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ORIGINAL PAPER

Correlation between Docosahexaeoic Acid Intake and It's Content in Breast Milk of Lactating Mothers in Jakarta

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Abstract

Objective: Docosahexaenoic acid (DHA) is the predominant structural fatty acid in the brain and crucial for cognitive development in early life. Newborn DHA intake completely depends on preformed DHA in mother's breast milk. In advancing years, globalization has been declining the fish intake of Asian countries. This study aims to determine DHA intake among lactating mothers in Jakarta and its association with breast milk's DHA.

Method: This cross-sectional study was conducted in Grogol Petamburan and Cilincing Public Health Centers, Jakarta. Eighty healthy lactating mothers aged 20–35 years old in 1–6 months postpartum were taken using consecutive sampling method. Characteristics data were taken by interviews and DHA intake was assessed with the semiquantitative food frequency questionnaire. Breast milk specimens were collected in the morning and its DHA content was analyzed using Gas Chromatography with Mass Spectrometry. Descriptive analyses and Spearman rho test were used with a 95% confidence level.

Result: This study showed the median of subjects' DHA intake was 158.5(13.9–719.7) mg/day, i.e., 67.5% of the subjects was below Food and Agriculture Organization (FAO) recommendation. The median of breast milk DHA was 51.7(19–184.7) mg/day, only 42.5 % of the subjects had breast milk DHA to meet the minimal requirement of their infants. A moderate positive correlation was found between maternal DHA intake with breast milk DHA ($r = 0.478$, $p < 0.001$).

Conclusion: Maternal DHA intake has moderate positive correlation with breast milk DHA, more than half of the subject had DHA intake below FAO recommendation.

Keywords: Lactation, Breast Milk, DHA, Nutrition, Indonesia

Introduction

Among all organs in the human body, the brain undergoes the fastest development in the first year of life. The brain volume will increase rapidly to reach 72% of adult brain volume in the first year. Optimal growth of the brain in this period is crucial because it will determine the intelligence of the future, whereas poor brain growth will result in a condition that may not be reversible by intervention after infancy. Because of the rapid growth and

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development, the brain has higher nutritional requirements and very vulnerable to nutritional deficiencies.[1,2]

One of the most critical nutrient that affects brain development is docosahexaenoic acid (DHA). In human cells, DHA is esterified to phospholipid of the membrane cells and serve as the primary structural fatty acid of human nerve cells, especially the brain. It is distributed in various parts of the human brain from the cortex, synaptic membranes, to retinal photoreceptors and serves critical functions of neurodevelopment and brain function, such as neurogenesis, proliferation, impulse transmission, membrane integrity, and gene expression.[3]

Humans were incapable of synthesizing alpha-linolenic acid (ALA) which is the parent of omega-3 fatty acid, but humans have various enzymes which are capable of lengthening and adding extra double bonds to the molecule to form DHA. During early life, this metabolic capability to convert ALA to DHA is very limited. Therefore, infant's requirements of DHA solely depend on the content of preformed DHA in mother's breast milk.[4,5]

The content of breast milk DHA is affected by maternal lipid intake. Dietary fatty absorbed to the bloodstream in the form of chylomicron then delivered to mammary glands to be used as the precursor of breast milk fatty acid. Fishes are rich in preformed DHA which is the best food source to increase breast milk DHA.[3] However, it has been a concerning matter that in many Asian countries, which are known to be fish eaters, are undergoing a reduction of fish intake and increment of the red meat and prepared food intake in the advancing years.[6] In Indonesia, besides the influence of western diet, fish and seafood also have been considered as food taboo for pregnant and lactating mothers.[7] A study in East Jakarta, Indonesia, showed that the adequacy of omega-3 fatty acid intake among pregnant mothers was only 35.8% and 28.4% for DHA intake.[8]

Inadequate maternal DHA intake of lactating mothers could affect the availability of DHA in their breast milk, which is needed for optimal brain development of the infant. However, recent study in Indonesia by Wibowo et al shows that more than 70% of first-trimester pregnant woman had deficient blood DHA concentration.[9] Little is known about the DHA intake of lactating mothers in Indonesia. Only one study by Nahrowi that shows a correlation between fish intake of lactating mothers and breast milk DHA[10]. This study aims to determine the DHA intake of lactating mothers in Jakarta and its association with breast milk DHA content.

Methods

This cross-sectional study was conducted in Grogol Petamburan Public Health Center, West Jakarta, and Cilincing Public Health Center, North Jakarta, from February to April 2019. This study was part of a larger study of nutrient concentration, inflammation status, and oxidative stress in breast milk: specific assessment to DHA, β-carotene, Zinc, C-Reactive Protein (CRP), Superoxide Dismutase, and Malondialdehyde.

This study has been approved by the Committee for Ethics in Research of the Faculty of Medicine Universitas Indonesia (No.1129/UN2.F1/ETIK/2018, protocol number 18-10-1242).

Subjects

The subjects for this research were lactating mothers aged 20–35 years old at 1–6 months postpartum who had single term delivery and consented to join the study. Lactating mothers who had a history of diabetes mellitus, suffered from mastitis or breast tumor, using drugs to inhibit nutrient absorption (lipase and glucosidase inhibitor, laxative agent), statin, and corticosteroid in the previous 2 weeks, and undergo weight loss diet program were excluded. A total of 95 subjects were recruited using consecutive sampling method, but 15 subjects didn't finish the data collection process, thus only 80 samples were analyzed.

Materials and Specimen

The specimen was post-feed mature breast milk. The mothers were asked to empty either breast 2 hours before breast milk extraction. The selected breast cleaned with sterile distillated water, after that about 30 ml of breast milk were collected with manual milk pump Real Bubee® using non-powdered latex gloves into a sterile container. Collected breast milk then stirred lightly and 6 ml of breast milk was separated to a sterile breast milk bag, stored in -70°C until analysis. The rest of the breast milk was returned to the mother.

Characteristic data
Characteristics data, e.g. identity, age, education, ethnicity, infant sex, postpartum duration, family income, parity, were taken by interview.

Nutrition Status and Dietary Intake Assessment
Anthropometry measurement was taken using Seca 703s digital scale and stadiometer. They were measured according to standardized height and weight measurement protocol. Body mass index (BMI) calculated as weight divided by height squared (kg/m^2). Energy and macronutrient intake were assessed using 24-hour recall for two non-consecutive days, one in the weekday and one in the weekend. Omega-3 and DHA intake were assessed using semi-quantitative food frequency questionnaire.

Laboratory Assessment
Breast milk specimens were analyzed in Prodia Esoteric and Research Laboratory, Kramat, Senen, Central Jakarta.

Docosahexaenoic Acid
Breast milk DHA content was analyzed with Gas Chromatography (GC) and Mass Spectrometry (MS) using a method that was modified from Ren et al,[11]and Lagerstedt et al.[12] The frozen breast milk specimens were thawed and homogenized, then the liquid-liquid extraction with methanol-water-chloroform mixture was used to separate the fat from another macronutrient. The separated fat then undergo transesterification using boron trifluoride and methanol to form their associated fatty acids methyl esters (FAMEs). The separation and identification of FAMEs were performed using Agilent Gas Chromatography System 7890B and Agilent Mass Selective Detector 5977A. Authentic standards were used to identify DHA based on its retention time and mass distribution was calculated from the peak area.

Data Analysis
Data were analyzed using IBM statistical package for the social sciences (SPSS) statistic software version 20.0. Kolmogorov-Smirnov test was used to determine data distribution. It is considered normal if the p-value is above 0.05. Spearman correlation (1-tail) was used to determine the correlation between maternal DHA intake and numerical subject characteristic with breast milk DHA and Chi-square test was used to determine the odds ratio between DHA intake and the adequacy of breast milk DHA, p-value <0.05 was considered significant.

Nutrients database was constructed using Nutrisurvey 2007 by incorporating value from Indonesian Food Composition Table 2017, Indonesian fatty acid composition book,[13] Food Composition Table from United State Department of Agriculture, food composition research form Sukarsa,[14] Jacoeb et al,[15] and Swastawati et al.[16]

Results

A total of 80 subjects data were analyzed. The average age of subjects was 28 ± 4 years old. Most of the subjects had a moderate level of education (67.5%), were Sundanese and Javanese, and were overweight and obese (51.2%). The characteristics can be seen in Table 1.

The adequacy of energy and macronutrient intakes were compared to Indonesian Recommended Dietary Allowances (RDA) 2013 and DHA intake were compared to Food and Agriculture recommendation for lactating mothers (200mg/day).[17,18] Most of the subjects had energy and macronutrient intake below Indonesian RDA. Median of subjects DHA intake was 158.5 (13.9–719.7) mg/day, only 32.5 % of the subjects meet the FAO recommendation. The dietary intakes of the subjects can be seen in Table 2.

The median of breast milk DHA content was 59.6 (22–213) mg/day. There was a weak positive correlation between family income and breast milk DHA (r = 0.220, p <0.025). Correlations between subject characteristics and Breast Milk DHA can be seen in Table 3.

Table 1. Baseline characteristics of subjects (n=80)

Basic Characteristics	Values
Age (years)	28 ± 4†
Education level n (%)	
Low	11 (13.8)
Moderate	54 (67.5)
High	15 (18.8)
Ethnicity n (%)	
Sundanese	20 (25)
Javanese	27 (33.8)
Betawi	13 (16.3)
Melayu	9 (11.3)
Other	11 (13.8)
Infant's sex n (%)	
Male	41 (51.3)
Female	39 (48.8)
Postpartum duration (weeks)	14 (4–24) ‡
Family Income (Rp/months)	3,900,000 (200,000–18,000,000)
Parity (child)	2 (1–4) ‡
BMI (kg/m^2)	23.96 ± 4.19†
Nutritional Status n (%)	
Underweight	3 (3.8)
Normal	36 (45)
Overweight	16 (20)
Obese	25 (31.2)

†: mean ± standard deviation. ‡ : median (minimum–maximum)

Table 2. Dietary intakes of subjects

Dietary Intakes	Value	
	24 hours recall	SQ-FFQ
Energy (kcal/day)	1838 (1055–3375) ‡	
Energy adequacy n (%)		
Low	67 (83,8)	
Adequate	13 (16,3)	
Carbohydrate (gr/day)	229.5 (104–420) ‡	
Carbohydrate adequacy n (%)		
Low	74 (92.5)	
Adequate	6 (7.5)	
Protein (gr/day)	67.5 (41–149) ‡	
Protein adequacy n (%)		
Low	53 (66.2)	
Adequate	27 (33.8)	
Fat (gr/day)	77.1 (27–130) ‡	
Fat adequacy n (%)		
Low	43 (53.8)	
Adequacy	37 (46.2)	
DHA (mg/day)		158.5 (13.9–719.7)‡
DHA adequacy n (%)		
Low		26 (32.5)
Adequacy		54 (67.5)
Omega-3 fatty acids (mg/day)		1089 (209–7154) ‡

†: mean ± standard deviation. ‡ : median (minimum–maximum)

Table 3. Correlations between subject characteristics and breast milk DHA

Characteristics	Breast Milk DHA	
	r	p (*1-tailed*)
Age	- 0.009	0.468
Postpartum duration	- 0.025	0.414
Family Income	0.220	0.025*
Parity	- 0.113	0.160
Body Mass Index	- 0.38	0.370
Energy Intake	0.117	0.151
Carbohydrate Intake	0.109	0.168
Protein Intake	0.013	0.455
Fat Intake	0.050	0.329

*: statistically significant.

Correlation between subjects fatty acid intakes and breast milk DHA showed in Table 4. There was a moderate positive correlation between maternal DHA intake and breast milk DHA (r = 0.479, p <0.001). Omega-3 intake seems to be positively correlated to breast milk DHA, although its not statistically significant.

Table 4. Correlation between fatty acid intakes and breast milk DHA

Fatty acid Intakes	Breast Milk DHA	
	r	p (1-tailed)
DHA	0.479†	<0.001*
Omega-3	0.176†	0.059

Based on the average breast milk intake of Indonesian 0–6 months infant by Winarno, et al.[19], which is 750ml, the minimum value of FAO recommendation for 0–6 months infant DHA requirements (0.1–0 ,18% of total energy intake), and Indonesian RDA 2013 for 0–6 months infant calories intake (550 kcal), we calculated the adequacy of breast milk DHA to meet the recommendation.

We used two different cutoffs (minimum and maximum) from FAO recommendation for DHA intake of 0–6 months infant (Table 5). Only 15% of the subjects had adequate breast milk DHA if the maximum cutoff (0.18% of total energy or 110mg DHA/day) was used compared to 50% of the subjects for the minimum cutoff (0.1% of total energy or 61.1mg DHA/day). There were higher odds to have adequate breast milk DHA if maternal intake exceeds 200 mg/day. Using the minimum cutoff (61.1 mg/day) the odds to have adequate DHA in the breastmilk is 4.265 times higher if maternal DHA intake was above 200 mg/day and it was 5.556 times higher if the maximum cutoff (110mg/day) was used.

Discussion

More than half of the subject in this (51.2%) were overweight and obese. The study by Makela et al.[20] showed that overweight and obese mother have lower breast milk DHA. Obesity has been known to cause low-grade inflammation in the body. This condition could increase the proportion of omega-3 fatty acids converted to anti-inflammatory eicosanoids, reducing the availability of DHA to be transferred into breast milk lipid.[21,22] This study result showed a negative correlation between BMI and breast milk DHA, but it was not statistically significant.

This study found that there is a weak positive correlation between family income and breast milk DHA. Higher family income will ensure food security and the availability of DHA food source for lactating mother. This result is similar with a study by Forsyth et al.[18] showed that food security correlates with omega-3 intake, including DHA, and high-income countries have a better estimation of DHA intake. High DHA intake is a strong factor that determines breast milk DHA content.

The median value of subjects DHA intake in this study (158.5 mg/day) was higher than the FAO

Table 5. Association between DHA intake and Breast Milk DHA adequacy of different cutoff.

DHA intake	Breast Milk DHA Cutoff 61.1 mg/day		p-value^{C,1}	Breast Milk DHA Cutoff 110 mg/day		p-value^{F,2}
	Adequate	Not Adequate		Adequate	Not Adequate	
Adequate	19	7	0.004	8	18	0.01
Not Adequate	21	33		4	50	

^{C}: Chi-Square test; ^{1}: Odds Ratio = 4.265 (1.531–11.886); ^{F}: Fisher's exact test
^{2}: Odds Ratio = 5.556 (1.491–20.705)

estimation of DHA intake for developing country in South East Asia (134 mg/day), it is also higher than Kim et al.[23] in South Korea. However, it was still below FAO recommendation for lactating mother (200mg/day), only 32.5% of the study subjects meet the recommendation. The median value of omega-3 fatty acid intake in this study was 1.1 (0.2–7.2) gr/day and it was also below the Indonesian RDA for lactating mothers (1.4 gr/day).

This study found a significant correlation between DHA but not omega-3 intake with breast milk DHA. As stated before, this result showed that preformed DHA was a better source of breast milk DHA compared to its precursors. Study done by Nahrowi.[10] in Indonesia, where majority of the lactating mothers lived in coastal areas and had high intake of saltwater fish also have higher average breast milk DHA. On the other hand, study done in Nepal by Henjum et al.[24] showed a lower breast milk DHA that possibly caused by their fatty acids intake mostly came from soybean and sunflower oils, which were abundant in ALA but did not contain preformed DHA.

Indonesia is one of the largest maritime country in the world and it is surprising to see that more than half of our subjects didn't have adequate DHA intake. We suspect that globalization have influenced westernization in Indonesian diet. The other factor that caused this was there were several ethnics in Indonesia that considered fishes and seafoods as a taboo food for among pregnant and lactating. They believe that eating fishes could make their breastmilk smell fishy or delayed the delivery wound healing.[7]

The availability of DHA for brain development in early life solely depends on the mother's breast milk DHA content. Half of the study subjects have breast milk DHA below the FAO minimum recommendation of DHA intake for infants aged 0–6 months, which is 61.1 mg/day. Even though DHA can be stored by infants during pregnancy and we didn't measured the DHA status of the infants, from availability of DHA in their mother breast milk alone we can roughly say that at least half of the subject's infant were at risk of DHA deficiencies.

Adequate DHA is needed to ensure optimal brain development in the early life, especially for cognitive function.[6] Optimal brain growth in this golden period, not only beneficial for the infant in the early age but also affect their future. A study of Lassek and Gaulin.[2] showed that maternal breast milk DHA as strong predictor of the cognitive performance that even greater than educational expenditures.

Breast milk was the primary food and the only natural DHA source for newborn. Breast milk lipid contains a considerable amount of phospholipids in micelle like form, in addition it is also packed with endogenous lipase that make it easier to digest.[25] A study by Meldrum, et al.[26] showed those characteristics of maternal breast milk DHA made it a better predictor to red blood cell DHA of the infant compared to DHA fish oil supplement that contains nearly threefold amounts of DHA. This result suggests that by increasing breast milk DHA content we could secure more DHA for infant's brain development. It also encourages mothers to exclusively breastfed their infants as breast milk will provide higher bioavailability DHA than another source.

This study shows a positive moderate correlation between maternal DHA intake and breast milk DHA. We believe that increasing maternal DHA intake is an effective way to ensure DHA availability in mothers' breast milk. We also found that mothers with DHA intake exceeds 200 mg/day was 4 to 5 times more likely to have adequate DHA in their breast milk to meet their infants' requirement.

There were several limitations to this study. First, there was a possibility of recall and social desirability biases related to nutritional assessment using 24-hour recall and SQ-FFQ. Second, there were limited data of trans fatty acid, omega-3, and DHA in Indonesian food composition table. Indonesian Food Photo Book was used and the standardized procedure has been done during the interview to minimize the possibility and magnitude of biases. Adaptation of nutrient content of several key foods from USDA and food technology research was done to complete the food database.

We conclude that maternal DHA intake during the lactation period positively correlated with breast milk DHA. More than half of our subjects had DHA intake below the FAO recommendation and half of them had inadequate amount of DHA to meet their infants daily intake. Maternal DHA intake below FAO recommendation associated with higher risk to have inadequate DHA to meet the infant's requirement. Because DHA is important nutrients for brain development in early life. It is crucial for lactating mothers to exclusively breastfed their infants while paying attention to their fatty acid intake to fulfill the infant's DHA requirements.

Conflict of Interest

Authors declared no conflict of interest regarding this study.

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