited abilities to infectious challenges to respond effectively and quickly, which explain infants ongoing susceptibility to infections. Passive immunity is provided for infants through IgA and other antimicrobial peptides in breast milk, particularly colostrum. The purpose of this study is to determine the differences between the levels of secretory immunoglobulin A from faeces of full breastfeeding and mixed feeding infants. Design of this study was pre-test and post-test design. The sample size was 38 neonates in community health center work areas, selected by consecutive sampling. The level of secretory IgA was measured by ELISA method, then analyzed using t-test. The results showed that no significant differences levels of secretory IgA on 0 day from faeces of full breastfeeding and mixed feeding (p-value=0.141), the levels of secretory IgA on 28 days was higher in infants of full breastfeeding than mixed feeding (p-value=0.000), delta levels of secretory IgA was higher full breastfeeding infants than mixed feeding (p=0.000). Levels of secretory immunoglobulin A of full breastfeeding infants faeces was higher than mixed feeding infants.

Keywords: Secretory immunoglobulin A, Full breastfeeding, Mixed feeding

INTRODUCTION

Background

Neonatal Mortality Rate in 2010 in Indonesia reached 19 per 1.000 births. These numbers were the same as in 2007 and only 1 point decline compared to Indonesia Demographic and Health Survey (*SDKI*) in 2002-2003, which was 20 per 1.000 births. The concern to reduce neonatal mortality (0-28 days) becomes important because it had a contribution to the 59% of infant mortality[(1)]. Based on the *SDKI* in 2012, the highest proportion of the death cause of infants aged 0-12 months was diarrhea (42%), followed by pneumonia (24%), meningitis/encephalitis (9%), digestive tract disorders (7%), heart abnormalities and hydrocephalus (6%), sepsis (4%), tetanus (3%), and others (malnutrition, TB, measles) (5%)[(2)].

Newborn have immature and inadequate immune systems at the time of birth. The human immune system begin to form and develope during the fetus period. The baby's immune system develops during at least the first 2 years of life. Overall, the baby has a limited capability to respond effectively and quickly to the microorganisms and that cause the baby continuously vulnerable to infections[(3)].

Secretory IgA intestinal mucosa was important in the early life to eliminate pathogens and the development of oral tolerance against commensal bacteria in the gut. Although the IgA can be detected in faeces of infants breastfeeding in the first few weeks of life, the baby's ability to produce IgA during this period are still limited. Passive immunity is provided for infants by the IgA and other antimicrobial peptides in breastfeeding, especially colostrum. Breastfeeding also provides a source of living bacteria that helps in the formation of early intestinal microbiota. There is a mutual relationship between the intestinal IgA and commensal intestinal bacteria because the microbial exposure stimulates the production of IgA in infants and in turn the IgA plays a role to control the

composition of the infant microbiota. The production of IgA in infants who was given the formula milk was slower and have lower levels in the first year of life[4].

The benefits of breastfeeding for neonates showed in baby's immunity. Nurmiati and Besral[5] reported that the duration of breast feeding greatly affects the survival rate of infants in Indonesia. The baby who have breastfeeding within six months duration or more have survival rate 33.3 times better than infants who are breastfed less than 4 months, and babies who are breastfed for 4-5 month has survivability 2.6 times better than the babies who are breastfed less than 4 months after controlled by the number of toddlers in the family and the place to live. Jafarzadeh[6] reported that the children who were given breastfeeding had the levels of IgA saliva significantly higher than children who were given formula milk. Bridgman[7] reported that there was a relation between the levels of fecal IgA of infant within 4 months after birth with the breastfeeding status, IgA levels rise with exclusive breast feeding. Also, according to IDAI[8], breastfeeding contains different anti substance cellular or humoral, so neonatal who were given breastfeeding have lower mortality and morbidity than the neonatal who were given formula milk.

Based on *Sumatera Barat* Health Profile in 2014[9], the rate of exclusive breastfeeding was 62.6%, and was higher than national (54.3%) and global rate (39%). However, we still need more effort to reach the target rate for exclusive breastfeeding, that is 80%. Based on Padang Public Health Office report in 2015, Koto Tangah and Kuranji are two districts with the highest rate of exclusive breastfeeding and highest incidence of diarrhea. In Koto Tangah, exclusive breastfeeding rate was 69.58% and diarrhea incidence was 766 cases. While in Kuranji, exclusive breastfeeding rate was 75% and diarrhea incidence was 389 cases.

High rate of gastrointestinal infection, caused by neonate's gastrointestinal immaturity, high rate of consuming formula milk, or both, is a health problem that the solution must be found immediately. This study aims to investigate the difference of immunoglobulin A secretory level between neonate's fecal who received exclusive breastfeeding and who did not receive exclusive breastfeeding.

Purpose

The purpose of this study is to determine the differences between the levels of secretory immunoglobulin A from faeces of full breastfeeding and mixed feeding infants.

METHODS

This was an study with pre-test dan post-test with control group design. The sample of this study was neonates aged 0 to 28 days who received exclusive breastfeeding and did not receive it, in the working area of Koto Tangah and Kuranji Community Health Center. Sample size was 38, consist of 19 neonates who received exclusive breastfeeding and 19 neonates who did not receive it. Sample was selected using consecutive sampling technique. Instrument used in this study was questionnaire and ELISA. The neonates fecal was collected 100 mg and put into a sterile bottle, and then carried to Biomedical Laboratory, Faculty of Medicine, Universitas Andalas to be saved at -80^0C until total sample was fulfilled. The data obtained was analyzed used independent sample t-test.

RESULTS

The Characteristics of The Subject

Table 1. The Characteristics of respondents

Characteristics	Full Breastfeeding n=19		Mixed Feeding n=19	
	n(%)	Mean±SD	n(%)	Mean±SD
Sex				
Man	10 (52.6)		6 (31.6)	
Women	9 (47.4)		13 (68.4)	
Birth weight (gr)		3305±363		3126±369

Based on the table 1, there were no statistical differences between gender and birth weight of neonates who full breastfeeding and mixed feeding.

The Difference Secretory Immunoglobulin A between Faeces Sample of Full Breastfeeding and Mixed Feeding Infant

Table 2. Secretory Immunoglobulin A between faeces sample of full breastfeeding and mixed feeding infant

	Group		
	Full Breastfeeding n=19 Mean±SD	Mixed Feeding n=19 Mean±SD	p-value
sIgA age 0 day (ug/ml)	0.854±0.193	0.928±0.226	p=0.141
sIgA age 28 days (ug/ml)	0.783±0.154	0.383±0.209	p=0.000
Delta sIgA (ug/ml)	-0.071±0.171	-0.545±0.261	p=0.000

There was no difference in average levels of fecal sIgA age 0 day between infant who got breastfeeding and formula with p-value > 0.05. There was a difference in average levels of fecal sIgA in age 28 days between infant who got full breastfeeding and mixed feeding with p-value < 0.05. There was a difference in average delta levels of fecal sIgA between both groups with the p value < 0.05.

DISCUSSION

The Difference Secretory Immunoglobulin A Age 0 Day between Faeces Sample of Full Breastfeeding and Mixed Feeding Infant

Based on the statistical tests the p-value = 0.141. This means that there is no significant difference in the levels of fecal secretory immunoglobulin A between the neonates who received only breastfeeding and the one who had not breastfeeding only.

There is no difference in the level of sIgA in both groups because the neonatal stool at age 0 day taken after the baby gets the intake of breastfeeding only or a mixture of breastfeeding and formula. At the age of 0 day the neonatal was yet to produce the immune system itself, so the all components of immune system in neonates was received from mothers. After birth the neonates got the breastfeeding which contained complete antibodies, so there is no difference in the levels of sIgA age 0 days because the neonates hasn't been able to produce endogen sIgA and the only sIgA source was breastfeeding[(8)].

The breastfeeding sIgA role in the early stages of life was in the maturing process of the digestive tract epithelium. The commensal bacteria associated directly with the GALT development such as lymphoid follicles or secretion of sIgA with unknown specification called natural sIgA. The existence of sIgA can initiate colonization of microbiota and allows the development of the immune system in a non-inflammatory conditions. In normal conditions the commensal bacteria can be recognized by TLR, the interaction seems to be important to maintain the intestinal epithelium homeostasis[(8)].

Breast milk contains epithelial growth factor that stimulates the maturation of gastrointestinal barrier so it can inhibit the penetration of microorganisms as well as macromolecules. Breastfeeding stimulates the mucosal immune system to produce secretory IgA, which is a marker for the maturation of the immune system and may provide protection against environmental antigens. TGF-β induced over the isotope IgA and is found in breast milk. TGF-β in breast milk stimulates the production of IgA in infant[(8.10).]

The Difference Secretory Immunoglobulin A age 28 days between faeces sample of full breastfeeding and Mixed feeding infant faeces

The results of the statistical tests obtained the p-value = 0.000. This means that there was a significant difference of fecal secretory immunoglobulin A levels between the neonates who received full breastfeeding or mixture breastfeeding. The level of fecal sIgA in neonates who received full breastfeeding was higher compared to neonates who got the mixture breastfeeding.

The babies who got the breastfeeding has more secretory immunoglobulin A levels than the babies who got the formula milk because the fecal secretory immunoglobulin A was influenced by the composition of breastfeeding which is rich in secretory immunoglobulin A, the growth factors and the bifidogenic factors such as oligosakarida. During the 28 days the Epidermal Growth Factor (EGF) can improve the maturation of gastrointestinal barriers to prevent the penetration of pathogenic agent to the intestinal mucosal epithelium. Bifidogenic factors can increase local commensal bacteria colonization which is useful for the maturation of neonatal immune system. Also, there is already a sIgA in breastfeeding that can improve the intestinal mucosa's immune system. That caused the difference between the sIgA levels of baby who got full breastfeeding and mixed

breastfeeding. Immunoglobulin in breast milk cannot be imitated by formula milk. Breastfeeding contained molecules with the antimicrobial activity and also the probiotic bacteria Lactobacillus gasseri and Lactobacillus fermentum[8.11].

This study is in line with a study conducted to baby aged under 2 months, which the result of the study showed that fecal sIgA level in exclusive breastfeeding group is significantly higher than formula milk-feeding group[12].

There is difference of baby's fecal sIgA level in both groups caused by the decreasing of breastfeeding practice in mix-feeding group, led to decreasing of breastfeeding intake which is rich of immunoglobulin A secretory. Mix-feeding group who received formula milk had risk to exposed by pathogen bacteria which could affect baby mucosal immunity. A study by Cooke el al., [13] in sample aged 0-5 days and 5 weeks, showed that in baby aged 6 weeks, Escherichia coli bacteria was more common to find in baby who consumed formula milk ($p = 0.042$), while Bifidobacterium was more common to find in GI tract of breastfeeding baby.

Breast milk provides living bacteria sources which could help forming intestinal microbiota at early stage. There is mutualism relationship between intestinal IgA and commensal intestine bacteria, that is microbe exposure could stimulate IgA production on babies, and subsequently IgA will regulate the composition of baby microbiota. The production of IgA on formula milk-fed baby is slower and has lower level in first year of life[4,7]. Exclusive breastfeeding group had higher fecal sIgA level because baby just received breast milk without other complementary food/drink. In other hand, breast milk sIgA could also stimulate neonates immune system.

The Difference Delta Secretory Immunoglobulin A between Faeces Sample of Full Breastfeeding and Mixed Feeding Infant

Based on statistical analysis, we got p-value = 0.000. It means there is significant difference of the delta of immunoglobulin A secretory level between breastfeeding neonates faeces and mix feeding neonates faeces. The delta of sIgA level in breastfeeding neonates faeces is higher tan sIgA level in mix-feeding neonates.

Delta of fecal sIgA in both groups were negative that showed there were the decrease of fecal sIgA level. The decrease of fecal sIgA level on exclusive breastfeeding group was lower than mix-feeding group. This decrease was due to the amount of sIgA id different in every lactational stage. In colostrums breast milk, the amount of sIgA was 335.9 mg/100ml, while in transitional breast milk, sIgA was not found, and in mature breast milk, sIgA level decreased into 119.6 mg/100ml[11].

Delta of neonates fecal sIgA level who received who received exclusive breast milk was higher than sIgA level of mix-feeding neonates because the primary source of sIgA for neonates is breast milk sIgA. The factors affecting baby's intestinal mucosal immune system are nutrition, microbe, and maternal immunity. The exposure of breast and formula milk during neonatal ages will affect sIgA level. Exclusive breastfeeding neonates will receive more breast milk sIgA compared to mix-fed neonates. Intestinal microbiota can ferment carbohydrates, so that it will produce lactate acid that will led the intestinal PH became more acid. The acid environment formed was the signal to produce baby mucosal sIgA[4,8].

This study is in line with a study conducted by Bridgman et al[7], which showed that there was correlation between fecal IgA level of babies aged 4 months, breastfeeding status, and exposure of before/after birth. Babies who received breast milk and newborn babies have higher average of fecal IgA level (23.11 ± 9.34 μg/g and 22.19 ± 8.23 ug/g, p-value = 0.04).

The same result was also found by Man-Chin et al[15] who performed fecal sIgA level examination for babies who received breast milk and who received formula milk at the age 5 days, 2 months, and 4 months. Breastfeeding babies had significantly higher fecal sIgA level compared to babies who received formula milk (p-value < 0.05). Fecal sIgA level decreased at the age of 5 days to 2 months, and then increased at the age of 4 months. The increasing at the age of 4 months showed that babies have already had the ability to produce sIgA endogenly. Breast milk composition changes and varies among mothers[16]. Colostrums breast milk is rich of immunology component and growth factor. Colostrums can affect development and maturity of baby's mucosal immune system. Generally, breast milk gives protection from infection.

Intestinal sIgA secretion does not occur during neonatal ages, but starting to increase at the age of 4 to 12 months[3]. IgA secretory is an important component in mucosal immune response which protecting intestinal tract form infection. There are several factors affecting baby's intestine mucosal system, include nutrition, microbe, and maternal immunity. Baby's immune system is very dependent to maternal factors. After birth, babies receive IgA from colostrums breast milk, so breast milk immunity factor can directly act at the surface of baby's intestinal mucosa. Exposure of mother's commensal bacteria antigene to newborn babies is transfer aspect of mother's immunity system to the babies. This exposure can occur through genitalia tract during vaginal delivery and during skin contact between mothers and babies (mainly when breastfeeding). These factors are expected to affect the development of baby's microbiome and change baby's intestine mucosal immunity system[4].

Babies who already received breast milk have higher fecal IgA level. There is correlation between exclusive breastfeeding and baby's fecal sIgA level, so that sIgA level of breastfeeding babies are different to

babies who received formula milk. Higher fecal sIgA level is followed by increasing of breastfeeding babies amount, because breast milk is the primary source of IgA for babies during several first months[8,11].

CONCLUSION

Levels of secretory Immunoglobulin A full breastfeeding infants faeces was higher than mixed feeding infants.

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