Curr Pediatr Res 2021; 25 (8): 833-842 *ISSN 0971-9032*
www.currentpediatrics.com

The effect of docosahexaenoic acid supplementation in preterm infants on the incidence of necrotizing enterocolitis and its correlation with the level of platelet activating factor.

Noha M Kamal[1], Hisham A Awad[1], Ola G Badr[1], Sally M Hassan[1], Heba E Hashem[1], Wafaa O Ahmed[2*]

[1]Department of Pediatric, Ain Shams University, Cairo, Egypt

[2]Department of Clinical Pathology, Ain Shams University, Cairo, Egypt

Abstract

Background: Necrotizing Enterocolitis (NEC) is a serious intestinal infection that primarily affects premature and low birth weight babies. Both prematurity and enteral feeding are major risk factors for NEC. This interventional two armed, double blinded randomized controlled clinical trial (IIb) study was to determine the role of Docosahexaenoic Acid (DHA) in preventing or lessening the severity of necrotizing enterocolitis in preterm/low birth weight neonates.
Methods: One group (n=20) was assigned to receive DHA and the other received the placebo.
Results: Platelets activating factor was significantly higher in neonates who received DHA supplements ($p<0.001$). As regards the incidence of feeding intolerance, our results demonstrated a significant statistical improvement among neonates of DHA group; regarding abdominal girth, intestinal sound, gastric residual, passage of stool and modified NEC BELL'S criteria ($P<0.05$). SNAP score for evaluation of sepsis was evaluated initially and 10 days later for both groups where, it increased from 9.6 to 19.2 among patients of group B and decreased from 14.4 to 8.3 among neonates of group A ($P<0.001$ and 0.001) respectively.
Conclusion: DHA has an apparent role in improving growth of preterm neonates, decrease severity and progression of NEC.

Keywords: DHA, Proinflammatory cytokines, Platelets activating factor, Necrotizing enterocolitis, Low birth weight neonates, Preterm neonates.

Accepted on 27th August, 2021

Introduction

Necrotizing Enterocolitis (NEC) is the most lethal gastrointestinal disease in newborns. The pathogenesis of NEC is not well understood, but evidence strongly suggests that its occurrence is multifactorial. Prematurity and enteral feeding are significant risk factors for NEC. Excessive inflammatory response by the immature intestinal function to external stimuli, as well as impaired intestinal barrier integrity and abnormal bacterial colonization, are thought to be factors involved in the pathophysiology of NEC [1]. In Egypt, the prevalence of feeding intolerance is 2.6%, while the prevalence of NEC is 9.6%, with mortality rates ranging from 20% to 30% [2]. Current evidence would suggest that immature innate immunity mediated by the TLR4 signaling pathway may contribute to an excessive intestinal inflammatory response in NEC [3,4].

NEC is the most serious intestinal emergency condition, and it is primarily considered a disease of preterm infants. Because of the rapid progression from nonspecific signs to extensive small intestinal necrosis, primary prevention is also the top priority. Several immune-regulatory nutrients, including bovine colostrum's, prebiotics, probiotics, Long Chain Poly Unsaturated Fatty Acids (LCPUFA), and amino acids have recently been shown to play an important role in the primary prevention of NEC (glutamine, cysteine, L-arginine, and N-acetylcysteine)[5]. Platelets Activating Factor (PAF) is a key inflammatory mediator associated with NEC. Platelet Activating Factor Receptor (PAFR) inhibition or increased intestinal PAF degradation *via* PAF Acetylhydrolase (PAF-AH) supplementation reduces the occurrence of NEC. We hypothesised that Poly Unsaturated Fatty Acids' (PUFA) protective effect on NEC was due to PUFA's ability to suppress PAFR gene expression [6]. Premature infants are more likely to develop NEC because LCPUFA accretion occurs primarily during the third trimester of pregnancy, when maternal serum levels are high and brain growth and development is at its peak. Premature infants who are not exposed to PUFA in their gut have higher levels of microbiota and are more likely to develop NEC [7].

Preterm babies have an immature immune system, as well as dysregulation of inflammatory responses, which play a critical role in the occurrence of many life-threatening neonatal diseases, including NEC. Furthermore, preterm defense is heavily reliant on the nonspecific innate immune response. Preterm infants' antigen-specific adaptive immune systems are also underdeveloped at birth, particularly T-cells that mediate inflammatory responses [8]. Docosahexanoic Acid (DHA) supplementation may improve intestinal cell integrity by decreasing bacterial or endotoxin translocation and/or mucosal PAF formation and receptor activation [9]. DHA acts as a

mediator in the development of oral tolerance and modulates the developing immune response, allowing neonates to respond effectively to self and pathogenic environmental stimuli. DHA promotes the colonisation of beneficial bacteria while inhibiting the growth of pathogenic bacteria. In addition, decreased PAF receptor and Toll Like Receptors (TLR) gene expression in intestinal epithelial cells plays an important role in the initiation of NEC [10].

Previous research has shown that the full effect of omega-3 fatty acids takes weeks to become effective. Furthermore, when administered at the onset of developing feeding intolerance, some human studies have reported beneficial effects of acute omega-3 fatty acid supplementation [11]. Previous research has shown that circulating DHA levels are a deficiency marker and that premature infants with higher circulating DHA levels have a lower risk of disease, including NEC Retinopathy of Prematurity (ROP) and Broncho Pulmonary Dysplasia (BPD) as well as improved vision and neurodevelopmental outcomes [12-14,7]. Positive health benefits have been found most consistently using “high dose” DHA supplementation, which more closely mimics in utero accretion rates [15]. This study provides evidence that DHA supplementation can protect preterm infants from developing NEC and may even reduce the severity of cases and sepsis, as dietary supplementation with long chain fatty acids suppresses cytokine production by mononuclear leukocytes, or improves the course of inflammatory diseases [16,17].

Methods

The current study was a two-arm; double-blind, randomised controlled clinical trial (IIb) conducted in Neonatal Intensive Care Units (NICU) of Ain-Shams university hospitals from September 2017 to September 2020. Preterm/very low birth weight neonates were used in the study. After considering inclusion and exclusion criteria and obtaining informed consent from parents or caregivers, patients were enrolled in the study.

Patients are eligible for inclusion if they are preterm neonates with a gestational age of 32 weeks or less at birth, admitted to the NICU, have a weight (less than or equal to 1.500 KG), are clinically stable to begin enteral feeding, and are both males and females. According to SNAP 11, sepsis was not considered an exclusion criterion (this score considers six physiologic variables such as blood pressure, temperature, the Po2/Fio2 ratio, serum PH, seizures, and urine output, Töllner score, and Rodwell score)[18]. The current study excluded any preterm with persistent bleeding at any level, receiving anticoagulant medication, persistent vomiting, or both Gastrointestinal malformations or surgery and mother taking n-3 supplements.

The enrolled patients were divided into two groups: group A (n=40) received DHA, while group B (n=40) received placebo (physically matched solution). Forty preterm infants were included in the study and received 100mg DHA daily via enteral route for ten days. Antenatal history including rupture of membrane, Chorioamnionitis, history of urinary tract infection, natal history including mode of delivery, place of delivery, need for resuscitation, recorded Apgar score at 1 minute and 5 minutes, and postnatal history including: age of admission in neonatal intensive care unit, symptoms suggest infection

Comprehensive clinical evaluation, including weight, length, and OFC (twice weekly), cardiovascular, respiratory, neurological and abdominal examination was performed, with particular attention paid to: indications of food intolerance and NEC. One group was labeled by yellow card and the other by red card. One color was assigned to receive DHA and the other receive the placebo; where their information were kept hidden and unknown till results of lab was received. A computer-generated list of random numbers is used. The nurses were not informed by the type of the medication they give to patients.

All infants received standard neonatal care and were followed up on until they were 37 weeks corrected gestational age, discharged, or died, whichever came first. All neonates were fed as soon as they could tolerate it (when hemodynamically stable).

Begin with trophic feeding on 10-20 ml/kg and then begin subtracting feds from total fluid intake. Increase the feeds from 10-20 ml/kg/day [19]. All neonates in group A received 100 mg DHA as soon as possible with enteral feeding for 10 days and the patients were followed up for signs of feeding intolerance or NEC, where in the latter case the patient was kept Nothing Per Os (NPO). While group (B) received primarily a place to (physically matched solution that was distilled water) in addition to their regular feeding.

Laboratory investigations

Before start of DHA supplements, the recruited neonates were had been investigated by the following:

Complete blood count: One ml of the whole blood sample was added to EDTA (K3EDTA) vacutainer with concentration of 1.2 mg of anhydrous salt per ml of blood. The CBC was done immediately and results were documented quantitative C-Reactive Protein (CRP), two millimetres of the sample was left to clot for 30 minutes in sterile dry venipuncture tubes without additives or gel barrier at room temperature then sera were separated by centrifugation at 3000 xg for 10 minutes. Part of serum of each sample was analyzed immediately after centrifugation. Both cases and controls were subjected to a quantitative ELISA technique for PAF. The patients were further followed up clinically for development of NEC and the second samples were withdrawn 10 days later to assess CBC, CRP, bleeding profile to assess the decrease in PAF levels

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human PAF. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human PAF and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate

well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human PAF, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow.

The Optical Density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human PAF. You can calculate the concentration of Human PAF in the samples by comparing the OD of the samples to the standard curve. Detection Range: 78.13-5000 pg/mL NEC was diagnosed clinically as well as by Abdominal X-ray (Erect & Supine), when necrotizing enterocolitis is suspected and further staging using the modified BELL's score. An abdominal ultrasound was performed to confirm the presence of NEC [20]. The current study was approved by the local ethical committee (MD 308/2017) and registered in Clinical Trial.gov as NCT04746885.

Statistical analysis

The collected data will be revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). P-value: level of significance: $P > 0.05$: Non significant (NS), $P < 0.05$: Significant (S), $P < 0.01$: Highly significant (HS).

Results

The study was conducted as an interventional two armed double blinded randomized control trial. The study recruited 80 neonates, they were divided blindly in to two groups (40 each) where the patients of group A had 35% males and group B had 37.5% females. Both groups were age and sex matched. Antenatal risk factors were recorded in 75% and 65% of group B and A respectively, they included; preeclampsia, maternal diabetes, twinning and maternal diseases and fever. Neonatal resuscitation was needed in 72.5% of both cases and controls. The neonates were further evaluated clinically by measuring baseline anthropometric measurements Table1.

			Study group		X2	P value
			Group B (control group / Placebo) N=40	Group A (interventional group/ DHA) N=40		
Gender	Male	N.	15	14	0.054	0.816
		%	37.50%	35.00%		
	Female	N.	25	26		
		%	62.50%	65.00%		
Lactation	BF	N.	24	28		0.61
		%	60.00%	70.00%		
	Supplemtary BF with AF	N.	16	12		0.342
		%	40.00%	30%		
Mode of Delivery	VD	N.	14	16	0.213	0.644
		%	35.00%	40.00%		
	LSCS	N.	26	24		
		%	65.00%	60.00%		
Maternal risk factors (Antenatal risk factors) PROM, UTI, Chorioamnionitis, PET	No	N.	10	14	0.952	0.329
		%	25.00%	35.00%		
	Yes	N.	30	26		
		%	75.00%	65.00%		
Gestational age		Mean	31.4	31.3	0.381	0.704
		SD	1.4	1.5		
Age of Admission (days)		Mean	1.2	1.1	1.732	0.087
		SD	0.5	0.3		

Table 1. Demographic data distribution among studied groups. Significant<0.05, highly significant<0.001.

It was noticed that weight was significant difference between group B and A at baseline weight measurements being lower in group A ($p<0.05$) while it became statistically non-significant

in follow up, being higher in group A. The length noticed to be become significantly higher in group A after 10 days follow up. Regarding the OFC; there was no significant difference regarding neither the baseline nor the follow up recordings. It was of at most importance to stress on giving breast milk for all neonates, so the 80 patients were breast fed in Table 2. According to the definition of feeding intolerance; regarding the abdominal distension, passage of stool, audible intestinal sounds and bloody stool were observed to occur in higher incidence at baseline evaluation that was improved at 10 days follow up in Tables 3 and 4.

		group(N=40, each)	Mean	SD	t	P value
Weight	Baseline	B	1362.5	104.2	2.133	0.036
		A	1299.8	154.1		
	Follow-up	B	1330	95.9	-1.902	0.061
		A	1389.5	173.1		
Length	Baseline	B	39.4	1.2	-1.888	0.063
		A	40.1	1.9		
	Follow-up	B	40.4	1.2	-3.895	0
		A	41.9	2		
OFC	Baseline	B	28.3	1	0.737	0.463
		A	28.1	1.9		
	Follow-up	B	28.8	1	-0.825	0.412
		A	29.1	1.9		

Table 2. Anthropometric measurements of the two studied groups. OFC: Occipito Frontal Circumference, significant<0.05, highly significant<0.001.

	Study group N=40, each	Mean	SD	t	P value
Abdominal Girth Baseline	B	24.83	1.999	-6.492	0
	A	27.4	1.516		
Abdominal Girth 10 Days	B	27.13	1.713	4.576	0
	A	24.78	2.759		

Table 3. Abdominal distension as a sign of feeding intolerance in the two studied groups. Significant<0.05, highly significant<0.001.

				Study group		X2	P value
				Group B. n=40	Group A. n=40		
Intestinal Sounds	Baseline	No	N.	0	15	40.99	0
			%	0.00%	37.50%		
		Yes	N.	28	2		
			%	70.00%	5.00%		
		Decreased	N.	12	23		
			%	30.00%	57.50%		
	10 Days	No	N.	25	5	29.333	0
			%	62.50%	12.50%		

		Yes	N.	8	32		
			%	20.00%	80.00%		
		Decreased	N.	7	3		
			%	17.50%	7.50%		
Passage of stool	Baseline	Bloody	N.	0	5	5.333	0.021
			%	0.00%	12.50%		
		Positive	N.	40	35		
			%	100.00%	87.50%		
	10 Days	Bloody	N.	9	0	13.373	0.001
			%	22.50%	0.00%		
		Positive	N.	31	36		
			%	77.50%	90.00%		
		Absent	N.	0	4		
			%	0.00%	10.00%		
Gastric Residual	Baseline	No	N.	28	0	43.077	0.021
			%	70.00%	0.00%		
		Yes	N.	12	40		
			%	30.00%	100.00%		
	10 Days	No	N.	5	29	29.463	0.021
			%	12.50%	72.50%		
		Yes	N.	35	11		
			%	87.50%	27.50%		
Modified NEC Bell'S criteria	Baseline	0	N.	28	1	43.296	0.001
			%	70.00%	2.50%		
		1A	N.	12	26		
			%	30.00%	65.00%		
		1B	N.	0	13		
			%	0.00%	32.50%		
	10 Days	0	N.	5	29	46.141	0
			%	12.50%	72.50%		
		1A	N.	14	6		
			%	35.00%	15.00%		
		1B	N.	21	0		
			%	52.50%	0.00%		
		2A	N.	0	2		
			%	0.00%	5.00%		
		2B	N.	0	3		
			%	0.00%	7.50%		

Table 4. Other signs of feeding intolerance (intestinal sounds & Passage of stool & gastric residual and modified NEC Bell's criteria) in the two studied groups. Significant<0.05, highly significant<0.001.

On evaluation of our studied groups regarding the incidence of NEC; 30% go group B were staged as stage 1A of NEC while in group A; 97.5% of patients had signs of NEC where, 65% had stage 1A and 32.5% had stage 1B. After 10 days; evaluation denoted progression of group B with both occasions having a high significant difference among neonates of both groups (p=0.001 and <0.001) respectively in Table 5. SNAP score for evaluation of sepsis was evaluated initially and 10 days later for both groups where, it increased from 9.6 to 19.2 among patients of group B and decreased from 14.4 to 8.3 among neonates of group A (P<0.001 and 0.001) respectively, this reflects the improvement of group A. There was high statistical difference regarding the length of hospital stay being higher in group B (25.1 days) than in group A (22 days) (p=0.02) mortality was higher in group A (12.5%) than in group B (10%)however, it was statistically insignificant (p=0.723) Table 6.

		Study group n=40; each	Mean	SD	t	P value
HB	Baseline	B	12.4	1.1	1.34	0.184
		A	12	1.6		
	10 Days	B	11.1	1.7	-1.79	0.077
		A	11.9	1.9		
WBC	Baseline	B	12.1	3.2	-1.74	0.086
		A	13.8	5.1		
	10 Days	B	17.1	5	6.18	0
		A	11.2	3.4		
PLT	Baseline	B	199	102	6.07	0
		A	93.7	40.6		
	10 Days	B	123	73.8	-3.51	0.001
		A	193	101		
PT	Baseline	B	14.8	1.8	-2.39	0.019
		A	16.1	2.8		
	10 Days	B	17.9	3.3	4.77	0
		A	15	1.8		
PTT	Baseline	B	38	9.1	-3.27	0.002
		A	49.7	20.7		
	10 Days	B	61.6	17.6	6.47	0
		A	40.9	10		
INR	Baseline	B	1.1	0.1	-3.64	0
		A	1.3	0.3		
	10 Days	B	1.3	0.2	3.46	0.001
		A	1.2	0.2		
PAF (pg/mL)	Baseline	B	5049	1303	-1.8	0.076
		A	5612	1491		
	10 days	B	5600	1066	6.08	0
		A	3238	2102		

Table 5. Lab evaluation of the studied groups; baseline and on follow up. Significant<0.05, highly significant<0.001,

Hemoglobin: HB, White Blood Cells: WBC, Platelets: PLT, Prothrombin: PT, Partial Thromboplastin: PTT, International Normalised Ratio: INR, Platelets Activating Factor: PAF.

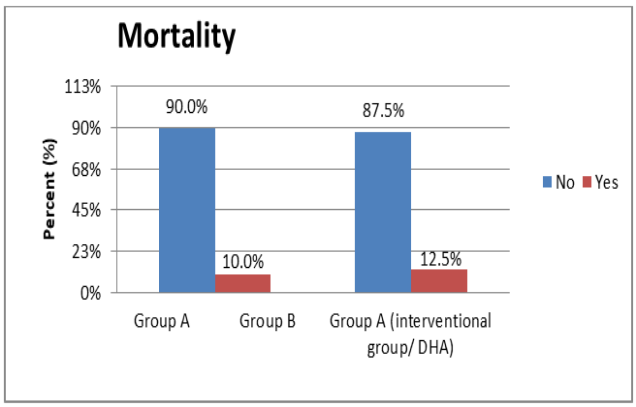
		Study group n=40; each	Mean	SD	t	P value
SNAP II	Baseline	B	9.6	4.7	-3.53	0.001
		A	14.4	7.1		
	10 Days	B	19.2	6.7	6.66	0
		A	8.3	7.9		
Duration of Hospitalization (Days)		B	25.1	4.4	2.38	0.02
		A	22	7.1		

Table 6. SNAP II score and length of hospital admission in the two studied groups. Significant<0.05, highly significant<0.001, Premature Rupture of Membranes: PROM.

Correlation between clinical and laboratory investigation among neonates of group A were illustrated in Table 7 showing marked improvement. Neonatal mortality and morbidities percentages were illustrated in Figure 1.

Group A, n=40,		Mean	SD	t	P value
Weight	Baseline	1299.8	154.1	-6.089	0
	Follow-up	1389.5	173.1		
Length	Baseline	40.1	1.9	-21.162	0
	Follow-up	41.9	2		
OFC	Baseline	28.1	1.9	-41	0
	Follow-up	29.1	1.9		
Abdominal girth	Baseline	27.4	1.5	7.131	0
	Follow-up	24.8	2.8		
WBC	Baseline	13.8	5.1	2.796	0.008
	Follow-up	11.2	3.4		
PLT	Baseline	93.7	40.6	-6.369	0
	Follow-up	192.8	101.4		
PT	Baseline	16.1	2.8	2.287	0.028
	Follow-up	15	1.8		
PTT	Baseline	49.7	20.7	2.336	0.025
	Follow-up	40.9	10		
INR	Baseline	1.3	0.3	2.039	0.048
	Follow-up	1.2	0.2		
SNAP II	Baseline	14.4	7.1	3.987	0
	Follow-up	8.3	7.9		
PAF {pg/mL)	Baseline	5611.9	1491.2	6.708	0
	Follow-up	3237.6	2102.2		

Table 7. Comparison of different studied parameters during follow among neonates Group A (interventional group/DHA). Significant<0.05, highly significant<0.001, White Blood Cells:WBC, Platelets: PLT, Prothrombin: PT, Partial Thromboplastin: PTT, International Normalised Ratio: INR, Platelets Activating Factor: PAF.



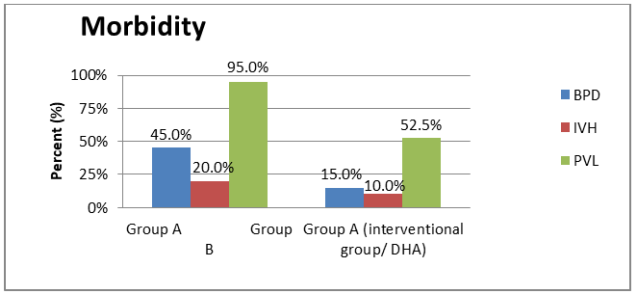


Figure 1. Mortality and Morbidity in cases (Group A) & control groups (Group B).

Discussion

DHA supplementation may improve epithelial cell integrity by decreasing bacterial or endotoxin translocation and/or mucosal PAF synthesis and receptor activation [9]. DHA functions as a mediator in the promotion of oral tolerance while also modulating the developing immune response, allowing infants to respond effectively and appropriately to both self and pathogenic environmental stimuli. DHA encourages the growth of beneficial bacteria while inhibiting the growth of pathogenic bacteria. PAF receptor and TLR gene expression in intestinal epithelial cells was also significantly reduced, despite the fact that both of these genes play important roles in the initiation of NEC [10].

With this study, we present evidence that administering DHA to preterm infants with NEC via the orogastric pathway in the acute phase may provide a benefit. The feasibility, tolerability, and efficacy of daily enteral DHA supplementation for premature infants are demonstrated in this study. Our study enrolled 80 neonates, who were randomly assigned to one of two groups (40 each). Both groups were matched in terms of age and gender. When the baseline characteristics of the two study groups were compared, it was discovered that infants randomized to receive DHA were significantly smaller than infants randomized to the placebo group. The neonates were further evaluated clinically by measuring anthropometric measurements at baseline & after 10 days follow-up evaluation.

Our study enrolled 80 neonates, who were randomly assigned to one of two groups (40 each) weight was recorded for both cases and controls, with mean weights of 1.36 and 1.29 kg in both groups B and A, respectively. After 10 days, weight was recorded again, with mean weights of 1.33 kg and 1.38 kg in groups B and A, respectively. Weight gain was found to be greater in DHA-treated infants than in the placebo group; however, this difference was not statistically significant (P=0.061). On follow-up evaluation, the weight of group A was statistically increased (P=0.001), whereas the weight loss in the placebo group was also statistically significant (P=0.003). Also, it was noticed that those who did not attain weight gain had initially higher weight in the placebo group (1.389 *vs.* 1.330 mm) (p=0.061).

It is noteworthy to mention that placebo neonates who did not gain weight during the follow-up stage were presented with higher weight proportions at baseline. These findings are similar to those of Alarcón et al. who studied 27 neonates (16 cases and 11 controls) and found that neonates who received DHA had a significant increase in total body mass and fat mass, whereas placebo neonates did not [21].

This finding supports an independent effect of supplementation, most likely because DHA administered during the acute phase of infection is quickly available to leukocytes, allowing for a better-modulated inflammatory response with fewer metabolic side effects. In terms of other anthropometric measurements taken at baseline and at the end of follow-up, the mean lengths at admission and 10 days later were 39.4 cm and 40.1 cm in groups B and A, respectively, and 40.4 cm and 41.9 cm at follow-up in groups B and A, respectively. After a 10 day follow-up, the length in group A increased significantly (P=0.000). A similar study on preterm neonates found that DHA supplemented infants had an increased rate of linear growth compared to placebo supplemented infants [22].

In terms of the OFC, there was no significant difference between the baseline and follow-up recordings. Whereas it was initially 28.3 and 28.1 cm in groups B and A, respectively, it then becomes 28.8 cm and 29.1 cm (P=0.41). Our findings were comparable to those of a previous study [23].

Although length and head circumference increased in both groups, length gain tended to be greater in infants who received DHA than in the placebo group (25 mm *vs.* 10 mm) (P =0.07). It confirms the results of our study which showed that length gain was significantly increased (P=0.000) but there was no difference in head circumference between groups (20 *vs.* 25 mm) (P=0.37). Yet there is single difference between the two studies. In our study, changes in length are significantly greater than the weight gain in the DHA group (P=0.000 *vs.* P=0.061). On the contrary, the other study demonstrated that changes in fat mass were significantly greater that the length gain in the same group (P=0.03 *vs.* P=0.07). In line with the current study was the study of Clandinin et al. who supported increased growth among neonates received DHA supplementation. On the contrary, study by Rayan et al. found that only males had restricted growth parameters when fed DHA supplemented formula [24,25].

Gastric residual was also assessed as a sign of feeding intolerance; 30% versus 100% of patients in groups B and A,

respectively, had repeated attacks of having gastric residual. On the second evaluation after 10 days, 87.5 % and 22.5 percent of both groups B and A, respectively, showed gastric residual, resulting in a statistically significant difference (p=0.02) between the case and control groups.

In terms of the incidence of NEC in our studied groups, 30% of those in group B were staged as stage 1A of NEC staging, while 70% were stage zero. Group A had 97.5% of patients with NEC, with 65% having stage 1A and 32.5% having stage 1B. After 10 days, evaluation revealed that group B (52.2 percent) had progressed to stage 1B, while 35 percent remained in stage 1A and 12.5% remained in stage zero. On the other hand, although some patients in Group A progressed, it was a small percentage; 7.5% and 5% progressed to 2B and 2A, respectively, and only 15% remained in stage 1A. A high percentage of signs improved (72.5%), which was regarded as a significant improvement in the clinical condition. On both occasions a high significant difference was observed among neonates of both groups (p=0.001 and <0.001) respectively. Notably, this was near to that reported by Zhang et al. in their study of 900 infants born at less than 32 weeks gestation. A recent meta-analysis disclosed that n-3 LCPUFAs supplementation were associated with a trend toward reduced risk of NEC [25]. Also Garcia et al and Innis et al. confirmed that NEC cases was lower in neonates from the DHA compared with the control group on the other side two studies reported no effect of formulas supplemented with DHA for preventing NEC in preterm neonates[27-30].

Furthermore, the platelet count also decreased in group B from 199.3 to 123.1 while; it increased from 93.7 to 192.8 among neonates of group A, yielding a high significant difference at further evaluation and demonstrates the improvement occurred in the neonates taking DHA. These findings signify the possible role of DHA during sepsis in preterm infants. On the other side the study of García et al. showed that median of platelet counts was not statistically significant when compared the DHA and control groups at the baseline and after the intervention (p=0.647) García et al. gave DHA to preterm babies on dose 75mg/kg/day [27].

In terms of the bleeding profile, it was assessed for both groups and found to be increased from 1.1 to 1.3 and decreased from 1.3 to 1.2 in neonates from groups B and A, respectively. The study found a statistically significant difference between the two groups (P 0.001). The SNAP score was evaluated for both groups initially and 10 days later, and it increased from 9.6 to 19.2 among patients in group B and decreased from 14.4 to 8.3 among neonates in group A (P0.001 and 0.001, respectively). This reflects group A's progress.

It is worth noting that one of the secondary outcome findings in our study was a significant statistical difference in the length of hospital stay between groups B (25.1 days) and A (22 days) (p=0.02). It was discovered that the length of hospital stay of group A who received DHA was significantly shorter than that of group B who received a placebo. In comparison to a clinical trial conducted in Mexico to determine whether DHA is effective in preventing or reducing the severity of Necrotizing Enterocolitis (NEC) in preterm neonates weighing 1500 gm at birth and beginning enteral feeding. One of their findings in the study was a lower stay at neonatal intensive care [29].

PAF was measured initially and it was 5048.7+1302.5 (mean + SD) in group B, while it was 5611.9 + 1491.2 (mean +SD) in group A, (p=0.07). While on follow up it becomes more higher in group B than in group A (p<0.000). A previous randomized, controlled clinical trial showed that PUFA supplementation for preterm infants reduced the incidence of NEC by having beneficial effects on epithelial cell integrity, reducing bacterial or endotoxin translocation and/or reducing mucosal PAF synthesis and receptor activation[12,31,32].

Mortality was higher among the control group (12.5% *vs.* 10%) in the current study, on the contrary Garcia et al. found that no difference in death between the DHA and control (p=0.119) [27].

Conclusion

DHA has not been used as unique intervention at a high but physiological dose; in addition, our previous results found an anti-inflammatory effect in neonates. Therefore, we expect that preterm infants may have a reduced bowel inflammatory response and lower NEC events and or severity. Large-scale intervention studies are necessary to further define the clinical benefits of DHA supplementation in preterm infants, but before this can be done, a dosing method that is both safe and efficacious (increases DHA levels) must be better developed. Studies are needed to investigate this mechanism by comparing cytokine production in DHA-supplemented and non-supplemented septic neonates, as well as DHA incorporation into leukocytes. To the best of our knowledge this is the first study that measure PAF in neonates received DHA supplements.

References

1. Niño DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016; 13(10): 590-600.
2. Khashana A, Moussa R. Incidence of feeding intolerance in preterm neonates in neonatal intensive care units, Port Said, Egypt. J Clin Neonatol 2016; 5(4):230-2.
3. Timothy L. Denning, Amina M. Bhatia, Andrea F. Kane et al. Pathogenesis of NEC: Role of the innate and adaptive immune response. Semin Perinatol 2017; 41(1): 15-28.
4. Lee JY, Plakidas A, Lee WH, Heikkinen A et al. Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. J Lipid Res 2003; 44(3): 479-86.
5. Ballard O, Morrow AL. Human milk composition: Nutrients and bioactive factors. Pediatr Clin North Am 2013; 60(1):49-74.
6. Caplan MS, Lickerman M, Adler L, Dietsch GN, Yu A. The role of recombinant platelet-activating factor acetylhydrolase in a neonatal rat model of necrotizing enterocolitis. Pediatr Res 1997; 42(6): 779-83.

7. Martin CR, DaSilva DA, Cluette-Brown JE, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. J Pediatr 2011; 159(5): 743-9.
8. Aderem A and Ulevitch R. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406(6797): 782-7.
9. Caplan MS, Russell T, Xiao Y, Amer M, Kaup S, Jilling T. Effect of polyunsaturated fatty acid (PUFA) supplementation on intestinal inflammation and necrotizing enterocolitis (NEC) in a neonatal rat model. Pediatr Res 2001; 49(5): 647-52.
10. Lee JY, Zhao L, Youn HS, et al. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. J Biol Chem 2004; 279(17): 16971-9.
11. Mayer K, Seeger W, Grimminger F. Clinical use of lipids to control inflammatory disease. Curr Opin Clin Nutr Metab Care 1998; 1(2): 179-84.
12. Carlson SE, Montalto MB, Ponder DL, et al. Lower incidence of necrotizing enterocolitis in infants fed a preterm formula with egg phospholipids. Pediatr Res 1998; 44(4):491-8.
13. Pawlik D, Lauterbach R, Walczak M, et al. Fish-oil fat emulsion supplementation reduces the risk of retinopathy in very low birth weight infants: A prospective, randomized study. JPEN J Parenter Enteral Nutr 2014; 38(6): 711-6.
14. Harris WS, Baack ML. Beyond building better brains: bridging the docosahexaenoic acid (DHA) gap of prematurity. J Perinatol 2015; 35(1):1-7.
15. Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. Jama 2009; 301(2): 175-82.
16. Endres S, Ghorbani R, Kelley VE et al. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989; 320(5): 265-71.
17. Calder PC. Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Braz J Med Biol Res 1998; 31(4): 467-90.
18. Makhlouf A-MA, Mahmoud AM, Ibrahim RG, et al. Effects of vitamin D and simvastatin on inflammatory and oxidative stress markers of high-fat diet-induced obese rats. J Sci Res Med Biol Sci 2021; 2(3): 39-50.
19. Richardson DK, Corcoran JD, Escobar GJ, et al. (2001): SNAP-II and SNAPPE-II: simplified newborn illness severity and mortality risk scores. J Pediatr 2001; 138(1): 92-100.
20. Olsen SL, Park ND, Tracy K, et al. Implementing Standardized Feeding Guidelines, Challenges, and Results. Neonatal Netw 2018; 37(4): 218-23.
21. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33(1): 179-201.
22. López-Alarcón M, Bernabe-García M, Prado MD, et al. Docosahexaenoic acid administered in the acute phase protects the nutritional status of septic neonates. Appl Nutr Invest 2006; 22(7-8): 731-7.
23. Baack ML, Puumala SE, Messier SE et al. Daily enteral DHA supplementation alleviates deficiency in premature infants. Lipids. 2016; 51 (4): 423-33.
24. López-Alarcón M, Bernabe-García M, Del Prado M et al. Docosahexaenoic acid administered in the acute phase protects the nutritional status of septic neonates. Nutrition 2006; 22(7-8):731-7.
25. Clandinin MT, Aerde JEV, Merkel KL, et al. Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. J Pediatr 2005; 146(4): 461-8.
26. Ryan AS, Montalto MB, Groh-Wargo S, et al. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. Am J Hum Biol 1999; 11(4): 457-67.
27. Zhang P, Lavoie PM, Lacaze-Masmonteil T, et al. Omega-3 long-chain polyunsaturated fatty acids for extremely preterm infants: a systematic review. Pediatrics 2014; 134(1): 120-34.
28. Bernabe-García M, Calder PC, Villegas-Silva R, et al. Efficacy of docosahexaenoic acid for the prevention of necrotizing enterocolitis in preterm infants: A randomized clinical trial. Nutrients 2021; 13(2): 648.
29. Innis SM, Adamkin DH, Hall RT, et al. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. J Pediatr 2002; 140(5): 547-54.
30. Smithers LG, Gibson RA, McPhee A, et al. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: A systematic review of randomized controlled trials. Am J Clin Nutr 2008; 87(4): 912-20.
31. Henriksen C, Haugholt, K, Lindgren, M et al. Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. Pediatrics 2008; 121(6): 1137-45.
32. Rabinowitz SS, Dzakpasu P, Piecuch S. Platelet-activating factor in infants at risk for necrotizing enterocolitis. J Pediatr 2001; 138(1): 81-6.
33. Michael S Caplan, Dyan Simon and Tamas Jilling The role of PAF, TLR, and the inflammatory response in neonatal necrotizing enterocoliti. Semin Pediatr Surg 2005; 14(3): 145-51.
34. Shoukry LR, Mohamed AN, Sharaf AEA, et al. Diagnostic markers for early detection of neonatal sepsis. J Sci Res Med Biol Sci 2021; 2(3): 13-26.