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Article

COMPARISON OF SERUM ALBUMIN LEVELS IN THE BREAST MILK OF BREASTFEEDING INFANTS AGED 1-3 MONTHS AND 4-6 MONTHS

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Abstract: (1) Background: Serum albumin is the most abundant plasma protein in the blood, contributing to maintaining colloid osmotic pressure and binding substances that are poorly soluble in plasma so that they can be distributed throughout the body. Serum albumin levels in breast milk can vary, influenced by various factors such as the lactation phase (age of the baby), number of parities, age and body mass index (BMI) of the mother. This study aims to determine the comparison of serum albumin levels in breast milk of mothers who breastfeed infants aged 1-3 months and 4-6 months and to find the relationship with the number of parities, age and BMI of the mother. (2) Methods: Cross-sectional design experimental study, breast milk samples as stored biological fluids were obtained from 58 mothers at the Petamburan and Cilincing Health Centers. Serum albumin levels were measured with the Bromocresol Green (BCG) kit. (3) Results: The results showed that breast milk in the earlier lactation period, namely at 1-3 months, had significantly higher serum albumin levels compared to the serum albumin levels in the 4-6 months age group (p=0.002). Serum albumin levels in breast milk for infants aged 1-3 months did not correlate with mother's parity (p=0.428) and mother's age (p=0.881), but had a significant positive correlation with mother's BMI (p=000). Serum albumin levels in breast milk in the 4-6 months age group did not correlate with mother's parity (p=0.823) and mother's age (p=0.581) but had a strong positive correlation with maternal BMI (p=0.000). (4) Conclusions: Breast milk serum albumin levels are affected by the lactation phase (age of the baby), namely at the age of 1-3 months the baby increases significantly compared to the age of 4-6 months. The level of serum albumin in breast milk is related to the mother's BMI, which increases with increasing mother's BMI.

Keywords: Breast milk; Infants aged 1-3 months; Infants aged 4-6 months; Serum albumin levels; Maternal age; Parity; Maternal BMI

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1. Introduction

Breast milk is a dynamic, complex biological fluid that is designated as the gold standard in infant nutrition. Breast milk is uniquely shaped according to the needs of each baby at a certain time, both in nutritional composition and non-nutritional bioactive factors. The World Health Organization (WHO) and other national advisory bodies from many countries actively support and promote programs of exclusive breastfeeding for the first 6 months of life as the only source of nutrition, and continued for 1 to 2 years or more and assisted with complementary foods, as a normative standard for the fulfillment of infant nutrition [1-10]. Breast milk has a dynamic composition which is influenced by many things, one of which is the lactation phase. Breastfeeding aims to meet the nutritional needs of infants according to their developmental period [1-8]. Exclusive breast-

feeding has many benefits for the health of infants, including prevention of infection, optimal development of the nervous system, may be able to limit the probability of occurrence of allergies, obesity, and diabetes in later day.

Breast milk can be divided into 4 periods: colostrum, transitional milk, mature milk, and involutional milk [5]. There are differences in the nutritional composition of breast milk in different lactation phases [2,5,11]. The first milk produced by the breasts after giving birth until about 5 days after giving birth is called colostrum [1,12]. Colostrum is produced in low quantities during the first few days after delivery, its composition is rich in immunoprotective components, this is because the antibodies passively obtained by the baby while still in the mother's womb are only temporary, and cannot last until Infants can form c components by themselves [1,5,10,12,13]. In addition, the concentration of fat and lactose is relatively low, which indicates that the main function of colostrum is to improve and maintain the immunology of the baby's body which is still vulnerable compared to nutrition [1,9,10,12]. Transitional milk has some of the characteristics of colostrum but is produced in larger quantities. In this case it aims to support the nutritional needs of babies who are in a period of growth and development [1]. At 4-6 weeks after delivery, breast milk will be considered fully mature. The composition of mature milk will remain relatively the same despite small changes during breastfeeding and is clearly different from the composition of breast milk in the first month of breastfeeding [1]. Involutional milk is produced during the late lactation phase [5].

The protein content in breast milk will vary in each phase depending on the baby's needs. Various kinds of protein contained in breast milk contribute to the dynamic quality possessed by breast milk. Protein content in breast milk is divided into 3 categories: whey, casein, and mucin complex [4,10]. Alpha-lactalbumin and serum albumin are the major proteins found in breast milk [14]. Serum albumin in addition acts as a modulator of plasma oncotic pressure, it also functions to transport various substances such as bilirubin, ions, fatty acids, and exogenous ligands such as drugs [15]. Serum albumin can also help insight into human liver function related biosynthesize proteins and factors vital to total body homeostasis [16,17,18]. Most proteins are synthesized by the mammary epithelium, but serum albumin is a protein that is obtained directly from the mother's blood circulation [6,7,10]. This protein is channeled from the mother's blood circulation to breast milk through blood-milk barrier. Tight junctions are important in the blood-milk barrier, because they play a role in maintaining milk secretion and preventing paracellular transport of ions and small molecules between the blood and breast milk compartments. The integrity of the tight junctions in the mammary gland epithelium will change throughout the lactation phase which is one of the causes of variations in the composition of breast milk [11]. Understanding the dynamic composition of breast milk is important, because it can be an indicator that helps in managing the feeding of infants who need more nutritional intake to meet their growth and development needs [1,2].

One of the factors that influence the comparison of serum albumin levels in breast milk is the lactation phase. The lactation phase reflects the baby's needs at certain times so that differences can be found in the composition of serum albumin contained in breast milk. However, it is not yet known whether the difference in serum albumin levels in breast milk increases with the age of the baby, and whether the total age parity and maternal BMI affect it. Therefore, this study aims to determine differences in mother's serum albumin levels in different infant age groups and to analyze the relationship between breast milk serum albumin levels on parity, age and mother's BMI.

2. Results

2.1 Serum Albumin Levels in Breast Milk for Infants aged 1-3 Months and 4-6 Months

The results of measuring serum albumin levels in the breast milk group for infants aged 1-3 months showed a median value of 5.22 mg/mL with the lowest and highest levels (2.87-14.27)mg/mL, while in the breast milk group infants aged 4-6 months obtained a median value of 4.04 mg/mL with the lowest and highest levels (1.69-11.67)mg/mL. The

distribution of the data was not normal (Kolmogrov-Smirnov; P <0.05), so the Mann-Whitney test was used to determine the difference between serum albumin levels in breast milk between groups of infants aged 1-3 months and 4-6 months. The results of the analysis showed that the serum albumin level of breast milk in the 1-3 months old group was significantly higher (P = 0.002) compared to the 4-6 months old group. The results of measuring breast milk serum albumin levels and statistical test analysis are shown in Table 1.

Table 1. Serum Albumin Levels in Breast Milk Group of Infants Aged 1-3 Months and 4-6 Months.

	Median (Minimum-Maximum) mg/mL	***P*-value[a]**
Serum Albumin Levels of Breastfeeding Mothers Infants Age 1-3 Months (n=29)	5,22 (2,87-14,27)	0,002*
Serum Albumin Levels of Breastfeeding Mothers Infants Age 4-6 Months (n=29)	4,04 (1,69-11,67)	

2.2 Subject Characteristics

To find out what factors affect serum albumin levels in breast milk, a correlation analysis of serum albumin levels with data on subject characteristics was carried out, namely the number of parities, age and maternal BMI. Table 2. describes the characteristics of research subjects.

Table 2. Subject Characteristics.

	Mother's Breast Milk Breastfeeding Babies Age 1-3 Months ***N = 29*** **(% (n/*N*))**	***Mother's Breast Milk Breastfeeding Babies Age 4-6 Months*** ***N = 29*** ***(% (n/N))***
Number of Parities		
• Primiparous	37.93% (11/29)	34.48% (10/29)
• Multipara	62.07% (18/29)	65.52% (19/29)
Mother's Age		
• 20-29 Years Old	55.17% (16/29)	68.97% (20/29)
• 30-39 Years Old	44.83% (13/29)	31.03% (9/29)
Maternal Body Mass Index During Pregnancy		
• Low Weight	non	6.90% (2/29)
• Normal	51.72% (15/29)	37.94% (11/29)
• Excess Weight	20.69% (6/29)	34.48% (10/29)
• Obesity Type 1	17.24% (5/29)	10.34% (3/29)
	10.35% (3/29)	10.34% (3/29)

2.3 Correlation Results of Breast Milk Serum Albumin Levels with Subject Characteristic Data

Breast milk serum albumin levels for infants aged 1-3 months and 4-6 months were correlated with data on the characteristics of the study subjects, namely: number of parities, age and maternal BMI. The results of the correlation test of serum albumin levels in the group of infants aged 1-3 months to the number of parities and the age of the mother obtained a negative correlation but not significant with weak strength. However, in the correlation test for the group of infants aged 4-6 months to the number of parity and mother's age, the results of a positive correlation were not significant with weak strength. The

results of the correlation test for serum albumin levels in the groups of infants aged 1-3 months and 4-6 months on the maternal BMI obtained a strong and significant positive correlation. These results are shown in Table 3.

Table 3. Correlation of Serum Albumin Levels in Breast Milk with Research Subject Characteristics

	Levels of Serum Albumin in Breast Milk for Infants Aged 1-3 Months N=29	***Levels of Serum Albumin in Breast Milk for Infants Aged 4-6 Months N= 29***
Mother's Age		
• Correlation (rs)	-0,029	0,107
• *P*-value[b]	0,881	0,581
Parity		
• Correlation (rs)	-0,153	0,043
• *P*-value[b]	0,428	0,823
Body Mass Index		
• Correlation (rs)	0,672**	0,685**
• *P*-value[b]	0,000*	0,000*

[b] Spearman correlation test
* significantly different
** strong correlation test

3. Discussion

3.1 Effect of Infant Age on Breast Milk Serum Albumin Levels

The results showed that the serum albumin of breast milk for infants in the 3-4 months age group increased significantly compared to the 4-6 months age group. These results are consistent with a cohort study conducted in Copenhagen which proved that protein concentrations tended to decrease with increasing age [8]. However, a study conducted by Bo Lonnerdal (1976) in infants aged 0-6.5 months showed that serum albumin levels in breast milk tended to be constant during the lactation period. There is a slight decrease in this, possibly caused by a decrease in the passive transport of serum albumin from mother to baby through the blood-milk barrier [6,7]. Previous researchers stated that the first few months of life, the composition of breast milk will contain more serum albumin. This is because serum albumin is a protein that functions to maintain the immune system in infants who have not been able to form their own immune cells [5,11,19].

3.2 Correlation between Total Parity and Levels of Serum Albumin in Breast Milk

Primiparous mothers are known to have higher levels of total protein and immunoprotein concentrations than multiparous mothers. In this study, a negative correlation was found with a weak and not significant relationship between parity numbers and breast milk serum albumin levels in the group of infants aged 1-3 months. There was also a negative correlation with a weak and insignificant relationship between parity numbers and breast milk albumin levels in the 4-6 months old group. The results of the correlation in the group of infants aged 1-3 months and 4-6 months were not significant, but the pattern as parity increased, the levels of serum albumin in breast milk decreased. This is in line with the results of a study conducted by Prentice (1996) which concluded that multiparous mothers tend to produce lower quality breast milk [7].

3.3 Correlation between Maternal Age and Breast Milk Serum Albumin Level

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Increasing maternal age is associated with a decrease in protein concentration. This is because at the age of 1-3 months babies are not fully able to form proteins that are useful in maintaining their body's immunity, one of these proteins is serum albumin [2,4,5]. The results of this study found a negative correlation with a weak but not significant relationship between mother's age with serum albumin levels in breast milk in the group of infants aged 1-3 months. There was also a positive correlation with a weak and insignificant relationship between maternal age and breast milk albumin levels in the 4-6 months old group.

The results of the correlation between breast milk serum albumin levels on maternal age in the group of infants aged 1-3 months, although not significant, are in line with the results of a study conducted by Prentice (1996) which concluded that older mothers tend to produce lower quality breast milk. However, the results of the correlation in the group of infants aged 4-6 months did not match the research by Prentice (1996). This is probably caused by the majority of the subjects of this study, the majority of whom are under 35 years of age.

4. Materials and Methods

Primary data was obtained through experimental research in the form of measurements of serum albumin levels in breast milk which were grouped into 2 groups, namely 1-3 months and 4-6 months. Secondary data is the number of parities, age and maternal BMI taken from previous studies.

Breast milk serum albumin levels were measured using a spectrophotometric with the Bromocresol Blue Kit. The initial step for measuring serum albumin levels in breast milk was a blank in the form of distilled water and 50 μL samples from each test group were put into a test tube and 10 μL of 1% acetic acid was added. Furthermore, all samples and blanks were centrifuged at 3500 rpm for 10 minutes. Supernatant of 5 μL from each sample and blank was taken and transferred to a new test tube which was labeled according to the name of the sample. 1250 μL of BCG reagent was added to each test tube and blank tube, mixed well and incubated for 10 minutes. The last step, the absorbance of the blank and all samples was read on the spectrophotometer with a wavelength of 628 nm. The results of measuring the absorbance value of breast milk serum albumin levels are calculated and converted into units of mg/mL, with the following formula:

$$Cu = \frac{Ru}{Rs}\ x\ Ks$$

Cu = Albumin concentration value (mg/mL)
Ru = Absorbance Test
Rs = Standard Absorbance
Ks = Albumin standard concentration

Primary data, namely serum albumin levels in infants aged 1-3 months and 4-6 months, and secondary data were processed using SPSS version 20.0. The normality test was carried out using the Kolmogrov-Smirnov test because the number of samples was more than 30 subjects. If the data distribution is normal, the t-test is used to compare serum albumin levels in infants aged 1-3 months and 4-6 months. Conversely, if the data is not normal, the Mann-Whitney test is carried out. Correlation test of albumin levels in breast milk for infants aged 1-3 months and 4-6 months to parity of number, age and maternal BMI uses the Pearson test if the distribution is normal, whereas if it is not normal the Spearman test is used.

5. Conclusions

Breast milk serum albumin levels are affected by the lactation phase (age of the baby), namely at the age of 1-3 months the baby increases significantly compared to the age of 4-

6 months. The level of serum albumin in breast milk is related to the maternal BMI, which increases with increasing maternal BMI.

Institutional Review Board Statement: This research has obtained ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia with Number: KET 007/UN2.F1/ETIK/ PPM.00.02/2020.

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