

# Alpha-Lactalbumin Levels in Breastmilk of Breastfeeding Mothers in Makassar City, Indonesia

Citrakesumasari<sup>1\*</sup> and Yessy Kurniati<sup>2</sup>

<sup>1</sup>Department of Nutrition, Faculty of Public Health, Hasanuddin University, Jl. Perintis Kemerdekaan, Tamalanrea Indah, Tamalanrea Subdistrict, Makassar City, South Sulawesi 90245.

<sup>2</sup>Public Health Study Program, Faculty of Medicine and Health, Alauddin Islamic State University Makassar, Jl. Sultan Alauddin, Romangpolong, Somba Opu Subdistrict, Gowa Regency, South Sulawesi 92113.

\*Corresponding Author E-mail: citeku@gmail.com

<https://dx.doi.org/10.13005/bpj/2570>

(Received: 20 October 2022; accepted: 01 December 2022)

Alpha-lactalbumin level is only one part of a complex component of HAMLET (Human alpha-lactalbumin made lethal to tumor cell) in breastmilk which became an anti-cancer perspective. This research aimed to measure the breastmilk alpha-lactalbumin levels in breastfeeding mothers according to a group of children under two years-old with assessing the nutritional status of the breastfeeding mothers. The research used analytical survey method with cross sectional study design and performed in Sudiang Raya and Sudiang Health Centers working areas in Makassar City. Research population consisted of all breastfeeding mothers in those areas. Samples were recruited purposively regarding to the inclusion criteria including mothers of breastfed children aged 2 weeks-24 years who lived in research area and approved the informed consent. We obtained 146 samples by this method and the parameters assessed were maternal and children characteristics, as well as the alpha-lactalbumin levels of the breastmilk. Data analysis were performed using SPSS according to the data types with significance of  $p < 0.05$ . The alpha-lactalbumin levels were measured using ELISA method in the Laboratory of Hasanuddin University Hospital. The results showed that alpha-lactalbumin levels were significantly different in each toddler levels, with the highest level was found in children <6 months old. However, alpha-lactalbumin levels were not significantly different in based on the maternal nutritional status. Further research is needed to investigate the correlation between alpha-lactalbumin levels with nutritional intake.

**Keywords:** Alpha-Lactalbumin; Breastmilk; Children Under 2 Years Old; Maternal Nutritional Status.

Exclusive breastfeeding is highly recommended by World Health Organization and American Academy of Pediatrics. Breastfeeding is the most effective method to maintain mother's health, as well as encouraging healthy growth and optimal development in children. Empowering and enabling women to breastfeed must be the key point of nation's effort to keep every children alive and to build a healthy, intelligent, and productive community.<sup>1</sup>

Breastmilk contains nutritional component such as carbohydrate, protein (in the form of alpha-lactalbumin) and fat. Protein contributed 22% of breastmilk components and 36% are composed of whey protein. Whey protein of the breastmilk is very benefitable on toddlers, especially those under 2 years old, regarding its smoother deposition compared to casein, which is why whey protein is so much easier to digest. One of the whey protein components is alpha-lactalbumin.<sup>2</sup>

The discovery of growth factor, cytokines, and heterogenous cell population (stem cells, probiotics, dan HAMLET complex (Human alpha-lactalbumin made lethal to tumor cell)) in the breastmilk has increasing the concern of studies on breastmilk as a natural medicine.<sup>3</sup>

Research reveals that alpha-lactalbumin concentrations are 37% higher in mature milk than in colostrum and increase during lactation even when the total protein concentration decreases.<sup>4</sup> The content of alpha-lactalbumin in breast milk shows a fairly large variation. This variation is related to factors such as lactation, and maternal nutritional status.<sup>5</sup>

A study conducted in India and Guatemala revealed that low protein concentrations in breast milk were influenced by poor nutritional status in breastfeeding mothers.<sup>6</sup> This is similar to a study that investigated the correlation between maternal nutrition and body composition with the nutritional quality of breast milk, the study showed that food intake did not affect the quality of breast milk, but maternal body composition had a strong correlation with the nutritional quality of breast milk.<sup>7</sup>

The Nutrition Status Assessment Report states that the prevalence of chronic energy deficiency in pregnant women in Indonesia 2017 was 14.8%. South Sulawesi Province is higher than the national figure, with the prevalence

reached 15.9%.<sup>8</sup> Based on data from the Makassar City Health Office, it was revealed that of the 24 regencies/cities in South Sulawesi, Makassar City had the most CED (Chronic Energy Deficiency) cases, including 3,373 cases in 2018 and it was also reported that out of 10 health centers in Makassar City, Sudiang Raya Health Center had the most cases of CED (218 cases, 16.12%).<sup>9</sup>

The benefits of the alpha-lactalbumin component in breast milk and the high cases of CED in the working area of the health centers were what encouraged researchers to investigate the differences of alpha-lactalbumin levels in CED and normal mothers based on the age group of under two years old in the working area of the Sudiang Raya and Sudiang Health Centers in Makassar City.

## MATERIALS AND METHODS

### Study Location and Design

This study was performed in Sudiang Raya and Sudiang Health Centers working areas in Makassar City using analytical survey method and cross sectional study design.

### Study Period

The research was conducted for two months, starting from June to August 2019, consisting of one month of field research and one month of laboratory research.



**Fig. 1.** The location of Research in Sudiang Raya and Sudiang Health Centres

### Study Population and Samples

The population in this study were all breastfeeding mothers in the Sudiang Raya and Sudiang Health Centers in Makassar City. 146 samples were recruited purposively according to the inclusion criteria which included breastfeeding children aged 2 weeks-24 months and lived in the study area and approved the informed consent. This research has been approved by the Ethics Committee of the Faculty of Public Health, Hasanuddin University Makassar number 8956/UN4.14.7/TP.01.02/2019 and protocol number 0411192162 dated November 18, 2019.

### Data Collection

Data on respondent characteristics including maternal age, education, and parity obtained through direct interviews with the subjects. Maternal nutritional status (CED or normal) was obtained by measuring the mother's arm circumference. Breastmilk was collected by using an electric breast pump which is carried out by the mother independently on one breast until the mother's breast feels empty. The breast milk samples were brought to the Laboratory of Hasanuddin University Hospital using a cool box. From these breastmilk samples, researchers only took 5-10 ml to assess the levels of alpha-lactalbumin using the ELISA method. To avoid bias in this study, all samples were collected at the same time (during the day).

The formula used to determine the sample size is the Dahlan formula (2013):

$$n_1 = n_2 = 2 \left[ \frac{(Z\alpha + Z\beta) S}{(X1 - X2)} \right]^2$$

### Information :

$n_1 = n_2$  = minimum sample size

$Z\alpha$  = standard deviation of alpha (type 1) error of 1% = 2,326

$Z\beta$  = beta standard deviation (type 2) error of 10% = 1.28

$X_1$  = mean value of oleic acid in normal mother's milk = 1.5 (Buts A, 2018)

$X_2$  = mean value of oleic acid in SEZ mother's milk = 0.75 (Buts A, 2018)

$S$  = standard deviation of oleic acid at = 0.36 (Buts A, 2018)

### Data Analysis

Differences in breastmilk alpha-

lactalbumin levels in mothers with CED and normal nutritional status were analyzed using the T-Independent and Kruskal Wallis tests and performed using the Windows version of the SPSS 24 program.

## RESULTS

### Sample Characteristics

Table 1 showed that the sample in this study was mostly aged 20-35 years (87.0%), graduated from high school (41.1%), without differences in CED and normal nutritional status (50%), the sex of children under two years was female (54, 8%) with the most age being >6-12 months (35.6%).

Table 2 shows that alpha-lactalbumin levels are highest in the breast milk of mothers who breastfeed children aged 0-6 months and the lowest at the age of >6-12 months. The levels of alpha lactalbumin differ significantly based on the age of the children under the age of two,

**Table 1.** Mother and Children Under Two Years Old Characteristics in Sudiang Raya and Sudiang Working Areas in 2019

Mother and Children Under Two Years Old Characteristics	Total	
	N	%
Maternal age		
<20 years old	1	0.7
20-35 years old	127	87.0
>35 years old	18	12.3
Maternal education		
Elementary	15	10.3
Junior high	35	24.0
Senior high	60	41.1
Diploma	18	12.3
Bachelor	18	12.3
Maternal nutritional status		
Chronic energy deficiency	73	50.0
Normal	73	50.0
Children's gender		
laki-laki	66	45.2
Perempuan	80	54.8
Children age		
0-6 months	46	31.2
6-12 months	52	35.6
12-24 months	48	32.9

Source: Primary data, 2019

both in CED mothers and in normal mothers. Breastfeeding mothers who are 0-6 months old with normal nutritional status have higher levels of alpha-lactalbumin. Meanwhile, in children aged >6-12 months and 12-24 months, levels of alpha lactalbumin in breast milk were found to be higher in CED mothers than in normal mothers. However, based on the nutritional status of the mother, in each age group the levels of alpha-lactalbumin were not significant.

**DISCUSSION**

Based on research conducted by <sup>10</sup> which compared the concentration of alpha-lactalbumin levels in nine countries including: Australia, Canada, Chile, China, Japan, Mexico, the Philippines, England and America, it was

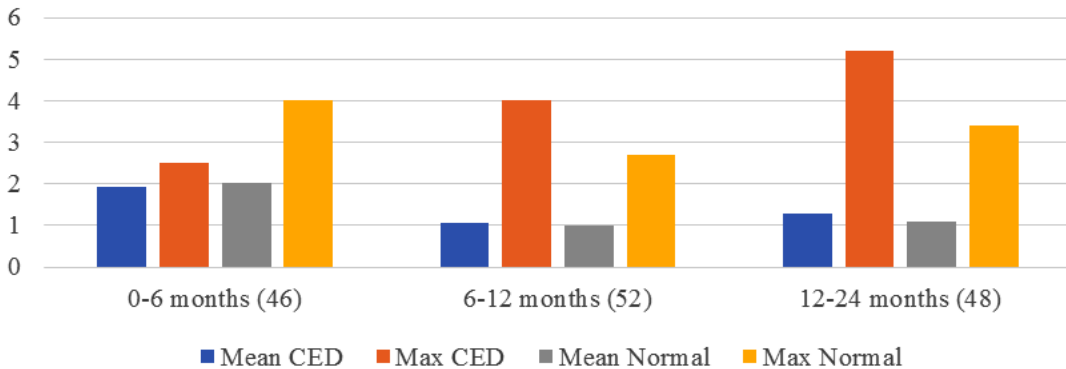
found that the United States had the highest levels of 4.0 g/ L and Mexico is the lowest at 2.0 g/L. Meanwhile, in this study, it was found that the levels of alpha-lactalbumin in breastfeeding mothers were still low, both in breastfeeding mothers with normal nutritional status and in CED nutritional status, namely the nutritional status of normal breastfeeding mothers reached 2.03 g/L (<6 months), 0.99 g/ L (6-12 months) and 1.1 g/L (12-24 months). In the nutritional status of CED breastfeeding mothers, alpha-lactalbumin levels reached 1.94 g/L (<6 months), 1.06 g/L (6-12 months) and 1.3 g/L (12-24 months). However, 4 g/L was also found in the samples. One study found that the content of alpha-lactalbumin in breast milk was associated with factors such as lactation and nutritional status of the mother, but the results of this study were based on maternal nutritional

**Table 2.** Comparison of Alpha-Lactalbumin Levels in Various Age Group Based on Maternal and Children Nutritional Status in Sudiang and Sudiang Raya Health Care Centers Working Areas in 2019

Children age (n)	Alpha-Lactalbumin Levels Based on Maternal Nutritional Status (gram/L)								P value
	CED				Normal				
	Mean	SD	Min	Max	Mean	SD	Min	Max	
0-6 months (46)	1.94	0.25	1.49	2.51	2.03	0.56	1.63	4	0.448*
6-12 months (52)	1.065	0.74	0.3	4	0.992	0.57	0.5	2.7	0.692*
12-24 months (48)	1.3	1.1	0.5	5.2	1.1	0.6	0.5	3.4	0.456*
nilai p		0,000**					0,000		

\*T-Independent    \*\*Kruskal Walis

**Alpha Lactalbumin Concentration in Makassar City**



**Fig. 2.** Graphics of Alpha Lactalbumin Concentration in Makassar City

status, in each age group of under two years the alpha-lactalbumin level was not significant.<sup>5</sup> The main factors that can affect the composition and quality of breast milk are the stages of lactation (colostrum breast milk, transition milk, mature milk) and premature birth.<sup>11</sup> This study only used mature breast milk and normal birth and normal birth weight. Research by Jura et al (2018) states that food intake factors do not affect the quality of breast milk, while maternal body composition has a close relationship with the nutritional quality of breast milk, but this study did not measure the body composition of breastfeeding mothers and intake of breastfeeding mothers was only limited to macronutrient intake which includes protein intake, where protein intake for breastfeeding mothers with normal nutritional status reaches 80% of the RDA while breastfeeding mothers with CED are 80% of the RDA.

Another study conducted in Egypt showed similar results, it was found that the volume and quality of macronutrient breast milk produced by malnourished mothers were not much different from well-nourished mothers.<sup>12</sup> Another study conducted on Nutrition Recommendations in Pregnancy and Lactation explained that weight loss during breastfeeding, BMI, body fat percentage, and weight gain during pregnancy did not affect breast milk production and did not affect the quantity or quality of breast milk.<sup>13</sup>

Previous research has also revealed that although determinants of breastfeeding mothers such as intake and good nutritional status can affect breast milk composition, most studies have found a weak or no relationship of such parameters on breast milk composition.<sup>14</sup>

The composition of breast milk varies greatly among mothers and even within a single breast milk. This multidimensional variation in composition is believed to reflect the needs of children under two years of age, geographic area, and food supply. Variations in breast milk composition between mothers are reported as a response to cultural differences such as diet and lifestyle factors as well as environmental factors such as soil mineral content which is then reflected in the mineral density of the food grown there, and the presence of human genetic differences.<sup>15</sup>

Alpha-lactalbumin is the largest part of the total protein in breast milk with a concentration

of 10-20%, where alpha-lactalbumin plays a major role in lactose synthesis, an activator in the movement of water from circulating maternal nutrients through breast milk, and a rich source of essential amino acids for the growth and development of toddlers.<sup>10</sup> Recently, studies related to the anticancer properties of breast milk have shown that HAMLET is a complex relationship between alpha-lactalbumin and oleic acid which has antitumor activity.<sup>16</sup> HAMLET can be a natural medicine from an anticancer perspective because its mechanism of action is in several targets on tumor or cancer cells so that it is symbolized like a hydra animal and does not damage normal cells around it.<sup>17</sup> not much information has been obtained regarding the process of formation of HAMLET such as how much alpha-lactalbumin is needed for its formation, so it is not known whether the levels found in this study are optimal for forming HAMLET.

The composition of breast milk varies among nursing mothers. This multidimensional variation in composition is believed to be a reflection of baduta/toddlers needs, geographic area, and food supply. Variations in breast milk composition between mothers were reported in response to cultural differences such as diet and lifestyle and environmental factors.<sup>15</sup>

The results of this study did not show the relationship between alpha-lactalbumin levels and the nutritional status of breastfeeding mothers, so it is still advisable to continue to promote exclusive breastfeeding campaigns even though the mother's nutritional status category is CED because of the function of breast milk which is good for health and as a natural medicine.

## **CONCLUSION AND FUTURE DIRECTIONS**

The average level of alpha-lactalbumin in breast milk for breastfeeding mothers is still low. Based on the nutritional status of the mother, there was no difference in the levels of alpha-lactalbumin in breast milk. However, based on the age group of children under two years old, there were significant differences in alpha-lactalbumin levels in both the nutritional status of normal breastfeeding mothers and the nutritional status of CED breastfeeding mothers.

Further analytical research is needed regarding alpha-lactalbumin levels with levels 4g/L to be used as the basis for research interventions that increase alpha-lactalbumin levels in breastfeeding mothers by intervening determinant factors that improve alpha-lactalbumin levels.

#### Conflicts of Interest

The authors have no conflicts of interest to declare.

#### Funding Sources

Authors state no funding involved.

### REFERENCES

- UNICEF. Breastfeeding: A mother's gift, for every child. Nutrition Section, Programme Division, Data and Analytics Section, Division of Data. *Res Policy, Div Commun.* 2018;3.
- Layman DK, Lönnerdal B, Fernstrom JD. Applications for  $\alpha$ -lactalbumin in human nutrition. *Nutr Rev.* 2018;76(6):444-460.
- Witkowska-Zimny M, Kamińska-El-Hassan E, Wróbel E. Milk therapy: unexpected uses for human breast milk. *Nutrients.* 2019;11(5):944.
- Goonatilleke E, Huang J, Xu G, et al. Human milk proteins and their glycosylation exhibit quantitative dynamic variations during lactation. *J Nutr.* 2019;149(8):1317-1325.
- Forsum E. Determination of  $\alpha$ -lactalbumin in human milk. *J Dairy Sci.* 1976;59(1):14-18.
- Dias B, Nakhawa P. Effect of maternal nutritional status on the biochemical composition of human milk. *Int J Res Med Sci.* 2016;4:4541-4543.
- Bzikowska-Jura A, Czerwonogrodzka-Senczyna A, Olédzka G, Szostak-Węgierek D, Weker H, Wesołowska A. Maternal nutrition and body composition during breastfeeding: association with human milk composition. *Nutrients.* 2018;10(10):1379.
- Kemenkes RI. *Pemantauan Status Gizi.* Kementerian Kesehatan Republik Indonesia; 2017.
- Asmi AD, Citrakesumasari RI, Sirajuddin S, Nurudji Astuti Daud W. Fat Intake With Oleic Acid Levels In Breastfeeding Mother's Milk (6 Months-24 Months) In The Work Area Of Sudiang And Sudiang Raya Public Health Centers Makassar City. *NVEO-NATURAL VOLATILES Essent OILS Journal| NVEO.* Published online 2021:3606-3614.
- Jackson JG, Janszen DB, Lonnerdal B, Lien EL, Pramuk KP, Kuhlman CF. A multinational study of  $\alpha$ -lactalbumin concentrations in human milk. *J Nutr Biochem.* 2004;15(9):517-521.
- Wu T-C, Lau B-H, Chen P-H, Wu L-T, Tang R-B. Fatty acid composition of Taiwanese human milk. *J Chinese Med Assoc.* 2010;73(11):581-588.
- Soliman SM, Soliman AM, Bakr MS. Relationships between maternal nutritional status, quantity and composition of breast milk in Egypt. *Afr J Ag Sci Technol.* 2014;2:59-64.
- Kominiarek MA, Rajan P. Nutrition recommendations in pregnancy and lactation. *Med Clin.* 2016;100(6):1199-1215.
- Quinn EA, Largado FE, Power M, Kuzawa CW. Predictors of breast milk macronutrient composition in Filipino mothers. *Am J Hum Biol.* 2012;24(4):533-540.
- Butts CA, Hedderley DI, Herath TD, et al. Human milk composition and dietary intakes of breastfeeding women of different ethnicity from the Manawatu-Wanganui region of New Zealand. *Nutrients.* 2018;10(9):1231.
- Mossberg A, Hun Mok K, Morozova Roche LA, Svanborg C. Structure and function of human  $\alpha$  lactalbumin made lethal to tumor cells (HAMLET) type complexes. *FEBS J.* 2010;277(22):4614-4625.
- Svensson M, Düringer C, Hallgren O, et al. Hamlet—a complex from human milk that induces apoptosis in tumor cells but spares healthy cells. *Integr Popul outcomes, Biol Mech Res methods study Hum milk Lact.* Published online 2002:125-132.