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ROLE OF EXCLUSIVE BREASTFEEDING AND S-IGA ANTIBODIES ANTIROTAVIRUS BREAST MILK TOWARDS RISK OF ACUTE ROTAVIRUS DIARRHEA IN INFANTS AGE OF 1-6 MONTHS: DO THEY CORELATE TO BREASTFEEDING "DAILY DOSE" AND ANTIBODY TITERS?

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ABSTRACT

Exclusive breastfeeding reduces the incidence of diarrhea, especially in children who live in densely populated neighborhood. This study aims to determine the relationship between exclusive breastfeeding and breast milk contains antirotavirus s-IgA antibodies towards risk of acute rotavirus diarrhea in infants aged of 1-6 months. Case-control study design is applied to determine the relationship between exclusive breastfeeding and breast milk contains s-IgA antibodies antirotavirus with risk of acute rotavirus diarrhea. Cases in this study were patients with acute rotavirus diarrhea and controls were patients without acute rotavirus diarrhea. Cases and controls were matched based on age. There were 23 cases and 69 controls. The proportion who received exclusive breastfeeding was 34.8% in cases and 34.4% in controls, with OR of 1.21 (95% CI: 0.45 to 3.28) and p = 0.28. Breast milk contains sIgA antibodies antirotavirus for case was 17.39% and controls was 23.2%, OR was 1.12 (95% CI: 0.29 to 4.29), *p* = 0.203. In conclusion, exclusive breastfeeding and breast milk contains sIgA antibodies antirotavirus were not associated with risk of acute rotavirus diarrhea in infants 1-6 months. This may be caused by differences in population and demographic studies as well as low of milk sIgA antibody antirotavirus titters. Further research of breastfeeding regardless of antirotavirus containing high antibody titters sIgA is needed.

Keywords: exclusive breastfeeding, antirotavirus s-IgA antibodies, the risk of acute rotavirus diarrheal.

INTRODUCTION

Breast feeding, especially exclusive breast feeding is one of strategy to fulfil adequate nutrition, to prevent illness and death from diarrhea. These were related to breast milk nutrition, reduction of exposure to microbial pathogens and the presence of immune substances of milk. One of the primary antibodies is milk secretory immune globulin-A (s-IgA) antibody that has an ability to bind to antigene of microbial pathogens. This antibody also prevent adherence of microbe to intestinal mucosa, so that prevent the occurrence of diarrhea.[1,2,3]

Rotavirus is the most common cause of acute treated diarrhea to children aged below 5 years. the Prevalence of this diarrhea is on the ranges between 40-56%.[4,5,6] Rotavirus can be transmitted through contaminated food and beverage, as well as direct contact with people infected with rotavirus.[7] Acute rotavirus diarrhea occurs via several mechanisms. First mechanism, rotavirus replicates in intestinal villus epithelial cells which leads to damage of 'absorption' cells and resulted in secretion of excess of water and electrolyte absorption into small intestine lumen.

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Second mechanism, enzymatic disaccharides epithelial cells damaged which leads to osmotic diarrhea because of indigestive disaccharide. Third mechanism, stimulation of enteric nervous as a results of rotavirus antigen cell adhesion to enterocytes which leads to rise of consequences cascade of signals within the cell. This situation results in a variety of cytokines secretion that triggers secretory diarrhea.[8-10]

Breast milk acts as a carrier of passive immunity in infants who have breastfed, when local immune defense system in infants is still immature. Substance immune milk may serve as a factor in the innate and adaptive immune system, one of which is an S-IgA antibody bind to microbial pathogens in the lumen of the small intestine. S-IgA is the result of sensitization of B-lymphocyte cells in breast milk nodes "Peyer patches" by microbial pathogens that attack the intestinal mucosa of lactating mothers. B-lymphocyte cells that have been sensitive migrate through the blood circulation and lymph flow, and finally arrived at the mammary gland. Following B-lymphocyte cells turn into plasma cells which then produce antibodies and IgA secreted into breast milk in the form of S-IgA. S-IgA milk will have the specific properties of microbial pathogens that enter the small intestine into the lumen.[2,11-13]

Some results of previous studies showed that transfer antibodies from mother to baby through

placenta and breast milk. The mothers who deliver in New York public hospital, 30% had been infected with rotavirus group C and immunity and transferred to the baby through the cord bloodstream. In the post partum mothers whose colostrums was examined, 40% contain antirotaviral s-IgA antibodies, this could be a carrier of passive immunity in infants who have breast fed.[14,15]

The relationship between breastfeeding and acute diarrhea in children has been reported by several researchers. Exclusive breastfeeding until the baby 4 months old prevent diarrhea caused by rotavirus.[13,16] Breastfeeding anticholera contains of s-IgA antibodies prevent acute diarrhea caused by infection of cholera, but does not prevent bacterial colonization of the intestinal mucosa.[17] Intake of concentrated milk containing antibody antirotavirus s-IgA obtained from cow's milk "hyperimunized" shorten duration of rotavirus excretion in children treated for acute rotavirus diarrhea. This does not occur for non hyperimmunized concentrated dairy cows (low titters of sIgA antirotavirus).[18]

METHODS

This was an observational matched pair case-control study to determine the relationship between exclusive breastfeeding and breast milk contains antirotavirus s-IgA antibodies towards risk of acute rotavirus diarrhea in infants aged of 1-6 months. Affordable population is infant suffering from acute diarrhea and has breastfeeding, visit Sanjiwani Gianyar General Hospital and private practice. As inclusion criteria were a baby aged 1-6 months, full-term spontaneous birth with birth weight≥ 2500 g, has a good nutritional status, and suffers no major congenital abnormalities that can interfere with breastfeeding. Subjects are excluded if the mother suffered from severe malnutrition. The subjects who participated in this study were proven with inform consent signed by their parents.

Anamnesis, physical examination, and laboratory tests were conducted for selected samples. Laboratory tests consist of stool infant's feces and breast milk. Feces examination was carried out using "capture ELISA" to find rotavirus antigen.[19] Breast examination was performed using ELISA techniques, S-IgA antirotavirus antibody found positive if the titer observed around 28 units or more.[20] Based on feces examination, it was found two type of diarrhea, i.e. rotavirus diarrhea and non-rotavirus diarrhea, therefore, the samples were grouped into two groups. In both groups, infant's age, sexes, nutritional status, number of relatives live in one house, maternal age, parity, maternal nutritional status, education level, employment status and personal hygiene were registered.

Descriptive analysis is performed to assess the infant and maternal characteristics associated with risk of acute rotavirus diarrhea. Statistical analysis, "Mantel-Haenszel" was performed to assess the correlation between exclusive breastfeeding and breast milk containing antirotavirus s-IgA antibodies. Analytical results expressed as odds ratio (OR) with 95% of confidence interval (CI) and 5% of significance level. Correlation was considered significant when OR > 1 or <1 not including one with a value of p <0.05.

RESULTS

The study was carried out during period of November 2008 until January 2011, with 161 collected samples and 148 of them were eligible to be examined. The stool of 23 samples was positive of rotavirus antigen and 125 samples were not containing rotavirus antigen. In other words, there were 23 children suffering from acute rotavirus diarrhea and 125 children suffering from acute non-rotavirus diarrhea. Therefore, 23 children were grouped into case group and 69 children with non-rotavirus diarrhea (a control group).

Characteristics of the subjects

Subject characteristics of these two groups include age, sex, mean body weight, and number of brothers and sisters. Based on statistical analysis, the results showed no significant difference between the two groups. Similarly, characteristics of the subjects (mother), which consists of maternal age, number of parity, education level, employment status and personal hygiene in the two groups showed no significant difference (Table 1).

Characteristics of exclusivity of breastfeeding, feeding frequency, duration of breastfeeding, exclusive breastfeeding and longer antirotavirus sIgA antibodies in breast milk in patients with acute diarrheal were listed in Table 2.

The association between exclusive breast-feeding with risk of acute rotavirus diarrhea

In this study, subjects who received milk breast exclusively formed by eight children (34.8%) in the group of cases (rotavirus diarrhea), and 43 children (34.4%) in the control (rather than rotavirus diarrhea) group. The proportion of subjects who received exclusive breastfeeding in the two groups showed no significant differences. Based on the results of analysis of statistical value obtained from OR 1.21 (CI 95%: 0.45 to 3.28), p = 0.28 (Table 3).

The Association between breast milk contains s-IgA antibodies antirotavirus and risk of acute rotavirus diarrhea

In this study there were 4 samples (17.39%) antirotavirus milk contains s-IgA antibodies in the case group, whereas in the control group were 29 samples (23.2%). Of all breast milk samples tested contained 22% antirotavirus contains s-IgA antibodies. In the

group of subjects who received exclusive breastfeeding means antirotavirus milk sIgA antibody titters reached 30.64 ± 12.88 units.

Table 1
The relationship between risk factor characteristics of subjects Infants and Mothers with acute rotavirus diarrhea

Characteristics of Subjects	RV Acute Diarrhea n = 23	Non RV Acute Diarrhea n = 69
Infant Age		
1 – 2 months	3 (13.0%)	8 (12.0%)
2 – 3 months	3 (13.0%)	11 (15.2%)
3 – 4 months	6 (26.1%)	15 (21.7%)
4 – 5 months	4 (17.4%)	14 (20.3%)
5 – 6 months	7 (30.4%)	21 (30.4%)
Sex		
Male	11 (47.8%)	35 (50.7%)
Female	12 (52.2%)	34 (49.3%)
Infant's body weight (kg)		
Mean	6.58	6.67
SD	1.30	1.38
Total siblings		
No siblings	12 (52.2%)	37 (53.6%)
One sibling	8 (34.8%)	23 (33.4%)
Two or more siblings	3 (13.0%)	9 (13.0%)
Mother's Age		
Mean	28.22	28.51
SD	6.48	5.17
Parity		
1	12 (52.2%)	37 (53.6%)
2	8 (34.8%)	23 (33.4%)
3	3 (13.%)	9 (13.0%)
Level of education		
Completed SD	2 (8.7%)	5 (7.2%)
SLTP	5 (21.7%)	15 (21.7%)
SMU	12 (52.2%)	34 (49.3%)
PT	4 (17.4%)	15 (21.7%)
Occupation		
At home	7 (30.4%)	41 (59.4%)
Out of home	16 (69.5%)	28 (40.6%)
Personal hygiene		
Washes hands frequently	3 (13.0%)	11 (15.9%)
Seldom washes hands	12 (52.2%)	32 (46.4%)
No/Very seldom washes hands	8 (34.8%)	26 (37.7%)

Note: RV= rotavirus

While on the subject group receiving a non-exclusive breastfeeding, mean antibody titters of s-IgA milk reaches antirotavirus 21.00 ±15.45 units. Based on statistical analysis, no differences in the proportion of milk contains s-IgA antibodies antirotavirus between the two groups of subjects, OR 1.12 (95% CI: 0.29 to 4.29) and p-value = 0.203 (Table 4).

DISCUSSION

Exclusive breastfeeding associated with lower incidence of diarrhea in children under the age of 6 months. Some results of previous studies show an exclusive breastfeeding reduces the incidence of acute diarrhea that needs to be treated and the effect is more pronounced in children who live in dense settlements places.[1] Cohort studies were conducted in children aged of 0-8 months showed the effects of diarrhea, exclusive breastfeeding and the prevention of diarrhea that needs to be treated two times greater than the effect of the non-exclusive breastfeeding and four times greater than formula feeding.[21,22] Therefore, exclusive breastfeeding associated with decrease exposure to microbial pathogens that may be derived from dense neighbourhoods. Furthermore, breastfeeding, exclusively in relation to the prevention of diarrhea, the best effect compare to non-exclusive breast milk or infant formula, especially in children who live in dense neighbourhoods.

Table 2.
Characteristics of exclusivity of breastfeeding, feeding frequency, duration of breastfeeding.

Parameter	Rotavirus Acute Diarrheal (n=23)	Non-rotavirus Acute Diarrhea (n=125)
Exclusivity of breast-feeding:		
• Exclusive ASI	8 (34.8%)	43 (34.4%)
• Non exclusive ASI	15 (65.2%)	82 (65.6%)
• Feeding frequency	8.65±2.82	8.61±2.95
• Feeding frequency (minute)	15.65±7.73	16.22±8.79
• Exclusive feeding frequency (day)	116.6±48.27	104.6±45.45
Ab-sIgA antirotavirus of breastfeeding:		
• Positive	4 (17.39 %)	29 (23.2 %)
• Negative	19 (82.61 %)	96 (76.8 %)

The results of this study indicate differences to previous research, which is caused by several factors. First, there are differences in terms of housing density. In the subjects studied, more than fifty percent do not have relatives living in one house, so that his residence is not among dense occupancy. Second, effects-exclusive breastfeeding in this study were compared between rotavirus diarrhea and rotavirus diarrhea instead. As it is known that exclusive breastfeeding has a preventive effect against diarrheal disease caused by infection by various pathogens. [23] In other words, exclusive breastfeeding can prevent diarrhea caused by both rotavirus and rotavirus are not, so the difference in the level of protection against both exclusive breastfeeding is not so visible. Thirdly, demographic differences. Research carried out in several places with different levels of residential density, using the criteria of "Jarman", certainly not you can compare with this study.[1]

Level of protection against infectious diseases in the best-exclusive breastfeeding and predominant

breastfeeding breastfeeding, not associated with social status and covers all levels of society.[24.25] Non-exclusive breastfeeding consists of dominant breast milk, breastfeeding is balanced by the number of infant formula, and breastfeeding less than formula milk. In this study the non-exclusive breast milk is the dominant category occupying the largest portion. Therefore, the protective effect of breastfeeding against rotavirus diarrhea, which is categorized into exclusive breastfeeding, non-exclusive and will not show different results. For the record, research Quigley et al and Talayero et al,[1,22] the protective effect of exclusive breastfeeding, diarrhea that need to be treated than those who did not breast milk (formula milk only), show large differences in effects. So, the difference in the degree of protection associated with the frequency of breastfeeding is not directly related to frequency of exposure to microbial pathogens.

The average time feed exclusively on diarrhea rotavirus group and not by rotavirus, is almost less or same than 4 months, and therefore increase the incidence of diarrhea rotavirus or higher at the age of 4 months. This suggests that the protective effect of exclusive breastfeeding is more closely related to the "daily dose" of breastfeeding, in which a high frequency of breastfeeding related to less exposure to microbial pathogens.

Table 3.
The relationship between exclusive breastfeeding and the risk of acute rotavirus diarrhea (Mantel-Haenszel analysis)

Pairing	Frequency (N)	Percentage (%)
Post kr,total kr post=0	2	8.7
Post kr,total kr post=1	4	17.4
Post kr,total kr post=2	3	13.0
Post kr,total kr post=3	1	4.3
Neg kr,total kr post=0	3	13.0
Neg kr,total kr poat=1	7	30.4
Neg kr,total kr post=2	2	8.7
Neg kr,total kr post=3	1	4.3
Total	23	100

Note
Ks post: diarrhea rotavirus + Exclusive breastfeeding
Ks neg : diarrhea rotavirus + Non exclusive breastfeeding
Kr post: diarrhea non rotavirus + Exclusive breastfeeding
Kr neg : diarrhea non rotavirus + Non exclusive breastfeeding
Value of X2-Square Mantel-Haenszel is RO 1.21 (CI 95 %: 0.45-3.28) ,p= 0.28

The relationship between exclusive breast feeding, the risk of acute rotavirus diarrhea has been reported by several researchers. A case-control study in children aged <6 months treated for diarrhea compared to patients with untreated childhood diarrhea showing the relationship between exclusive breastfeeding, the risk of severe acute rotavirus diarrhea.[16] Kurugol, et al using the same method to compare patients with acute rotavirus diarrhea treated with the children cared for not because of diarrhea associated with exclusive breastfeeding.[26] Results showed that, there is a relationship between exclusive breastfeeding and the risk of rotavirus diarrhea. Different results reported by Wobudeya et al,[27] where exclusive breastfeeding, breastfeeding and non-exclusive breastfeeding is not the main categories associated with the incidence of rotavirus diarrhea. Prospective study in children aged <24 months showed that the incidence of rotavirus diarrhea was lower in children who received exclusive breastfeeding. But the proportion who received exclusive breastfeeding is very low, not representative enough to assess the strength of protection.[28]

Table 4.
The relationship between breastfeeding antirotavirus Ab-containing milk sIgA and Risk of acute rotavirus diarrhea (Mantel-Haenszel analysis)

Pairing	Frequency (N)	Percentage (%)
Ks pos,total.pos kr=0	1	4.2
Ks pos,total.pos kr=1	3	12.9
Ks neg,total.pos kr=0	13	56.4
Ks neg,total.pos kr=1	4	17.4
Ks neg,total.pos kr=2	2	8.7
Total	23	100

Note
Ks pos: diarrhea rotavirus + Ab-sIgA positive
Ks neg: diarrhea rotavirus + Ab-sIgA negative
Kr pos: diarrhea nonrotavirus + Ab-sIgA positive
Kr neg: diarrhea nonrotavirus + Ab-sIgA negative
Value of X2-Square Mantel-Haenszel is RO 1.12 (CI 95 %: 0.29-4.29), p= 0.203

However, Misra et al[29] by the same method, found no difference in the incidence of rotavirus diarrhea between exclusive breastfeeding and non-exclusive. Based on the results of these studies, the protective effect of exclusive breastfeeding on risk of acute rotavirus diarrhea varies depending on the method and criteria used for the subject. But there are similarities, that the protective effect of breastfeeding is even more evident in acute rotavirus diarrhea that requires treatment.

This study is a retrospective study, subjects were patients with mild acute rotavirus diarrhea by age range 1-6 months. In a retrospective study the possibility of bias derived from the data pattern of breastfeeding. Differences can also be caused by factors that control in this study was patients with rotavirus diarrhea. As already mentioned above that exclusive breastfeeding affect the overall incidence of infectious diarrhea. With the use of rotavirus diarrhea is not as a control, different levels of protection, exclusive breastfeeding will not be visible. The results

of this study indicate that the proportion who receives exclusive breastfeeding compared with those receiving non-exclusive breastfeeding in both groups on the same subject.

Antirotavirus milk antibody titters in acute rotavirus diarrhea s-IgA group who achieved a positive result was 17.39%, whereas in the non-rotavirus acute diarrhea 23.2%. There was no significant difference based on statistical calculations, OR 1.12 (95% CI: 0.29 to 4.29), p = 0.203. The average antibody titters in breast milk sIgA antirotavirus given exclusively to 30.46 (containing the rotavirus positive results when the ELISA titter> / = 28 units). In younger women with a smaller amount of parity, breast milk contains sIgA antibodies have a higher. [30] But research Henart et al [31], sIgA antibody levels were higher in older mothers with a large number of parity. In this research studied sIgA antibodies was associated with rotavirus. Most maternal age <30 years with parity mostly first-time mom, the results indicate antirotavirus sIgA antibody titters are low. So the difference in the results with previous studies because of differences in study subjects.

Breastfeeding contains sIgA antibodies specific for a particular microbe, can serve as an immunization to prevent diarrhea caused by microbes. Milk sIgA antibodies include antibodies neutralize microbial pathogens in the intestinal lumen of infants. If rotavirus antigen antibodies specific for rotavirus, which can be prevented then it is mounted so that diarrhea that does not happen.[32,33]

The relationship between the provisions of antirotavirus sIgA antibodies to the prevention of acute rotavirus diarrhea has been reported by Corthesy et al.[34] Using experimental animals given monoclonal antibody sIgA via oral, cannot prevent rotavirus replication in host cells. Feng et al [35] requires an antibody with a high level to achieve high protection. This suggests that the dose and mode of administration affects the level of protective antibodies. Type-specific antibodies against rotavirus protein VP4 and VP7, is the highest level of antibody protection when administered orally.[34,36]

Giving milk concentrate derived from cow's milk "hyper immunized" can reduce the duration of rotavirus excretion in children treated for acute rotavirus diarrhea. The results differ significantly on the provision of milk concentrate containing antibody antirotavirus sIgA antibody titters to "neutralize" low.[18] So in the supply of breast milk with the aim of prevent of acute rotavirus diarrhea in children, it takes antirotavirus milk contains sIgA antibodies with high titters, and or have a broad spectrum and specific nature of the rotavirus protein VP4 and VP7.

In this study the average antibody titters of sIgA antirotavirus breastfed infants who received exclusive breastfeeding is very low. This is one cause of exclusive breastfeeding in this study had no effect on the risk of acute rotavirus diarrhea. Other factors that could cause the level of specificity as the antibody "neutralize" milk is not examined in this study.

CONCLUSION

Exclusive breastfeeding in order to reduce the risk of diarrhea from severe rotavirus in children 1-6 months, the mechanism occurs through a reduction in the level of exposure to microbial pathogens. Protective effect of exclusive breast-feeding in the prevention of diarrhea in children 1-6 months and more pronounced in children who live in the dense residential area. Breast milk contains antibodies IGA antirotavirus will have a preventive effect against diarrhoea rotavirus when fairly high titter and has a wide range of properties and is specific or against rotavirus VP7 and VP4 protein.

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