Effect of DHA on pro inflammatory cytokines including Platelets Activating Factor (PAF) and it's role in prevention of necrotizing enterocolitis in preterm/very low birth weight neonates.

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Abstract

Background: Necrotizing enterocolitis (NEC) is a critical intestinal emergency condition, which mainly occurs in preterm low birth weight infants. Necrotizing enterocolitis (NEC) is the most devastating gastrointestinal disease in neonates. The pathogenesis of NEC is not well defined but evidence strongly suggests that it is multifactorial. Prematurity and enteral feeding are major risk factors for NEC.
Objectives: The purpose of this study is to determine whether DHA is effective in the prevention or reducing severity of necrotizing enterocolitis in preterm/low birth weight neonates.
Patients and Methods: We conducted an interventional two armed double blinded randomized control trial during the period from September 2017 to August 2020 in our tertiary level NICU. Our study recruited 80 neonates, they were divided blindly in to two groups (40 each) where; one group was labelled 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. The nurses were not informed by the type of the medication they give to patients.
Results: Our results showed a significant increase in the length of the neonates received DHA than those who received placebo (P=0.000). Additionally, although all other arthropometric measurements have been significantly increased in neonates of DHA at the follow-up evaluation, it was of no significant value when compared with the neonates of the opposite group. 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). It was of most important to monitor the effect of DHA On sepsis and clinical laboratory including CBC. 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. 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.
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.

Keywords: DHA, Proinflammatory Cytokines, Platelets activating factor, Necrotizing enterocolitis, Low birth weight neonates

Accepted on 07 August, 2021

Introduction

Necrotizing enterocolitis (NEC) is the most devastating gastrointestinal disease in neonatal period. The pathogenesis of NEC is not well understood, but evidence strongly suggests that it is multifactorial in incidence. Prematurity and enteral feeding are major predisposing risk factors for NEC. An excessive inflammatory reaction by the immature intestinal function to the external stimuli, together with intestinal barrier integrity impairment abnormal bacterial colonization are considered factors implicated in pathophysiology of NEC. In Egypt, the incidence of feeding intolerance reaches 2.6%, while the incidence of NEC is 9.6% with a mortality rate up to 20-30%.Current evidence strongly suggests that immaturity of the innate immunity mediated by the TLR4 signaling pathway may contribute to excessive intestinal inflammatory response in NEC. NEC is the most critical intestinal emergency condition, which mainly considered a disease of the Preterm infants. The fast progression from the nonspecific signs to the extensive small intestinal necrosis also makes primary prevention the first priority. Recently, increasing evidence has indicated the important role of several immuneregulatory nutrients in primary prevention of NEC including bovine colostrums, prebiotics, probiotics, LCPUFA, and amino acids (glutamine,

cysteine, L-arginine, and N-acetylcysteine) [1]. One of the key inflammatory mediators associated with NEC is platelets activating factor (PAF). Platelets activating factor receptor (PAFR) blockade or enhanced intestinal PAF degradation via PAF acetylhydrolase (PAF-AH) supplementation reduces the incidence of NEC. We hypothesized that the protective ef fect of PUFA on NEC may be due to the ability of PUFA to suppress PAFR gene expression. Premature infants are at increased risk of NEC because Long chain poly unsaturated fatty acids (LCPUFA) accretion occurs primarily during the third trimester of pregnancy, when maternal serum levels are high and also growth and development of brain is maximum. The lack of exposure of premature infant's gut to PUFA is associated with increased levels of their microbiota and risk for NEC. Preterm has an immature immuneregulatory system, added to the.dysregulation of the inflammatory responses that play a crucial role in the incidence of many life threatening neonatal diseases including NEC. Furthermore, preterm relies heavily on the non specific innate immune response for defense. The antigen-specific adaptive immune system of preterm infants is also underdeveloped at birth particularly with regard to T-cells mediating inflammatory responses. DHA supplementation may have beneficial effects on intestinal cell integrity by reducing bacterial or endotoxin translocation and/or reducing mucosal PAF formation and receptor activation. Docosahexanoic acid (DHA) serves as a mediator to promote oral tolerance and also modulates the developing immune response allowing the neonates to respond effectively to self and pathogenic environmental stimuli. DHA supports the colonization of beneficial bacteria and protects against growth of pathogenic bacteria. Also, both reduced PAF receptor and TLR gene expression in intestinal epithelial cells play a significant role in the initiation of NEC [2].

Aim of the work

Necrotizing enterocolitis (NEC) is a critical intestinal emergency condition, which mainly occurs in preterm low birth weight infants. The purpose of this study is to determine whether docosahexaenoic acid is effective in the prevention or reducing severity of necrotizing enterocolitis in preterm/low birth weight neonates.

Patients and methods

Type of Study: Interventional two arm, double blinded, randomized controlled clinical trial (IIb)

Study Setting : Neonatal intensive care units (NICU) of Ain-shams university hospitals

Study Period : September 2017 – September 2020

Study population

The study was performed on preterm/Very low birth weight neonates admitted in Ain-Shams University NICUs. Patients was enrolled in the study after consideration of inclusion and exclusion criteria and obtaining an informed consent from the parents or caregivers.

Patients are eligible for inclusion if they meet the following criteria: Preterm neonates having a gestational age equal or less than 32 weeks at birth, admitted in Ain Shams University NICU'S.

Weight (less than or equal 1.500 KG)

Clinically stable to begin enteral feeding

All Genders (Male, Female)

Written informed consent by parents

Exclusion criteria:

- Persistent bleeding at any level.
- Receiving medication to avoid coagulation.
- Persistent vomiting.
- Gastrointestinal malformations.
- Mother taking Omega-3 supplements and planning to breastfed.

The enrolled patients were subdivided into two groups; group A are infants receiving DHA, group B are infants receiving placebo (physically matched solution).

Our patients were classified into two groups

Group A (interventional group/ DHA): Forty preterm infants were included to receive 100mg DHA daily administered by enteral route for 10 days.

Group B (control group / Placebo): Forty of preterm infant controls were included to receive placebo (physically matched solution).

Sample size: The sample size is calculated for the two groups receiving DHA versus Placebo. The sample size is calculated using the incidence densities of NEC reported in the literature of 4.4/1000 and 17.3/1000 baby day. At least 20 babies should be included in each arm to reach an effect size of 0.8 with alpha error = 0.05 and power 0.80 (G*Power 3.1.7, Franz Faul, Universität Kiel, Germany, 2013) [3].

Materials and Methods

All infants received the standard neonatal care and underwent follow-up from birth until reach 37 weeks corrected gestational age, discharge or death whichever comes first.

Feeding protocol

Begin as early as the neonate can tolerate (when hemodynamically stable). Start with low volume 0.5 to 1 ml/6 hours, increasing gradually according to the tolerance (trophic feeding 10-20 ml/kg) start deduction of feds from total fluid intake thereafter. Gut priming is not used in patients with severe hemodynamic instability, suspected or confirmed NEC. Increment from 10 to 20 ml/kg/day.

DHA supplementation

Group (A): 100 mg DHA was given to all neonates in group A as soon as possible with the enteral feeding for 10 days (subtracted from the total volume of the feed).

Group (B): 1 Predominantly received placebo (physically matched solution) together with their regular feeding.

Data Collection: Careful history taking

Antenatal history including: Rupture of membrane, Chorioamnionitis, history of urinary tract infection.

Natal history including: Mode of delivery, place of delivery, the need for resuscitation, recorded Apgar score at 1minute and 5 minutes.

Postnatal history including: Age of admission in neonatal intensive care unit, symptoms suggest infection.

Thorough clinical assessment: Weight, length and OFC (twice weekly).

Complete examination including cardiovascular, respiratory, and neurological examination.

Abdominal examination was done focusing on: signs of feeding intolerance

Laboratory investigations: Complete blood picture, C-reactive protein, bleeding profile.

Quantitative ELISA technique for PAF done for both cases and controls done twice. Baseline sample withdrawn once NEC is suspected and the other one withdrawn 10 days from the first sample (to assess the decrease in PAF levels).

Radiological investigations: Abdominal X-ray Erect Supine (when necrotizing enterocolitis is suspected).

Abdominal ultrasound (when necrotizing enterocolitis is suspected).

1- Primary outcome: All infants underwent follow-up from birth until reach 37 week corrected gestational age, discharge or death whichever comes first. The following primary outcome data will be recorded clinical examination and radiological investigations when clinically indicated for evidence of NEC [4].

2- A secondary outcome measure

3- Tertiary outcome

Data Management and 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) (Table 1).

		Study group		X2	P value
		Group B (control group / Placebo)	Group A (interventional group/ DHA)		
Gender	Male	N.	15	14	0.054	0.816
		%	0.375	0.35		
	Female	N.	25	26		
		%	0.625	0.65		

Table 1. *Gender distribution among studied groups.*

Sepsis was not considered as exclusion criteria (according to SNAP 11; 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) (Table 2).

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

Table 2. *Anthropometric measurements of the two studied groups.*

The length showed significant statistical increase in the cases group compared to the control group in the follow up evaluation. P= 0.000. Also, weight of cases showed noticeable increase in the follow up stage (although being non-significant) (Table 3).

			Study group		X2	P value
			Group B	Group A		
Lactation	BF	N.	24	28	---	0.61
		%	0.6	0.7		
	Supplemtary BF with AF	N.	16	12	---	0.342
		%	0.4	0.3		
Mode of Delivery	VD	N.	14	16	0.213	0.644
		%	0.35	0.4		
	LSCS	N.	26	24		
		%	0.65	0.6		

Maternal risk factors (Antenatal risk factors) PROM, UTI, Chorioamnionitis, PET	No	N.	10	14	0.952	0.329
		%	0.25	0.35		
	Yes	N.	30	26		
		%	0.75	0.65		

Table 3. *Natal history between the 2 studied groups (cases and controls).*

DHA supplementation may have beneficial effects on epithelial cell integrity by reducing bacterial or endotoxin translocation and/or reducing mucosal PAF synthesis and receptor activation (Table 4).

	Study group	**Mean**	**SD**	**t**	**P value**
Abdominal Girth Baseline	Group B	24.83	1.999	-6.492	0
	Group A	27.4	1.516		
Abdominal Girth 10 Days	Group B	27.13	1.713	4.576	0
	Group A	24.78	2.759		

Table 4. *Abdominal distension as a sign of feeding intolerance in the two studied groups.*

With this study we are presenting evidence that administration of DHA to preterm infants with NEC may provide a benefit when administered by orogastric pathway in the acute phase. This study demonstrates the feasibility, tolerability and efficacy of daily enteral DHA supplementation for premature infants. Our results should aid in the development of larger multicenter studies aimed at improving both short- and long-term outcomes in premature infants through optimization of daily enteral DHA provision (Table 5).

				Study group		**X2**	**P value**
				Group B	**Group A**		
Intestinal Sounds	Baseline	No	N.	0	15	40.99	0
			%	0	0.375		
		Yes	N.	28	2		
			%	0.7	0.05		
		Decreased	N.	12	23		
			%	0.3	0.575		
	10 Days	No	N.	25	5	29.333	0
			%	0.625	0.125		
		Yes	N.	8	32		
			%	0.2	0.8		
		Decreased	N.	7	3		
			%	0.175	0.075		
Passage of stool	Baseline	Bloody	N.	0	5	5.333	0.021
			%	0	0.125		
		Positive	N.	40	35		
			%	1	0.875		
	10 Days	Bloody	N.	9	0	13.373	0.001
			%	0.225	0		
		Positive	N.	31	36		
			%	0.775	0.9		
		Absent	N.	0	4		
			%	0	0.1		
Gastric Residual	Baseline	No	N.	28	0	43.077	0.021
			%	0.7	0		
		Yes	N.	12	40		
			%	0.3	1		
	10 Days	No	N.	5	29	29.463	0.021
			%	0.125	0.725		
		Yes	N.	35	11		
			%	0.875	0.275		
Modified NEC BELL'S criteria	Baseline	0	N.	28	1	43.296	0.001
			%	0.7	0.025		
		1A	N.	12	26		
			%	0.3	0.65		
		1B	N.	0	13		
			%	0	0.325		
	10 Days	0	N.	5	29	46.141	0
			%	0.125	0.725		
		1A	N.	14	6		
			%	0.35	0.15		
		1B	N.	21	0		
			%	0.525	0		
		2A	N.	0	2		
			%	0	0.05		
		2B	N.	0	3		
			%	0	0.075		

Table 5. *Other signs of feeding intolerance (intestinal sounds and Passage of stool and gastric residual and modified NEC BELL's criteria) in the two studied groups.*

Studies in human beings have found that using dietary supplementation with long chain fatty acids suppresses cytokine production by mononuclear leukocytes (Endres et al., 1989). Or improves the course of inflammatory diseases (Table 6).

		Study group	Mean	SD	t	P value
HB	Baseline	Group B	12.4	1.1	1.34	0.184
		Group A	12	1.6		
	10 Days	Group B	11.1	1.7	-1.791	0.077
		Group A	11.9	1.9		
WBC	Baseline	Group B	12.1	3.2	-1.741	0.086
		Group A	13.8	5.1		
	10 Days	Group B	17.1	5	6.175	0
		Group A	11.2	3.4		
PLT	Baseline	Group B	199.3	102.3	6.068	0
		Group A	93.7	40.6		
	10 Days	Group B	123.1	73.8	-3.512	0.001
		Group A	192.8	101.4		

Table 6. *CBC findings among studied groups at baseline and 10 days follow-up evaluation.*

There was no significant difference between mean HB level neither initially nor at follow up among the 2 studied groups (p>0.05). The TLC showed no significant difference among neonates of both groups (p>0.05), while at 10 days follow up it was significantly higher in controls than in cases; reflecting the deterioration among them (p<0.001). Mean of TLC increased from 12.1 to 17.1 in group B while, it was improved in group A; decreased from 13.8 to 11.2 explaining the significant difference. The mean platelet count also decreased in group B from 199.3 to123.1 while; it increased from 93.7 to 192.8 among neonates of group A, yielding a high significant difference on baseline and further evaluation (P<0.001 and 0.001) respectively (Table 7).

		Study group	Mean	SD	t	P value
PT	Baseline	Group B	14.8	1.8	-2.391	0.019
		Group A	16.1	2.8		
	10 Days	Group B	17.9	3.3	4.765	0
		Group A	15	1.8		
PTT	Baseline	Group B	38	9.1	-3.274	0.002
		Group A	49.7	20.7		
	10 Days	Group B	61.6	17.6	6.473	0
		Group A	40.9	10		
INR	Baseline	Group B	1.1	0.1	-3.638	0
		Group A	1.3	0.3		
	10 Days	Group B	1.3	0.2	3.464	0.001
		Group A	1.2	0.2		

Table 7*. Coagulation profile in cases and control groups at baseline and follow-up evaluation.*

The bleeding profile was evaluated for both groups where, it was increased from 1.1 to 1.3 while decreased from 1.3 to 1.2 among neonates of group B and A respectively. The study had significant difference between both groups (P<0.001 and 0.001) respectively (Table 8).

		Study group	Mean	SD	t	P value
SNAP II	Baseline	Group B	9.6	4.7	-3.53	0.001
		Group A	14.4	7.1		
	10 Days	Group B	19.2	6.7	6.655	0
		Group A	8.3	7.9		

Table 8. *SNAP II score in the two studied groups.*

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 (Table 9).

	Study group	Mean	SD	t	P value
Duration of Hospitalization (Days)	Group B	25.1	4.4	2.376	0.02
	Group A	22	7.1		

Table 9. *Duration of Hospitalization in the two studied groups.*

Premature infants are at increased risk of NEC because Long chain poly unsaturated fatty acids (LCPUFA) accretion occurs primarily during the last trimester of pregnancy, when maternal levels are high and growth and brain development are rapid. The lack of exposure of premature infant's gut to PUFA is associated with their microbiota and risk for NEC (Table 10).

				Study group		X2	P value
				Group B	Group A		
Mortality		No	N.	36	35	0.125	0.723
			%	0.9	0.875		
		Yes	N.	4	5		
			%	0.1	0.125		
Morbidity	BPD	No	N.	22	34	8.571	0.003
			%	0.55	0.85		
		Yes	N.	18	6		
			%	0.45	0.15		
	IVH	No	N.	32	36	1.569	0.21
			%	0.8	0.9		
		Yes	N.	8	4		
			%	0.2	0.1		
	PVL	No	N.	2	19	18.66	0

			%	0.05	0.475		
		Yes	N.	38	21		
			%	0.95	0.525		

Table 10. *Mortality and Morbidity in cases and control groups.*

Mortality was higher in group A (12.5%) than in group B (10%) however, it was statistically insignificant (p=0.723) (Table 11).

		Study group	Mean	SD	t	P value
PAF	Baseline	Group B	5048.7	1302.5	-1.799	0.076
		Group A	5611.9	1491.2		
	10 days	Group B	5600.3	1065.8	6.075	0
		Group A	3237.6	2102.2		

Table 11*. Comparison of PAF at baseline and follow-up evaluation in the 2 studied groups.*

Despite being higher at baseline evaluation in cases group (5611.9), it become significantly much less at the follow- up evaluation (3237.6) indicating significant improvement. On the contrary, although PAF level was low in control group at the baseline evaluation (5048.7), it bcame much higher at the follow-up evaluation (Table 12).

Group A		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	Baseline	5611.9	1491.2	6.708	0
	Follow-up	3237.6	2102.2		

Table 12. *Comparison of different studied parameters during follow among neonates Group A (interventional group/DHA).*

All anthropometric measurements showed significant increase among the neonates of group A at the follow-up evaluation .However, there was no statistical significant difference between both groups at the follow-up evaluation except at length.

Discussion

NEC is a critical intestinal emergency condition, which mainly occurs in Preterm infants. The fast progression from nonspecific signs to extensive necrosis also makes primary prevention the first priority. Recently, increasing evidence has indicated the important role of several immunemodulatory nutrients in primary prevention of NEC including bovine colostrums, probiotics, prebiotics, LCPUFA, and amino acids (glutamine, cysteine, L-arginine, and N-acetylcysteine) [4]. One of the key inflammatory mediators associated with NEC is PAF. PAFR blockade or enhanced intestinal PAF degradation *via* PAF acetylhydrolase (PAF-AH) supplementation reduces the incidence of NEC. We hypothesized that the protective effect of PUFA on NEC may be due to the ability of PUFA to suppress platelet-activating factor receptor (PAFR) gene expression. Docosahexanoic acid (DHA) serve as mediator to promote oral tolerance and also modulate developing immune response allowing infants to respond effectively and appropriately to self and pathogenic environmental stimuli. DHA support the colonization of beneficial bacteria and protect against growth of pathogenic bacteria. Also significantly reduced PAF receptor and TLR gene expression in intestinal epithelial cells, both play a significant rule in the initiation of NEC. This study presents evidence that supplementation with DHA may protect the preterm neonates from development of NEC or even decrease severity of the case as well as sepsis. Such protection was achieved by providing the fatty acid as early as possible with the start of feeding or in the acute phase of infection. A study designed to demonstrate such positive effect of DHA supplementation is warranted. According to these studies, weeks are needed for the full effect of _-3 fatty acids to become effective. In addition, some studies in human beings have reported beneficial effects of acute supplementation with _-3 fatty acid when administered in the start of developing feeding intolerance. Previous studies demonstrate that circulating DHA levels are a marker of deficiency and that premature infants with higher circulating DHA levels have a lower risk of disease including necrotizing enterocolitis (NEC) (Carlson et al., 1998), retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD) as well as improved vision and neurodevelopmental outcomes . Positive health benefits are most consistently found in studies using “high dose” DHA supplementation that more closely mimic in utero accretion rates. 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 [5]. We conducted an interventional two armed double blinded randomized control trial during the period from September 2017 to August 2020 in our tertiary level NICU. Our study recruited 80 neonates, they were divided blindly in to two groups (40 each) where; one group was labelled 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. The nurses were not informed by the type of the medication they give to patients. The patients of group A had 35% males and group B had 37.5%; while group A and group B had 65% and 62.5% females respectively. The mean gestational age of group B was 31.4 weeks (SD = 1.4) and mean gestational age of group A was 31.3 weeks (SD=1.5). Both groups were age and gender matched. Baseline characteristics were compared between the two studied groups. Infants randomized to receive DHA were significantly smaller than infants randomized to the placebo group. The patients were recruited from newly admitted neonates (age at admission were 1.1 and 1.2 days in group A and B respectively. Antenatal risk factors, including Preeclampsia, chorioamnionitis and antipartum haemorrhage were recorded in 75% and 65% of group B and A respectively. Out of all patients: 35% and 40% were delivered by vaginal delivery respectively. The neonates were further evaluated clinically by measuring anthropometric measurements at baseline and after 10 days follow-up evaluation [2]. Weight was recorded for both cases and controls ;where their mean were 1.36 and 1.29 kg in both group B and A, then follow up recording of their weight was done after 10 days, where the mean weight were 1.33 and 1.38 kg in group B and A respectively. It was noticed that weight gain tend to be greater in infants who received DHA than in placebo group; however, this difference was non statistically significant (P=0.061). On follow-up evaluation, Weight of group A was statistically increased (P= 0.001), while there was weight loss in placebo group that also showed a significant difference (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 versus 1.330 mm) (P_0.061). It is important to recognize that placebo neonates who did not show an increase in their weight in the follow up stage being presented with higher proportions of weight at baseline. These findings are relatively close to those of Alarcón et al. (2006) who studied 27 neonates (16 cases and 11 controls) in which, neonates who received DHA showed a significant increase in total body mass and Fat Mass, whereas placebo neonates did not present any increment. This finding supports an independent effect of supplementation likely because DHA administered in the acute phase of infection is rapidly available for leukocytes for a better-modulated inflammatory response with lower metabolic side effects. Studies exploring this mechanism by comparing cytokine production in DHA-supplemented and non-supplemented septic neonates, and DHA incorporation to leukocytes, are underway. Regarding other Anthropometric measurements at baseline and at the end of follow-up, The length of was recorded at admission and 10 days after, the mean length were 39.4 cm and 40.1 cm in group B and A respectively and was 40.4 cm and 41.9 cm at follow up in group B and A respectively. It was noticed that length become significantly higher in group A after 10 days follow up (P_ 0.000). This come relatively close to Another study done on preterm neonates in April 2016 in united states which to determine feasibility, tolerability and efficacy of daily enteral DHA supplementation (50mg/d) in addition to standard nutrition for preterm infants (24–34 weeks GA) beginning in the first week of life [1].

The study included 60 preterm who were admitted to the NICU at Sanford Health in Sioux Falls. Infants were less than or equal to seven days of age at enrollment. The following clinical data were collected: birth weight, height, head circumference and growth chart percentile using the Fenton Growth Curve for preterm infants and the WHO Growth Chart for term infants. The results showed DHA supplemented infants had an increased rate of linear growth compared to placebo supplemented infant. Regarding the OFC; there was no significant difference regarding the baseline nor the follow up recordings. Where it initially was 28.3 and 28.1 in group B and A respectively, then it becomes 28.8 and 29.1 cm thereafter (P_0.41). Our results were relatively similar to the results of the study done by Alarcón et al. (2005). 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 versus 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 versus 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 Versus 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 versus P_0.07). It was of at most importance to stress on giving breast milk for all neonates, so fortunately; 52 patients were breast feed. According to the definition of feeding intolerance; follow up of patients for incidence of feeding intolerance was recorded where abdominal girth was measured and recorded at baseline and after 10 days. There was significant difference between group B and A regarding abdominal distension ($p<0.01$). As regards the incidence of feeding intolerance, patients were considered to have feeding intolerance if had decreased intestinal sounds. At initial evaluation; 70% of group B had normal intestinal sounds and the 30% had decreased intestinal sounds, while group A; 57.5 % had decreased intestinal sounds, 37.5% was non audible and 5% had normal intestinal sounds ($p<0.01$). So 30% of group B and 95% of group A were considered to have feeding intolerance. At follow up evaluation; the neonates of group B showed deterioration where; it was noticed that the percentage of patients having no intestinal sounds was 62.5% among group B, decreased sounds was evident in 17.5%, while 20% had normal intestinal sounds. On the other side, improvement of intestinal movements and

sounds was observed in 80% of group A, whereas it was decreased in 7.5% and non audible in 12.5%. So 80% of group A had improved intestinal sounds in the face of 80% of group B had progressed to feeding intolerance. So we found a statistical difference between both groups (p<0.01) [3]. Regarding the delayed passage of stool as a sign of feeding intolerance; 100% of group B had normal intestinal motions versus 87.5% had normal intestinal and 12.5% had bloody stool in group A. At 10 days follow up, worsening of group B was observed where 22.5% developed bloody stool, while the rest of the neonates had normal motions. On the contrary; improvement was so evident among patients of group A, where 12.5% had bloody stool initially, then improvement was evident after 10 days of follow up where, 10% had no passage of stool and the rest of the patients showed improvement. On both evaluations a significant statistical difference was evident between both groups (p =0.02 and 0.001 respectively). Gastric residual was also evaluated as a sign of feeding intolerance; 30% versus 100% of patients of both group B and A respectively had repeated attacks of having gastric residual. On the second evaluation after 10 days; 87.5% and 22.5% of both group B and A respectively showed gastric residual and this rendered a significant statistical difference (p=0.02) between the group of cases and controls. On evaluation of our studied groups regarding the incidence of NEC; 30% go group B were staged as stage 1A of NEC staging and 70% were staged as stage zero. 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 to 1B (52.2%) while, 35% remained in stage 1A and 12.5% remained in stage zero. On the other hand; although some patients of group A showed progression but in a small percentage; where, 7.5% and 5% progressed to 2B and 2A respectively and only 15% remained in stage 1A. So great percentage showed improvement of signs (72.5%) and this was considered a great improvement of 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. (2014) 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. In our study, there was no significant difference between mean HB level neither initially nor at follow up among the 2 studied groups (p>0.05). The TLC showed no significant difference among neonates of both groups (p>0.05), while at 10 days follow up it was significantly higher in controls than in cases; reflecting the deterioration among them (p<0.001). Mean of TLC increased from 12.1 to 17.1 in group B while, it was improved in group A; decreased from 13.8 to 11.2 explaining the significant difference. 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. As regard the bleeding profile, it was evaluated for both groups where, it was increased from 1.1 to 1.3 while decreased from 1.3 to 1.2 among neonates of group B and A respectively. The study had significant difference between both groups (P< 0.001). On the other hand, this come opposite to that published by Richardson et al. (2011) who demonstrate no difference in clotting factors: such as prothrombin time and partial thromboplastin time, suggesting no important effect of DHA on bleeding factors. Variation in sample sizes, ethnicity and duration of follow up might explain the different result. 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. this come into parallel to another study done by López-Alarcón et al. (2005) who found that the SNAP-II score and the leukocyte count tended to be higher and the platelet count lower in neonates who received the placebo than those received the DHA. It is worth mentioning that one of the secondary outcome finding in our study was the high statistical difference regarding the length of hospital stay being higher in group B(25.1days) than in group A(22 days) (p=0.02). It was noticed that the length of hospital stay of group A Who received DHA was much less than those receiving the placebo. In comparison to the clinical trial done in Mexico to determine whether DHA is effective in the prevention or reducing severity of necrotizing enterocolitis (NEC) in preterm neonates < 1500 gm at birth who are starting enteral feeding (López- Alarcón et al., 2017). One of their findings in the study was a lower stay at neonatal intensive care as long as lower Interleukin 1 beta in septic neonates, less IL-6, IL-10, increased weight, length and decreased organic failures in surgical neonates. In terms of mortality and morbidity in our study, mortality was higher in group A (12.5%) than in group B (10%); however, it was not statistically significant (p=0.723). This can be explained by many reasons which included the development of intracranial haemorrhage IVH (one of the most common complication of prematurity), beside the bad condition of these neonates who were suffering from stage 2 and 3 NEC, which interefere with the oral intake of DHA. Regarding BPD (one of the morbidity indicators in our study), it was higher in group B (45%) than in group A (15%), (p=0.003). Premature infants are at risk for inflammatory diseases such as bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC), which may be prevented by DHA's anti-inflammatory properties. Atopic sensitization is also known to occur early in life and it is thought that omega-3 may help prevent this. Martin et al. (2011) showed an association between DHA levels in the first four weeks of life and the development of chronic lung disease (CLD) in premature infants. Lower DHA levels in these infants were significantly associated with the development of CLD. his study showed that a 1% decrease in the total fatty acid mass of DHA in the blood gave a 2.5 fold increase in the odds of developing CLD. PVL also was higher in group B than in group A (95% versus 52.5%) respectively, (p<0.001). DHA is one of the most abundant LCPUFAs in the brain and has an important role in brain development including roles in neurotransmission and neurogenesis.

DHA is also found in high levels in the visual system, especially in rod photoreceptors and M retinal ganglion cells. From reviews of previous studies, the most consistent benefit from DHA supplementation was seen in earlier premature infants with lower birthweights, typically <1500 g, as these infants missed the majority of the third trimester with the highest fetal accretion rates of DHA. Janssen and Kiliaan (2014) Tam et al observed a cohort of 60 preterm infants between 24 and 32 weeks gestational age and noted an association between higher early red blood cell DHA levels and a decrease in both the incidence and severity of intraventricular hemorrhage (IVH). The authors showed that a 1% increase in early postnatal DHA was associated with a 4.3 fold decrease in the odds of intraventricular hemorrhage. At 30–36 months corrected age (mean age of 33 months corrected), neurodevelopment outcome was able to be assessed in 45 of the 60 enrolled infants using the Bayley Scales of Infant Development, 3rd edition (BSID-3). The study showed an association with higher early DHA levels and improved developmental outcome at 30–36 months old that was not fully explained by the decreased risk of IVH. Although we have agreed with the previous study on the effect of DHA on the Neurodevelopmental outcome of preterm neonates, our study showed a different result regarding IVH, where IVH was higher in group B than in group A (20% versus 10%) respectively, despite being statistically insignificant (p=0.2).This may be due to differences in the study design, Gestational age of participants, and the dose, route, duration of the DHA given. Another study was done in Australia called DINO trial. The DHA for the Improvement of Neurodevelopmental Outcome in preterm infants (DINO), which include premature infants delivered at <33 weeks gestation to mothers with a high-DHA or standard-DHA diet until they reach 40 weeks corrected age (Makrides et al., 2009). Mothers of infants in the high-DHA group took 6 capsules daily, containing DHA-rich tuna oil and the mothers of infants in the standard-DHA diet received placebo soy capsules. When analyzing the breast milk of the two groups, the high-DHA group had milk with a mean DHA concentration of 0.85% versus 0.25% in the standard DHA group. At 18 months the Mental Development Index (MDI) of the BSID-II was performed. Overall, there was no statistically significant difference between the high DHA and the standard DHA group. Although the MDI in infants with birth weights <1250 g was higher, it did not reach statistical significance when adjusted for gestational age, maternal education and birth order. When comparing only the girls in the high-DHA and standard-DHA groups, there was a statistically significant improvement in MDI scores in the high-DHA group. The proportion of infants with severe developmental delay was also significantly less in the high-DHA group. Premature infants are deficit in DHA at birth and while short-term developmental benefits have been seen with DHA supplementation, no effects on long-term development have been shown. Because studies vary in the dosage, delivery, and length of DHA supplementation, it remains difficult to come to a decisive conclusion on DHA and cognitive outcomes. Until an optimal method for supplementing DHA to a preterm infant is found, it will continue to be a challenge to determine the true effect DHA supplementation has on the cognitive development of a premature infant. 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 5600.3 +1065.8 (mean+SD) than in group A 3237.6+2102.2 (mean +SD) (p<0.000). A previous randomized, controlled clinical trial showed that PUFA supplementation for preterm infants reduced the incidence of NEC (Carlson et al., 1998) by having beneficial effects on epithelial cell integrity, reducing bacterial or endotoxin translocation and/or reducing mucosal PAF synthesis and receptor activation. In *vivo* studies have demonstrated that n-3 fatty acids protected mice from hypoxia-induced bowel necrosis by directly inhibiting endogenous PAF production and leukotriene B4 production. In addition, dietary supplementation of eicosatrienoic acid and DHA suppressed PAF generation in mouse peritoneal cells (Watanabe et al., 2001). Furthermore, DHA has been shown to reduce lipopolysaccharide (LPS)-induced IL-6 production (Moon and Pestka, 2003). LPS derived from Gram-negative bacteria is a dominant ligand for TLR4. Recent evidence showed that PUFA inhibited nuclear factor (NF)-κB activation and COX-2 expression induced by LPS or lipopeptide (TLR2 agonist) in macrophages. Therefore, there may be multiple pathways whereby PUFA impact intestinal inflammation and necrosis. Many studies have been done to study the effect of enteral administration of DHA on the proinflammatory cytokines including IL-1B, IL-6 and IL-10 other than PAF. Garcia et al have found that administration of DHA to the preterm neonates decrease the severity of NEC by lowering the levels of Interleukin (IL)-1 beta, IL-6 and IL-0 (Caplan et al., 2001).

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.

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Citation: *Wafaa Osman Ahmed Osman, Hisham Abd El Samie Awad, Ola Galal Badr El-Deen, Noha Mokhtar Kamal Mahmoud, Sally Mohamed Hassan Mohamed . Effect of DHA on pro inflammatory cytokines including Platelets Activating Factor (PAF) and it's role in prevention of necrotizing enterocolitis in preterm/very low birth weight neonates. Curr Pediatr Res 2021;25(10):01-10.*

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