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Comparison of DHA Levels in Breast Milk of Indian Mothers of Term and Preterm Neonates

Review Article

Mani B[1]*, Gonsalves R[1], Rao S[2], Mani I[3], Bannikopa P[3] and Janet V[1]

[1]*St. John's Medical College Hospital, Bangalore, India*

[2]*Department of Neonatology, St. John's Medical College Hospital, Bangalore, India*

[3]*Department of Nutrition, St. John's Research Institute, Bangalore, India*

***Corresponding author:** Mani B, St. John's Medical College Hospital, Bangalore, India; E-mail: bhavupaveen@gmail.com

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Abstract

Objectives: Docosahexaenoic acid (DHA, 22:6 n-3) is a fatty acid, which plays an important role in growth and neurodevelopment. There is wide variability of DHA levels in breast milk based on gestational age, geographical location, and diet. Indian diets contain low levels of DHA, implying lower breast milk DHA levels. However there is dearth of information on the fatty acid profile of the breast milk of Indian mothers.

In this study we compared the fatty acid composition of the breast milk of Indian mothers with term and preterm neonates and determined the relationship of breast milk DHA with dietary intake of DHA.

Study Design: This observational study included postnatal mothers (Days 4-7) from the NICU and postnatal wards of a tertiary care hospital in South India. Expressed breast milk from 23 term and 24 preterm mothers was analyzed for fat composition by gas chromatography. Dietary history was taken by a food record questionnaire. BMI of mothers was noted. The sample size of 48 was calculated with 90% power and α error of 5%.

Results: DHA was significantly higher in term breast milk as compared to preterm breast milk (0.18 ± 0.08 ug % Vs 0.12 ± 0.09 ug % p = 0.025). The levels of arachidonic acid and also overall PUFA were significantly higher in term milk. This difference was seen even though the dietary intake of fatty acids in both groups was similar. Whether this has long term consequences needs to be investigated.

Conclusion: The levels of DHA was significantly lower among preterm infants' mothers' breast milk, compared to term infants, despite there being no significant dietary differences between the two groups. Whether supplementation of DHA to pregnant women or preterm infants could improve neurological and retinal outcomes is yet to be studied.

Keywords: DHA; Preterm; Breast milk; Fatty acid

Introduction

Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is a primary structural component of the human brain, cerebral cortex, skin, and retina [1]. DHA comprises 40% of the polyunsaturated fatty acids (PUFAs) in the brain and 60% of the PUFAs in the retina [2]. The time of the most rapid neural and retinal development occurs mainly during the third trimester (28-40 weeks of gestation). Inadequacy of long chain n-3 fatty acids such as DHA during this period can compromise brain function [3]. Preterm infants may be disadvantaged by virtue of their shortened gestation and thereby shortened transplacental transfer of these essential fatty acids (EFAs). Preterm neonates are also more likely to suffer complications including cold stress and hypoglycemia. Even after recovering from neonatal complications, they remain more prone to poor physical growth and poor neurodevelopmental outcome [4]. Thus early nutritional support of preterm infants is important because it influences long-term health and development. Postpartum, the major source of these EFAs is human breast milk, which is universally considered as the optimal food for both term and preterm infants [5]. It has been a matter of debate whether the fatty acid composition of human milk differs in preterm as compared to term breast milk. The breast milk content of LCPUFA is highly variable, depending on geographic location, dietary habits and genetic influences. Brenna et al report a descriptive meta-analysis that included 65 studies of 2474 women. The highest DHA concentrations were primarily in coastal populations and were associated with marine food consumption [6]. This meta-analysis showed the variability of DHA worldwide. However there is a dearth of studies quantifying the micronutrient profile of the breast milk of Indian mothers. This study is designed to compare the fatty acid composition in breast milk of Indian mothers with preterm and full-term newborns and to determine the relationships of dietary intake of docosahexaenoic acid (DHA) of mothers and the content of this fatty acid in their milk.

Materials & Methods

Type of study: This observational, analytical study was conducted in the postnatal wards and NICU of St. John's Medical College Hospital, Bangalore.

Subjects: Sample size (n=24 in each group) was calculated using the Nmaster software to provide a power of 90% with an alpha error of 5%. Institutional Ethical Review Board approval was obtained. Samples were collected from mothers of 4-7 days old preterm (<37 completed weeks of gestation) and term neonates. The exclusion criteria were multiple gestations, prior delivery less than 2 years from the date of current delivery, diabetes in pregnancy, use steroids/ anticonvulsants or DHA supplementation during period of gestation and maternal age less than 18 or greater than 40.

Sampling: The best gestational age of the neonate was calculated by the first trimester ultrasound (T1 USG). In the absence of T1 USG, mothers were included only if the gestational age based on last menstrual period and by Modified Ballard Score corroborated with each other. The first group (n = 23) included mothers delivered at term (gestational age from 37 to 42 weeks) and the second group (n = 24) included mothers who delivered preterm i.e.,< 37 weeks. The study was explained to the mothers and written consent was obtained before sample collection. The mother's age, parity, education status, and eating habits were recorded. Body mass index was calculated from prepregnancy weight / T1 weight (from the mother's records) and the mother's height. The mothers also completed a food record questionnaire (modified food frequency questionnaire) to determine their food intake (during pregnancy). Total energy, protein, carbohydrates and fatty acid composition were calculated using nutrition software. Mothers expressed foremilk and hind milk separately, and 2ml of hind milk was collected and stored for analysis. The rest of the milk was used for feeding the baby. Milk samples were stored at -70^{0}C till analysis.

Measurement of fatty acid status of breast milk: Fatty acids from breast milk total lipids (from 500 µl of breast milk) were analyzed using Gas chromatography with a flame ionization detector (Varian 3800; Varian, Palo Alto, CA, USA). Briefly, the procedure followed involved the extraction of total lipids and trans-methylation of all fatty acids from this fraction using BF3-methanol. 9 The fatty acid methyl esters were then separated by chain length and degree of saturation by injection onto a 50 m, 0.2 mm capillary column (FAME, Varian) with nitrogen as carrier gas. Individual fatty acids were identified against reference fatty acids.

The odd-chain FA - heptadecanoic acid, C17:0 was used as an internal standard in each sample. Based on the internal standard, total fatty acid contents of the samples were calculated and each identified fatty acid was expressed as a percentage of the total content of the identified fatty acids.

Statistical analysis: All analyses were performed using SPSS Version 20. Difference between means was assessed by independent samples t-test (DHA, other fatty acids). Correlation between quantitative variables was tested by Pearson's coefficient of correlation (DHA & BMI/ maternal dietary DHA). P values less than 0.05 were considered significant.

Results

Table 1 gives the anthropometric and demographic details of the women and their babies in both groups. No significant differences were seen between the mothers in the two groups.

Table 2 provides the information on the fat intakes in the two groups. No significant difference was seen in the dietary intakes of the two groups of women. Intakes of LCPUFA were relatively low in both groups.

The fatty acid profile of breast milk is presented in Table 3. The pre term milk was found to have relatively higher levels of SFA and significantly lower levels of total PUFA as well as both n6 as well as n3 PUFA. The n6/n3 ratio was seen to be high in both groups but not statistically different between the groups.

Table 4 provides a comparison of the fatty acid profile of full term *vs* pre term transitional milk from various countries. A high degree

Table 1: Characteristics of the mother and baby pairs in the groups with babies delivered at full term and those delivered pre term. *P< 0.01.

	Full term (n=23)	Pre-term (n=24)
Age (yrs)	25.4 ± 4.4	26 ± 4.0
Education of mother		
% high school or higher	54	42
Mother's height (cms)	157.1 ± 5.6	153.6 ± 7.2
Pre-pregnancy weight (kg)	54 ± 11	50.4 ± 10.8
BMI	22.0 ± 4.4	21.5 ± 4.5
Parity	1.4 ± 0.6	1.3 ± 0.6
Gestational age (days)	273.6 ± 6.8	234.3 ± 15.8*
Baby weight (kg)	2.92 ± 0.55	1.55 ± 0.48*
Baby length (cm)	48.6 ± 3.9	40.6 ± 4.5*
Head circumference (cm)	33.6 ± 1.7	30.2 ± 2.3*

Table 2: Intakes of fat and fatty acids (g/day) in the two groups of women.

	Full term (n=23)	Pre term (n=24)
FAT	57.1 ± 24.6	56.4 ± 38.9
SFA	20.9 ± 9.3	21.4 ± 14.9
MUFA	14.7 ± 6.6	15.4 ± 11.9
PUFA	11.7 ± 5.1	13 ± 9.1
LA (18:2 n6)	11.0 ± 4.8	12.2 ± 8.7
ALNA (18:3 n3)	0.63 ± 0.26	0.61 ± 0.37
AA (20:4 n6)	0.07 ± 0.04	0.08 ± 0.08
EPA (20:5 n3)	0.01 ± 0.01	0.01 ± 0.01
DHA (22:6 n3)	0.02 ± 0.02	0.02 ± 0.03

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Table 3: Fatty acid profile (% FA) of breast milk from mothers with full term and pre termbabies. $^{*}P < 0.05$.

	Full term (n=23)	Pre term (n=24)
C 8:0	0.08 ± 0.19	0.22 ± 0.42
C 10:0	0.8 ± 0.44	1.2 ± 0.8*
C 12:0	6.2 ± 3.34	7.6 ± 3.35
C 14:0	8.2 ± 3.32	9.1 ± 3.06
C 16:0	29.1 ± 2.18	29.4 ± 2.74
C 18:0	4.4 ± 0.63	4.6 ± 0.9
SFA	48.7 ± 7.0	52.1 ± 5.3
C 16:1	3.2 ± 0.89	3.1 ± 0.63
C 18:1	32.0 ± 4.7	31.4 ± 4.4
MUFA	35.2 ± 5.0	34.5 ± 4.5
C 18:2 n6	14.2 ± 4.05	12 ± 3.1*
C 18:3 n3	0.18 ± 0.14	0.11 ± 0.09*
C 20:3 n6	0.69 ± 0.2	0.55 ± 0.3
C 20:4 n6	0.75 ± 0.25	0.58 ± 0.22*
C 20:5 n3	0.01 ± 0.03	Nd
C 22:5 n3	0.01 ± 0.02	Nd
C 22:6 n3	0.19 ± 0.08	0.12 ± 0.09*
PUFA	16.1 ± 4.32	13.4 ± 3.3*
n-6	15.7 ± 4.29	13.1 ± 3.27*
n-3	0.38 ± 0.21	0.24 ± 0.13*
n-6/n-3	61.2 ± 46.6	69.2 ± 44.5

of variability in composition is seen across these countries and the results of the current study appear to be within these parameters.

Discussion

We enrolled mothers of 23 term and 24 preterm neonates. The groups were similar with respect to maternal characteristics like age, education, maternal height, pre-pregnancy BMI and parity.

Mothers in the preterm group had significantly lower levels of breast milk PUFA. This is finding is similar to findings in the study by Berenhauer et.al showing term mothers having a higher breast milk PUFA content compared to preterm mothers and in contrast to the study by Granot et.al showing no significant correlation [7,8]. Since there were no significant differences in the dietary PUFA intake between the mothers from the term and preterm group, the difference in PUFA levels in breast milk are likely to be attributed to other factors, including genetic factors. Maternal age, height and pre pregnancy weight were similar as well.

We also found that DHA in the breast milk of mothers of term infants was higher in comparison to that of mothers of preterm infants, despite there being no significant dietary differences between the two groups. Kovac et al. found values of docosahexaenoic acid

Table 4: Comparison of fatty acid profiles (% FA) of full term *vs* pre term transitional milk in different countries.

Full term	**Berenhauser 2012**	**Kovacs 2005**	**Bobiinski 2013**	**Granot 2015**	**Genzel- BoroviczeÂny 1997**	**Rueda 1998**	**Current study**
Country	Brazil	Hungary	Poland	Israel	Germany	Spain	India
C 10:0	-	-	0.35	0.69	0.5	0.45	0.8
C 12:0	2.02	3.12	2.3	4.06	3.42	5.5	6.2
C 14:0	5.27	5.43	6.1	6.04	6.93	8.21	8.2
C 16:0	27.4	25.5	23.6	25.1	24.56	19.59	29.1
C 18:0	7.39	5.29	5.8	7.36	7.23	6.28	4.4
C 16:1	0.51	1.71	2.96	2.07	-	1.57	3.2
C 18:1	35.6	35.8	39.9	29.4	32.16	39.6	32
C 18:2 n6	17.8	16.67	9.2	16.4	9.81	14.22	14.2
C 18:3 n3	0.74	0.32	0.75	1.37	0.66	nd	0.18
C 20:3 n6	-				-	-	0.69
C 20:4 n6	0.78	0.34	0.74	0.97	0.76	0.85	0.75
C 20:5 n3	0.02		0.08	0.11	0.11	0.1	0.01
C 22:5 n3	-	0.05	-	-	0.22	0.26	0.01
C 22:6 n3	0.09	0.13	0.42	0.68	0.45	0.41	0.19
Pre- term	**Berenhauser 2012**	**Kovacs 2005**	**Bobiinski 2013**	**Granot 2015**	**Genzel- Borovicze Âny 1997**	**Rueda 1998**	**Current study**
C 10:0		-	0.39	0.79	0.46	0.12	1.2
C 12:0	2.01	6.78	2.8	3.85	4.17	4.11	7.6
C 14:0	4.8	9.96	7.1	6.31	7.65	7.576	9.1
C 16:0	26.2	24.8	23.3	21.67	23.84	19.56	29.4
C 18:0	7.13	5.17	5.76	7.56	6.66	6.09	4.6
C 16:1	0.54	1.61	2.75	1.97	-	1.38	3.1
C 18:1	35.1	31.5	39.7	29.9	33.49	39.86	31.4
C 18:2 n6	20.1	13.27	9.2	17.36	9.86	13.07	12
C 18:3 n3	0.92	0.42	0.78	1.6	0.73	-	0.11
C 20:3 n6						-	0.55
C 20:4 n6	0.72	0.61	0.74	0.99	0.72	0.71	0.58
C 20:5 n3	0.02	-	0.1	0.15	0.04	0.19	-
C 22:5 n3	-	0.11	-	-	0.22	0.31	-
C 22:6 n3	0.1	0.26	0.45	1.1	0.45	0.48	0.12

were significantly higher in human milk samples of mothers of preterm as compared with full- term infants [9]. However Iranpour et al found no significant difference in DHA in breast milk of preterm and term mothers [10]. These contrasts could be attributed to the period we have chosen for sample collection (we studied day 4-7 milk) and different sampling methods.

In our study, breast milk DHA levels on average for the entire population (n=47) studied of 0.15±0.09. This was less than half of the global average as reported in a descriptive meta- analysis by Brenna et al [6]. The mean (+SD) concentration of DHA in breast milk (by weight) was 0.32 + 0.22%. The highest DHA concentrations were primarily in coastal populations and were associated with marine food consumption. Notably there were no studies from India as part of the review.

The mothers in our study consumed a predominantly vegetarian diet. This possibly contributed to the low DHA levels found in their breast milk. In a vegetarian diet, the DHA content is negligible. Although the body can convert alpha - linoeic acid to DHA, the maternal plasma levels produce lower concentrations of DHA in the breast milk compared to meat eaters, especially those who consume significant quantities of marine food.

However, a systematic review by Bravi et al. found only weak correlation between diet and breast milk DHA levels, further supporting the need for more systematic studies on this and the role of other factors, including genetics, in determining DHA levels of breastmilk [11]. A recent study by Sosa-Castillo et al. has also emphasized the role of the interaction between maternal diet and gene transcription mechanisms, resulting in variation in the proportion of fatty acids in milk. These mechanisms, yet to be completely elucidated, could explain the degree of variability in the differing findings in studies trying to infer the relation of breast milk composition with other variables.

The lower DHA levels in the breast milk of mothers are disconcerting, as preterm infants by virtue of their shortened gestation accumulate less DHA via transplacental transfer [12]. This is compounded by the fact a majority of that LCPUFA transfer occurs during the third trimester and that the infant's capacity to produce DHA de novo is limited [13]. This may put preterm infants at a disadvantage given the role DHA has been shown to have in the development of neuronal tissue. Babies born preterm require higher amounts of fatty acids than term babies for development. Few studies have shown that supplementation of the same in the neonatal period improves neuronal development. However, none of them were done in India. The European Food Safety Authority (EFSA) recommends 100 mg per day as the adequate intake of DHA for children less than 24 months of age [14]. However dearth of studies quantifying the micronutrient profile of breast milk of Indian mothers makes it difficult to objectively state whether the preterm infant is receiving the advised intake. Larger epidemiological studies would be required to assess breast milk fatty acid composition for the Indian population, and the impact of omega 3 fatty acid supplementation on breast milk composition. This is a promising area that could potentially improve the standard of care of preterm neonates, which could positively influence long term neurological outcomes.

Conclusion

The levels of DHA were significantly lower among preterm infants' mothers' breast milk, compared to term infants, despite there being no significant dietary differences between the two groups. Whether supplementation of DHA to pregnant women or preterm infants could improve neurological and retinal outcomes is yet to be studied. Also, mothers need to be educated about the importance of fats in their diet and consequences of deficiency of PUFAs and DHA in breast milk. In the long run, this would improve infant health and neurological outcomes.

Limitations and scope for further studies

Our sample size was small and consisted of patients from only one tertiary centre. This may not be representative of the entire population and hence further studies are needed.

Even though the importance of DHA in neurological and retinal development is known, more studies are needed to fully establish the need to supplement DHA to newborns whose mother's milk is deficient and better outcomes.

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