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Effect of omega-3 Fatty Acid Supplementation in Preterm Neonates

ShaimaaA.Belal[1], Rasha M.G EL shafie[1], Manal M.El batch[2], HebaSaid Elmahdy[1]

Departments of [1]Pediatrics, [2] Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt.
Corresponds to Shaimaa A. Belal MSc, Pediatric department, Faculty of Medicine, Tanta University , Tanta, Egypt. Tel,+201023747251, e-mail: dr.shaimaabilal2012@gmail.com.

Abstract
Background; *There is growing evidence that, in addition to effects on development, omega-3 LCPUFAs may reduce the incidence or severity of neonatal morbidities by affecting different steps of the immune and anti-inflammatory response. Also omega-3 LCPUFA have been linked to a favorable impact on lipidemic profile.*
Objectives; *The aim of our study was to assess the effect of omega-3 fatty acid supplementation in preterm neonates and their lipid profile.*
Patients and methods; *Our study included two groups: first group**:** included 25 preterm neonates whose birth weight was appropriate for gestational age, received omega-3 supplement (DHA 40 mg/kg day)with regular preterm formula within 5 days of the first enteral feeding for 21 days or until discharge, whatever comes first. Second groupincluded 25 preterm neonates matched to group 1 as regard gestational age and birth weight, received regular preterm formula without any supplement. CBC, liver enzymes, kidney function tests and lipid profile were done on enrollment, before omega-3 supplementation and after 21 days of initiation of therapy or before discharge of the case for the cases (group1) and for the control(group2) on enrollment and before discharge .*
Results; *There was significant decrease in serum cholesterol, triglycerides, LDL and VLDL while significant increase in serum HDL after omega-3 supplement in group 1. There was no statistical significant difference between two groups as regard growth, duration of respiratory support measures, secondary outcome of prematurity and duration of hospital stay.*
Conclusion; *Omega-3 supplementation in preterm infants in a dose **(40 mg/kg/day)** decreases serum level of cholesterol, triglycerides, LDL, VLDL and increases serum HDL, no effect of omega-3 supplementation on rate of growth, improving of feeding intolerance, duration of respiratory support during hospital stay, no significant difference as regard the total duration of hospital stay or secondary outcome(ROP, IVH, NEC, BPD and death) between the supplementation group and control group, omega-3 could be safe in preterm neonates for controlling serum lipid profile.*
Keywords; *Omega-3, preterm, lipid profile.*

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I. Introduction

Preterm infants are live born infants delivered before 37 weeks from the first day of last menstrual period [(1)].

Preterm delivery is a major cause of perinatal mortality and morbidity and sepsis remains common complication of prematurity[(2)].

Other comorbidities include respiratory distress syndrome (RDS), bronchopulmonary dysplasia, persistent pulmonary hypertension, intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP) as well as necrotizing enterocolitis (NEC) are due to the difficulty of extra uterine adaptation due to immaturity of organ systems [(3)].

Omega-3 fatty acids are essential poly unsaturated fatty acids (PUFAs) because they are required by our body to synthesize prostaglandins and other physiological regulators. They include eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and Alpha-linolenic acid [(4)].

Omega-3 fatty acids are essential for proper fetal development and supplementation during pregnancy has also been linked to decrease immune responses in infant including decreased incidence of allergies in infants [(5)].

. Omega-3 polyunsaturated fatty acids, in particular EPA and DHA have been implicated to have an

inhibitory effect on pro-inflammatory cytokines, such as tumor necrosis factor-a (TNF-a), interlukin-1and interlukin-6 [(6)].Fetal deficiency of omega-3 may place infant at risk for allergic diseases and suboptimum neuropsychiatric development[(7)].

The brain is particularly vulnerable to the effects of nutrition between 24 and 42 weeks of gestation. Thus, fetal and neonatal malnutrition may have global or isolated effects on the developing brain, depending on the requirements of the specific nutrients at the time of the deficit[(8)].

Both early and enriched supply of energy, protein and lipids have shown to be beneficial for growth and neurodevelopment[(9)].

Infants born prematurely have low total body long chain polyunsaturated fatty acid stores and the docosahexaenoic (DHA) and arachidonic acid (ARA) content of preterm human milk may be insufficient to meet increased needs, therefore the current ESPGHAN standard for preterm infants also recommends fortification of mothers own human milk as necessary to meet high LCPUFA requirements (11-27 mg DHA/ 100 kcal and 16-39mg ARA/100kcal) or use of infant formulas designed for premature infants [(10)]. Omega-3 LCPUFA have been linked to a favorable impact on lipidemic profile[(11)].

Patients and methods

The study was a case control study which was carried out in Neonatal Intensive Care Unit at Tanta University Hospital from the period between June 2016 to June 2018. The study was approved by Tanta Research and Ethical Committee and written informed consent was obtained from parents.

Infants included in the study were divided into two groups:

- Group 1**:** included 25 preterm neonates whose birth weights were appropriate for gestational age, received omega-3 supplement (DHA 40 mg/kg/day)[(12)](pure encapsulations)**,** Montana omega 300, each capsule contain 1000 mg of omega-3 only) with regular preterm formula within 5 days of the first enteral feeding for 21 days or until discharge, whatever comes first.
- Group (2):included 25 preterm neonates matched to group 1 as regard gestational age and birth weight, received regular preterm formula without any supplement.

Inclusion criteria:

Any preterm neonates with gestational age less than or equal 34 weeks and their birth weight appropriate for gestational age.

Exclusion criteria:

- Full term neonates.
- Preterm infants died before feeding started.
- Intrauterine growth restriction (IUGR).
- Multiple congenital anomalies including GIT anomalies.
- Congenital infection.
- Cholestasis.

Both groups were subjected to complete history taking including

Maternal age, parity, maternal diseases (hypertension, diabetes),history of PROM, history of maternal dietary intake of omega-3 during pregnancy, gestational age, gender, birth weight, birth order, APGAR score at 1minute and 5 minutes. Alsogeneral examination with special emphasis ongestational age assessment by New Ballard Scoring system[(13)]and anthropometric measurements(weight, length and head circumference) were taken on enrollment and before discharge.

We calculated weight gain daily, head circumference and length weekly and linear models were done to explore growth over time in both groups during NICU stay. Also general examination including

1-Eye examination for exclusion of ROP: was performed by ophthalmologist at 4 weeks of postnatal age. Also it was performed for preterm babies< 32 weeks or their birth weight <1500 gm and also for those 32-34 weeks and 1500-2000 gm and have risk factors for developing ROP (sepsis, respiratory distress syndrome, prolonged oxygen supplementation, hypotension, hypothermia and hypoxia) [(14)].

2- Chest examination: to detect signs of respiratory distress (tachypnea, retraction, grunting, cyanosis) [(15)].

3- Abdominal examination: to detect GIT problems like GERD, feeding intolerance (abdominal distention, constipation, gastric residual more than 25%, loose stool, repeated vomiting)[(16)].

We observed all the cases as regard:

1. Duration of mechanical ventilation, CPAP and other oxygen support.
2. Days on omega-3 fatty acid supplementation in group 1.
3. Days needed to reach full enteral feeding more than or equal 150 ml/kg/day.
4. Occurrence of adverse effects of prematurity (ROP, IVH, NEC, BPD, thrombocytopenia, sepsis, DIC and

death) during NICU stay.
5. Length of hospitalization.

Laboratory work up:
5ml of blood were withdrawn from each case for the following:
Complete blood count: blood was obtained from peripheral veins on EDTA tubes and it was assayed by the SYSMEX SF-3000 autoanalyzer system [(17),] **Liver enzymes(ALT, AST)and kidney function tests(blood urea, serum creatinine)**blood was obtained from peripheral veins and allowed to clot at room temperature for 20min. serum was separated by centrifugation (20 min, 2500r.p.m) and kept at -20^0c until analysis. They were assayed by Riele Photometer 5010[(18)].
Lipid profile: blood were obtained from peripheral veins and allowed to clot at room temperature for 20min. serum was separated by centrifugation (20 min, 2500r.p.m) and kept at -20^0c until analysis. **Serum cholesterol, triglycerides** and **HDL** were measured by enzymatic colorimetric method (PAP) using (BioSTC, High Performance Diagnostic Reagents) [(19)] .**LDL** was determined by direct enzymatic colorimetric method using SaluceaHaansberg 19 4874 NJ EttenLeur, The Netherlands [(20)].**VLDL** was calculated by friedewald'sformula [(21)].
ALL investigations were done on enrollment, before omega-3 supplementation and after 21 days of initiation of therapy or before discharge of the case for the cases and for the control on enrollment and before discharge.

Statistical analysis of the data
Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) **.**Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

II. Results

1. There was no statistically significant difference between the two studied groups as regard demographic data including gestational age, birth weight, gender, Apgar score at 1 minute and at 5 minutes (table 1).
2. There was no statistically significant difference between the two studied groups regarding maternal history including age, parity, diabetes, hypertension, history of PROM and maternal dietary intake of omega 3 during pregnancy (table 2).
3. There was no statistically significant difference between the two studied groups as regard the daily weight gain or weekly increase of length and head circumference (table 3).
4. There was no statistically significant difference between both groups as regard Hb %, WBCs and platelet count. But within the same group: there was significant drop of Hb % and WBCs count before discharge compared to that done on enrollment. But as regard platelet count: it was significantly increased in group I while nonsignificant increase in group II between enrollment and discharge levels (table 4).
5. There was no statistical significant difference between the two studied groups regarding liver enzymes including serum ALT and serum AST either on enrollment or before discharge. Within the same group there was significant decrease in them in both groups before discharge (table 5)
6. There was statistically significant increase in serum cholesterol in group I than in group II on enrollment while nonsignificant difference between them before discharge. As regard triglycerides level no significant changes between both groups on enrollment while before discharge, the triglycerides level was significantly lower in group I than in group II. Within the same group there were significant drop in serum cholesterol and triglycerides in group I while insignificant increase in group II before discharge(table 6).
7. Within group I there was insignificant increase in serum cholesterol and triglycerides before omega-3 supplement but after omega-3 supplement there was significant decrease in them (table 7).
8. There was no statistical significant difference between the two studied groups as regard serum LDL, HDL and VLDL on enrollment but before discharge there was significant decrease in serum LDLand VLDL. Also significant increase in serum HDL in **group I.** While in **group II** there was insignificant increase in serum LDL and VLDL also insignificant decrease in serum HDL (table 8).
9. Within **group I** there was insignificant increase in serum LDL and VLDL before omega-3 supplement while after omega -3 supplement there was significant decrease in them. Regarding serum HDL there was insignificant decrease in it before omega-3 supplement but after omega-3 supplement there was significant increase (table 9).
10. There was no statistical significant difference between the two studied groups regarding duration of respiratory support measures including mechanical ventilation, CPAP and other oxygen support measures (table 10).

11. There was no statistical significant difference between the two studied groups regarding adverse effects of prematurity including ROP, DIC and death, there were no cases of NEC, BPD and IVH (table 11).
12. Mean days needed to reach full enteral feeding in **group I** was 8.36 ± 3.26 and in**group II** was 9.92 ± 3.34 with no significant difference between both groups (table 12).
13. There was no statistical significant difference between the two studied groups regarding length of NICU stay (table 13).

Table 1: comparison between the two studied groups regarding demographic data:

	Group I (n = 25)		**Group II (n = 25)**		**Test of Sig.**	**p**
	No.	**%**	**No.**	**%**		
Sex						
Male	12	48.0	7	28.0	x^2=	0.145
Female	13	52.0	18	72.0	2.122	
Gestational age (weeks)						
Min. – Max.	31.0 – 34.0		32.0 – 34.0		t =	0.635
Mean ± SD.	32.80 ± 1.0		32.92 ± 0.76		0.478	
Median	33.0		33.0			
Mode of delivery						
CS	10	40.0	12	48.0	X^2=	0.569
NVD	15	60.0	13	52.0	0.325	
Birth weight (kg)						
Min. – Max.	1.10 – 1.72		1.150 – 1.70		t =	0.841
Mean ± SD.	1.60 ± 0.21		1.59 ± 0.14		0.202	
Median	1.70		1.60			
Apgar score at 1minute						
Min. – Max.	3.0 – 7.0		3.0 – 7.0		U=	0.871
Mean ± SD.	5.04 ± 1.06		5.0 ± 1.04		304.50	
Median	5.0		5.0			
Apgar score at 5 minutes						
Min. – Max.	3.0 – 7.0		4.0 – 7.0		U=	0.951
Mean ± SD.	5.84 ± 1.21		5.92 ± 0.91		309.50	
Median	6.0		6.0			

*χ^2, p: χ^2 and p values for **Chi square test** for comparing between the two groups; ^{FE}p: p value for **Fisher Exact** for Chi square test for comparing between the two groups; t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups*

Table 2 : Maternal history of the two studied groups:

	Group I (n = 25)		Group II (n = 25)		Test of Sig.	p
	No.	%	No.	%		
Maternal age (years)						
Min. – Max.	20.0 – 35.0		20.0 – 33.0			
Mean ± SD.	26.20 ± 4.22		26.08 ± 3.67		t=0.809	0.422
Median	25.0		27.0			
Parity						
P1	12	48.0	11	44.0		
P2	6	24.0	9	36.0		
P3	2	8.0	3	12.0	X^2=2.510	^{MC}p= 0.707
P4	4	16.0	2	8.0		
P5	1	4.0	0	0.0		
Maternal diabetes						
No	25	100.0	25	100.0	-	-
Yes	0	0.0	0	0.0		
Maternal dietary intake of omega-3						
None	21	84.0	23	92.0		
Irregular	2	8.0	1	4.0	X^2= 0.913	^{MC}p= 0.699
Regular	2	8.0	1	4.0		
History of PROM						
No	21	84.0	21	84.0	0.000	^{FE}p= 1.000
Yes	4	16.0	4	16.0		
Maternal hypertension						
No	23	92.0	22	88.0	x^2=	^{FE}p=
Yes	2	8.0	3	12.0	0.222	1.000

*χ^2, p: χ^2 and p values for **Chi square test** for comparing between the two groups; ^{FE}**p:** p value for **Fisher Exact** for Chi square test for comparing between the two groups; ^{MC}p: p value for **Monte Carlo** for Chi square test for comparing between the two groups; t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups*

Table 3: comparison between the two studied groups regarding rate of increment of anthropometric measurements:

	Group I (n = 25)	Group II (n = 25)	t	p
Weight (gm/day)				
Min. – Max.	15.0–20.0	13.50 –20.0		
Mean ± SD.	17.52±1.50	16.98 ±2.25	0.997	0.324
Median	17.0	17.40		
Length (cm/week)				
Min. – Max.	0.42 – 0.70	0.40 – 0.70		
Mean ± SD.	0.53± 0.06	0.50 ± 0.06	1.669	0.102
Median	0.55	0.49		
Head circumference (cm/week)				
Min. – Max.	0.20 – 0.40	0.18 – 0.40		
Mean ± SD.	0.36 ± 0.04	0.35 ± 0.05	1.210	0.232
Median	0.37	0.36		

*t, p: t and p values for **Student t-test** for comparing between the two groups; *: Statistically significant at $p \leq 0.05$*

Table 4 : CBC in both studied groups on enrollment and before discharge:

CBC		Group I (n = 25)	Group II (n = 25)	Test of Sig.	p
Hb (gm/dl)	**On enrollment**				
	Min. – Max.	12.50 – 16.10	10.70 – 16.0	t = 0.698	0.489
	Mean ± SD.	14.57 ± 0.97	14.34 ± 1.31		
	Median	14.80	15.0		
	Before discharge				
	Min. – Max.	10.90 – 13.0	11.0 – 13.0	t = 0.416	0.680
	Mean ± SD.	11.98 ± 0.62	11.90 ± 0.76		
	Median	11.87	12.0		
	% change	↓17.46 ± 6.26	↓16.30 ± 9.71	U=297.0	0.764
	^{t}p	<0.001*	<0.001*		
WBCs(Cx10^3/cmm)	**On enrollment**				
	Min. – Max.	8.0 – 25.0	6.0 – 26.0	U = 265.50	0.360
	Mean ± SD.	12.35 ± 3.85	14.65 ± 6.03		
	Median	11.50	12.0		
	Before discharge				
	Min. – Max.	8.0 – 12.0	8.0 – 15.30	U = 226.0	0.091
	Mean ± SD.	10.15 ± 1.10	11.73 ± 2.79		
	Median	10.10	12.0		
	% change	↓12.62 ± 21.49	↓12.61 ± 24.21	U=300.0	0.808
	^{Z}p	0.004*	0.003*		
Platelet(Cx10^3/cmm)	**On enrollment**				
	Min. – Max.	160.0 – 300.0	120.0 – 300.0	U =309.0	0.945
	Mean ± SD.	246.80 ± 43.47	239.40 ± 64.10		
	Median	250.0	260.0		
	Before discharge				
	Min. – Max.	170.0 – 300.0	150.0 – 300.0	U = 254.50	0.255
	Mean ± SD.	269.20 ± 33.03	250.40 ± 49.28		
	Median	280.0	270.0		
	% change	↑10.49 ± 12.40	↑7.63 ± 14.74	U=230.0	0.105
	^{Z}p	<0.001*	0.072		

*t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups; ^{t}p: p value for **paired t-test for** comparing between on enrollment and before discharge in each group; ^{Z}p: p values for **Wilcoxon signed ranks test** for comparing betweenon enrollment and before discharge in each group; *: Statistically significant at p ≤ 0.05*

Table 5: liver enzymes in both studied groups:

Liver Enzymes		Group I (n = 25)	Group II (n = 25)	Test of Sig.	p
Serum ALT (U/L)	**On enrollment**				
	Min. – Max.	13.0 – 40.0	12.0 – 54.0	t = 1.367	0.179
	Mean ± SD.	23.80 ± 7.02	27.60 ± 11.99		
	Median	23.0	25.0		
	Before discharge				
	Min. – Max.	12.0 – 32.0	12.0 – 40.0	t = 1.241	0.221
	Mean ± SD.	18.96 ± 5.50	21.32 ± 7.75		
	Median	18.0	19.0		
	% change	↓10.09 ± 49.51	↓13.02 ± 39.69	U=305.50	0.892
	^{t}p	0.028*	0.010*		
Serum AST (U/L)	**On enrollment**				
	Min. – Max.	15.0 – 40.0	20.0 – 40.0	U = 223.0	0.077
	Mean ± SD.	27.60 ± 8.86	31.28 ± 8.13		
	Median	29.0	30.0		
	Before discharge				
	Min. – Max.	12.0 – 25.0	16.0 – 30.0	U = 229.0	0.102
	Mean ± SD.	20.20 ± 4.75	22.52 ± 4.72		
	Median	21.0	23.0		
	% change	↓18.50 ± 35.38	↓24.14 ± 22.79	U=302.0	0.838
	^{Z}p	0.005*	<0.001*		

*t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups; ^{t}p: p values for **Paired t-test** for comparing between on enrollment and before discharge in each group; ^{Z}p: p values for **Wilcoxon signed ranks test** for comparing between on enrollment and before discharge in each group; *: Statistically significant at p ≤ 0.05*

Table 6: Serum cholesterol and triglycerides in both studied groups on enrollment and before discharge:

Lipid profile		Group I (n = 25)	Group II (n = 25)	Test of Sig.	p
Serum cholesterol (mg\dl)	**On enrollment**				
	Min. – Max.	65.0 – 120.0	65.35 – 120.0	t =2.747*	0.008*
	Mean ± SD.	95.12 ± 19.30	80.51 ± 18.28		
	Median	100.50	70.50		
	Before discharge				
	Min. – Max.	62.0 – 92.0	58.0 – 120.0	t=1.794	0.081
	Mean ± SD.	76.28 ± 9.81	84.28 ± 20.03		
	Median	73.0	85.0		
	% change	↓17.85 ± 12.02	↑8.48 ± 30.82	U=0.0*	<0.001*
	p_1	<0.001*	0.465		
Triglycerides 'TG' (mg\dl)	**On enrollment**				
	Min. – Max.	40.80 – 70.0	40.70 – 70.0	t =0.168	0.868
	Mean ± SD.	48.41 ± 8.46	48.03 ± 7.55		
	Median	44.50	45.0		
	Before discharge				
	Min. – Max.	34.0 – 48.50	40.0 – 48.94	t=5.751*	<0.001*
	Mean ± SD.	39.02 ± 3.87	49.94 ± 7.71		
	Median	38.50	46.0		
	% change	↓18.08 ± 10.19	↑1.99 ± 4.41	U=14.0*	<0.001*
	p_1	<0.001*	0.059		

*t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups; p_1: p values for **Paired t-test** for comparing between on enrollment and before discharge in each group; *: Statistically significant at p ≤ 0.05*

Table 7: Serum cholesterol and triglycerides level in group I at different time intervals:

	Group I (n = 25)			F	p
	On enrollment	**Before omega-3 supplement**	**Before discharge (After Omega 3 supplement)**		
Serum cholesterol (mg\dl)					
Min. – Max.	65.0 – 120.50	65.50 – 121.0	62.0 – 92.0		
Mean ± SD.	95.12 ± 19.30	96. 38 ± 18.88	76.28 ± 9.81	31.158*	<0.001*
Median	100.50	95.50	73.0		
Sig. bet. periods.	p_1=0.632,p_2<0.001*,p_3<0.001*				
Triglycerides 'TG' (mg\dl)					
Min. – Max.	40.80 – 70.0	41.0 – 70.50	34.0 – 48.50		
Mean ± SD.	48.41 ± 8.46	50.13 ±8.59	39.02 ± 3.87	33.611*	<0.001*
Median	44.50	48.30	38.50		
Sig. bet. period.	p_1=0.264,p_2<0.001*,p_3<0.001*				

*F: F test (**ANOVA) with repeated measures**, Sig. bet. Periods was done using **Post Hoc Test (LSD);** p_1: p value for comparing between on enrollment and before omega 3 supplement; p_2: p value for comparing between on enrollment and after Omega 3 supplement; p_3: p value for comparing between before omega 3 supplement and after Omega 3 supplement; *: Statistically significant at $p \leq 0.05$*

Table 8: Serum LDL, HDL and VLDL in both studied groups on enrollment and before discharge:

	Lipid profile	Group I (n = 25)	Group II (n = 25)	Test of Sig.	p
Serum LDL (mg/dl)	**On enrollment**				
	Min. – Max.	22.60 – 48.0	22.50 – 45.0		
	Mean ± SD.	29.21 ± 7.84	26.33 ± 6.07	t =1.450	0.154
	Median	23.60	23.60		
	Before discharge				
	Min. – Max.	15.50 – 29.0	22.0 – 46.50		
	Mean ± SD.	21.48 ± 4.27	26.64 ± 6.54	t =3.304*	0.002*
	Median	20.0	23.20		
	% change	↓24.88 ± 9.71	↑0.99 ± 4.29	U=0.0*	<0.001*
	p_1	<0.001*	0.162		
Serum HDL (mg/dl)	**On enrollment**				
	Min. – Max.	20.0 – 36.0	20.0 – 36.50		
	Mean ± SD.	27.54 ± 3.77	28.26 ± 3.18	t =0.727	0.470
	Median	27.50	28.70		
	Before discharge				
	Min. – Max.	32.50 – 42.0	19.50 – 35.20		
	Mean ± SD.	36.36 ± 2.21	27.94 ± 3.10	t=11.068*	<0.001*
	Median	36.50	28.0		
	% change	↑34.26 ± 18.97	↓1.06 ± 3.77	U=15.0*	<0.001*
	p_1	<0.001*	0.107		
Serum VLDL (mg/dl)	**On enrollment**				
	Min. – Max.	8.16 – 14.0	8.14 – 14.0		
	Mean ± SD.	9.66 ± 1.71	9.63 ± 1.60	t =0.123	0.903
	Median	8.90	9.0		
	Before discharge				
	Min. – Max.	6.80 – 9.70	8.0 – 9.78		
	Mean ± SD.	7.79± 0.79	9.66 ± 0.38	t=10.622*	<0.001*
	Median	7.70	9.78		
	% change	↓17.69±12.52	↑2.43± 13.44	U=88.0*	<0.001*
	p_1	<0.001*	0.867		

*t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups; p_1: p values for **Paired t-test** for comparing between on enrollment and before discharge in each group; *: Statistically significant at $p \leq 0.05$*

Table 9: Serum LDL, HDL and VLDL in group I at different time intervals:

Lipid profile	Group I (n = 25)			F	p
	On enrollment	Before omega 3 supplement	After Omega 3 supplement		
Serum LDL (mg/dl)					
Min. – Max.	22.60 – 48.0	22.70 –48.30	15.50 – 29.0		
Mean ± SD.	29.21 ± 7.84	31.08 ±7.16	21.48 ± 4.27	37.128*	<0.001*
Median	23.60	30.0	20.0		
Sig. bet. periods.	p_1=0.148,p_2<0.001*,p_3<0.001*				
Serum HDL (mg/dl)					
Min. – Max.	20.0 – 36.0	20.0 –35.50	32.50 – 42.0		
Mean ± SD.	27.54 ± 3.77	25.83±4.18	36.36 ± 2.21	75.543*	<0.001*
Median	27.50	25.10	36.50		
Sig. bet. period.	p_1=0. 105,p_2<0.001*,p_3<0.001*				
Serum VLDL (mg/dl)					
Min. – Max.	8.16 – 14.0	8.18 – 14.20	6.80 – 9.70		
Mean ± SD.	9.66 ± 1.71	10.51 ± 1.99	7.79 ± 0.79	24.262*	<0.001*
Median	8.90	11. 0	7.70		
Sig. bet. period.	p_1=0.104,p_2<0.001*,p_3<0.001*				

*F: F test **(ANOVA) with repeated measures**, Sig. bet. Periods was done using **Post Hoc Test (LSD);** p_1: p value for comparing between on enrollment and before omega 3 supplement; p_2: p value for comparing between on enrollment and After Omega 3 supplement; p_3: p value for comparing between before omega 3 supplement and After Omega 3 supplement; *: Statistically significant at $p \leq 0.05$*

Table 10: Duration of respiratory support measures in both studied groups

	Group I (n = 25)	Group II (n = 25)	Test of sig.	p
Days on mechanical ventilation and CPAP				
Min. – Max.	2.0-7.0	2.0-8.0		
Mean ± SD.	4.60 ±3.13	5.20 ± 2.49	U=12.50	1.000
Median	4.0	5.0		
Days on other oxygen support (nasal canula, head box, o2 in incubator)				
Min. – Max.	3.0–5.0	3.0 –4.0		
Mean ± SD.	3.71 ± 0.76	3.38 ± 0.52	U=21. 0	0.361
Median	4. 0	3.0		

*χ^2, p: χ^2 and p values for **Chi square test** for comparing between the two groups; ^{FE}p: p value for **Fisher Exact** for Chi square test for comparing between the two groups; t, p: t and p values for **Student t-test** for comparing between the two groups; U, p: U and p values for **Mann Whitney test** for comparing between the two groups*

Table 11 :Secondary outcome in both studied groups

	Group I (n = 25)		Group II (n = 25)		□□	p
	No.	%	No.	%		
ROP						
No ROP	25	100.0	23	92.0	□ □ □ □ □	^{FE}p=
Stage1 ROP	0	0.0	2	8.0		0.490
DIC						
No	25	100.0	24	96.0	1.020	^{FE}p=
Yes	0	0.0	1	4.0		1.000
Death						
No	25	100.0	24	96.0	1.020	^{FE}p=
Yes	0	0.0	1	4.0		1.000

Table 12: Comparison between the two studied groups regarding time needed to reach full enteral fed:

	Group I (n = 25)	Group II (n = 24)	U	p
Days needed to reach full enteral feeding				
Min. – Max.	5.0 – 15.0	6.0 – 17.0		
Mean ± SD.	8.36 ± 3.26	9.92 ± 3.34	203.0	0.050
Median	7.0	8.50		

*U, p: U and p values for **Mann Whitney test** for comparing between the two groups*

Table 13: Comparison between the two studied groups regarding length of NICU stay (days):

	Group I (n = 25)	Group II (n = 25)	t	p
Length of NICU stay (days)				
Min. – Max.	19.0 – 39.0	19.0 – 45.0		
Mean ± SD.	27.20 ± 7.45	30.16 ± 8.63	1.298	0.200
Median	25.0	30.0		

*t, p: t and p values for **Student t-test** for comparing between the two groups*

III. Discussion

Preterm infants are well recognized as being at risk of DHA dietary insufficiency [(22)].Newborn preterm infants have lower plasma and red cell concentrations of DHA compared to newborn term infants [(22)]. Also extremely premature infants are at increased risk of developing deficits in omega 3 LCPUFAs due to lack of adipose stores[(23)]. It has been shown that DHA supplementation has positive effects on growth, visual function and mental development in randomized controlled trials (RCT) [(23)].

Our study included two groups: first group**:** included 25 preterm neonates whose birth weight was appropriate for gestational age, received omega-3 supplement (DHA 40 mg/kg day)with regular preterm formula within 5 days of the first enteral feeding for 21 days or until discharge, whatever comes first. Second group included 25 preterm neonates matched to group 1 as regard gestational age and birth weight, received regular preterm formula without any supplement.

The two groups had no statistical significant difference as regard demographic data or maternal history.

Comparing between the two studied groups regarding mean increase in the anthropometric measurements during NICU stay we found that that there was no statistical significant difference regarding mean increase in daily weight gain between the two studied groups as mean increase in group I was 17.52±1.50 while in group II was 16.98 ±2.25 with (p value=0.324) also there was no statistical significant difference between the two studied groups regarding length increment as mean increase per week in group I was 0.53± 0.06 while in group II was 0.50 ± 0.06 with (p value= 0.102) and there was no statistical significant difference regarding head circumference increment per week between the two studied groups as mean increase in group I was 0.36 ± 0.04 while in group II was 0.35 ± 0.05 with (p value=0.232)(table 3).

This came in agreement with**D'Ascenzo et al.,** who made a clinical trial on 47 preterm infants whose birth weight<1250 gm, were divided into 2 groups the study group received fish oil lipid and control group received intralipid with no fish oil . Their results showed that the anthropometric data between the two groups were not significantly different [(24)].

Henriksenet al.,made a randomized, double–blind, placebo-controlled study on 141 infants with birth weight < 1500 gm. The intervention group received 32 mg of DHA and 31 mg of ARA per 100 ml of human milk started 1 week after birth and lasted until discharge from hospital compared to control group with no DHA. Their results showed that there was no statistical significant difference between the two groups as regard weight, length and head circumference. The mean daily weight gain was 23.3±2.5g/day in the intervention group and 22.8 ± 4.9 g/day in the control group. The mean daily length gain was 1.2 ± 0.5 mm in the intervention group and 1.3 ± 0.7 mm in the control group, the mean gain in head circumference was 1.2 ± 0.7 mm/day in the intervention group and 1.0 ± 0.4 mm/day in the control group [(25)].

But in contrast to our study **Innis et al.,** who made a double –blind, multi-center study on 194 premature infants divided them into control group given formula with no DHA or ARA, DHA formula group, DHA+ARA formula group for at least 28 days and then fed term formula (no DHA or ARA) to 57 weeks postmenstrual age (PMA), with 90 breast-fed infants as a reference group. Their results showed that in infants fed the DHA+ARA formula gained weight significantly faster during premature formula feeding than in infants fed the control formula (mean± SD, 34 ± 1.1 and 30.7 ± 1.1 gm/day respectively, p =0.004) [(26)].

Regarding the occurrence of GIT problems including GERD and signs of feeding intolerance during NICU stay we found that there was no statistical significant difference between the two studied groups regarding GIT problems including GERD and signs of feeding intolerance as in **group I** included 22 preterm had no signs of feeding intolerance (88%) and only 3 preterm showed one or more signs of feeding intolerance (12%) while in **group II** included 14 preterm had no signs of feeding intolerance (56%) and 11 preterm showed one or more signs of feeding intolerance (44%).This came in agreement with**Baack et al.,** who studied the effect ofdaily enteral DHA supplementation (50mg/day) of DHA liquid (pure encapsulations) in addition to standard nutrition for preterm infants (24-34 weeks) beginning in the first week of life until discharge in 31 preterm infants and results were compared with those found in placebo-supplemented group (receiving standard neonatal nutrition with medium chain triglycerides –MCT oil) (n=29). Their results showed that there were no reports about feeding intolerance, loose stool or other side effects. Also there were no significant differences in the number of infants with GIT problems during NICU stay (GERD, milk protein intolerance) [(27)].

Regarding CBC we found that there was no statistical significant difference between the two studied groups in HB level, WBCs and platelet count either on enrollment or before discharge. Within the same group there was significant decrease in HB level (p value <0.001) and WBCs count (p value<0.005) and increase in platelet count in the two groups before discharge and all values were within normal level. The decrement in HB level was not considered unusual because anemia is a common finding in premature infants [(28)].

Tomsits, et al. who made a clinical trial on sixty premature neonates (age 3-7 days, gestational age <34 weeks, birth weights 1000–2500 g) received parenteral nutrition, were divided into 2 groups, the study group received SMOF-lipid (containing fish oil 30 gm/l) and the control group received Intra-lipid (with no fish oil) for a minimum of 7 up to 14 days.

Their results revealed a drop in hemoglobin, hematocrit, and RBC count at the final observation. However, it is of note that the decline from day 0 to final in these parameters was comparable in both groups. Also insignificant increase in WBCs and platelet count at final observation on both groups [(28)].

Regarding liver enzymes including (serum ALT and AST) we found that there was no statistical significant difference between the two studied groups either on enrollment or before discharge. Within the same group there were significant decrease in them in both groups before discharge (but all levels were within normal).

Tomsits et al., found that there was decrease in serum ALT in both study group (fish oil group) and control group (no fish oil group) at final observation as mean serum ALT in the study group on day 0 was 14.33± 17.94 while at final observation was 11.96± 8.83 but in the control group on day 0 was 12.03± 9.64 while at final observation was 11.10± 7.56[(28)]. They explained the decrease in liver enzymes in fish oil group as fish oil preserve hepatic integrity and this indicating good liver tolerance [(29)].

Regarding renal function tests (blood urea and serum creatinine) we found that there was no statistical significant difference between the two studied groups either on enrollment or before discharge. Within the same group there was significant decrease in blood urea before discharge in both groups (p value<0.001) and insignificant increase in serum creatinine in group1, however significantly increased in group 2 (p value=0.014).

Skouroliakou et al. who made a double-blinded randomized clinical trial on 38 infants grouped into group A received SMOF lipid emulsion (containing fish oil 30gm/l) , while group B received standard fat emulsion Intralipid (containing no fish oil).clinical and biochemical data were collected on day 0, 14 and on discharge.Their results showed that serum creatinine decreased significantly in group B but within normal range[(30)].**Tomsits et al.** found that there was decrease in blood urea and serum creatinine in both study (fish oil group) and control group at final observation[(28)]. **D'Ascenzo et al.** Showed that there was no statistical significant difference between the study (fish oil group) and the control group regarding serum urea and creatinine on day 0 and on day 7 [(24)].

Regarding serum lipid profile:

We found that there was statistically significant difference between the two studied groups as regard serum cholesterol on enrollment which significantly lower in group 2 than in group 1 and no statistical significant difference between both groups before discharge. Within the same group there was significant decrease of serum cholesterol before discharge in comparison to enrollment level in group 1 while insignificant increase in group 2. Within group 1 the level of cholesterol was significantly decreased after omega-3 supplement in comparison to the level before omega-3 supplement. And this could be explained by fish oil induced clearance of cholesterol from the circulation and decreased de novo lipogenesis[(24)]. This came in agreement with **D'Ascenzo et al.,** who showed that serum cholesterol were significantly lower in the study group than in the control group (p value<0.05)[(24)]. **Tomsits et al.,**showed that there were no statistical significant difference between the two groups regarding serum cholesterol at final observation [(28)].

AS regard triglycerides level we found that there was no statistical significant difference between both groups on enrollment while before discharge, the triglycerides level was significantly lower in group 1 in comparison to group 2.Within the same group there was significant decrease in triglycerides level in group 1 while insignificant increase in group 2. Within group 1 the level of triglycerides was significantly decreased after omega 3 supplement in comparison to the level before omega 3 supplement.

This came in agreement with**Damsgaard et al .,** who made a clinical trial on healthy term 9 months old infants (n=83) were randomly assigned to receive 5 ml fish oil daily (intervention group) or no fish oil group (control group) from 9 to 12 months of age**.** Infants were also randomly assigned to drink either cow milk or standard infant formula with no LCPUFAs. Their results showed a significant decrease in plasma triglycerides in the fish oil group from 9 months to 12 months (p value=0.04) [(31)]. But in contrast to our study **Tomsits et al.,**who found that serum triglycerides increase slightly in the study group (SMOF Lipid containing fish oil) and in the control group (intralipid group with no fish oil) but this increase were comparable in both groups. And they explained this as premature infants are at higher risk for hypertriglyceridemia than term infants due to their limited muscle mass and decreased hydrolytic capacity of lipoprotein lipase [(28)].

Regarding serum LDL we found that there was no statistical significant difference between the two groups on enrollment but before discharge there was significant decrease in it in group 1 than in group 2. Within group 1 there was significant decrease in LDL level after omega-3 supplement in comparison to its level before omega-3 supplement. The increased level of LDL on enrollment and before omega-3 supplement in premature infants may be due to decreased activity of lipoprotein lipase, hepatic lipase and lecithin cholesterol acyl transferase enzymes and this might increase lipoprotein concentration and serum LDL [(32)].**Damsgaard et al .,** Found that there was significant increase in serum LDL in the fish oil group at 12 months (p value =0.02) [(31)].

Regarding serum HDL we found that there was no statistical significant difference between the two groups on enrollment but before discharge there was significant increase in it in group 1 than in group 2. Within group 1 there was significant increase in HDL level after omega-3 supplement in comparison to its level before omega-3 supplement.

This came in agreement with **Skouroliakou et al.,** who made a prospective, observational study composed of 2 groups of preterm neonates VLBW neonates group their number was 129 and LBW neonates their number was 153, each group were divided in 2 subgroups, subgroup 1 received parenteral lipid emulsion in the form of Medium chain triglycerides and omega-3 LCPUFAs, while subgroup 2 received intralipid emulsion with zero fish oil.Their results showed significant increase in serum HDL in omega-3 subgroup of VLBW neonates at time of discharge (p value =0.043) [(33)].**Tomsits et al.,** found that there was insignificant decrease in serum HDL in the study group (SMOF Lipid containing fish oil) at final observation[(28)].

As regard serum VLDL there was no statistically significant difference between both groups either on enrollment or before discharge. Within group 1 there was significant decrease in VLDL level after omega-3 supplement in comparison to its level before omega-3 supplement. And this could be explained as Fish oil decrease triglycerides and VLDL synthesis, may also modify VLDL lipid compositions in such a way that become a better substrate for lipoprotein lipase or liver receptor mediated reuptake [(34)]. This came in agreement with **Rosas-Nexticapa, et al.,** Who made a clinical study on 121 children aged 10-12 years old, were diagnosed as being overweight or obese and were divided in to 4 groups in order to daily supplemented with 2 or 3 gummies (70 or 105 mg DHA)and 10 or 15 gm of salmon per day. Supplementation was carried out for 3 months. Their results showed that there was significant decrease in serum VLDL when children were supplemented with 90 mg of DHA (3 gummies) (p value<0.05) [(35)].

Regarding duration of respiratory support measures including mechanical ventilation, CPAP, oxygen on nasal canula, head box and incubator oxygen.We found that there was no statistical significant difference between the two studied groups.This agree with **Manley et al.,** who made a randomized controlled trial compared the outcomes for preterm infants < 33 weeks of gestation ,consumed expressed breast milk from mother taking either tuna oil (high DHA) diet or Fish oil capsules . Their results showed that there were no statistical significant differences between the studied groups regarding duration of respiratory support measures [(36)].

Skouroliakou et al., Found that the days of ventilation support (Group A: 12.20±8.18 days, Group B: 9.11±6.19 days, p-value=0.371) [(30)].**Henriksen et al.,** found that there was no statistical significant difference regarding duration of respiratory support measures between the intervention and the control group[(25)].**Skouroliakou, et al .**showed that there was no statistical significant difference in between VLBW subgroup 1, 2 regarding days on mechanical ventilation (p value =0.150) [(33)].

Regarding secondary outcome in both groups:

Regarding occurrence of ROP, NEC, IVH, BPD, DIC and death rate during NICU stay there was no statistical significant difference between both groups. None of our preterm neonates suffered from NEC, BPD or IVH. One of our preterm neonates died in group 2 after development of sepsis and DIC.

This came in agreement with **Baack et al.** who found that there was no statistical significant difference in the incidence of adverse events of prematurity between DHA and placebo supplemented preterm infants. There were no cases of NEC, IVH or bleeding disorders and one death occurred during the study due to sepsis [(27)].

Collins et al., found that the incidence of major clinical morbidities was low. BPD was not present in infants receiving 40 or 60 mg/kg/day group but 2 infants (18%) with 120 mg/kg/day group, 1 infant (9%) with no supplementary DHA group and 4 infants (33%) with maternal supplementation group. NEC occurred in 1 infant in each of the 40 mg/kg/day, no supplementary DHA and maternal DHA supplementation groups with no NEC present in the 80 or 120 mg/kg/day groups[(37)].

Smithers, et al made a systematic review of randomized controlled trials involving preterm born < 37 weeks of gestation that compared infants fed a standard preterm formula containing no LCPUFAs with those fed a formula containing n-3 LCPUFAs for more than 1 month. Their results showed that the relative risk of sepsis and NEC did not differ between infants fed LCPUFA supplemented or control formula. There were no clear differences in ROP, IVH or BPD between preterm infants fed control or LCPUFA- supplemented formula [(22)].

Henriksen, et al found that there was no statistical significant difference in the registered adverse events (NEC, IVH and ROP) between the intervention (omega-3) group and the control group [25].

Innis, et al. Found that the incidence of serious adverse events was not different among the groups of preterm infants during hospitalization (control n=4, DHA n=3, DHA+ARA n=4). Similarly, no difference was found in the incidence of ROP, IVH, NEC, sepsis[26].

Regarding days needed to reach full enteral feeds we found that mean days needed to reach full enteral feeding in group I was 8.36 ± 3.26 and in group II was 9.92 ± 3.34 with no statistical significant difference in between both groups (p value = 0.050).

Collins, et al found that days needed to reach full enteral feeds, median (IQR), was 10 (6-15) in DHA (40 mg/kg/day) group, 14(12-19) in DHA (80 mg/kg/day) group, 11 (6-15) in DHA (120mg/kg/day), 10 (8-16) in {no supplementary DHA group } and 13 (9-15) in {maternal supplementation group} withno statistical significant difference in between them [37].

Baack et al., Found that the DHA supplemented infants had insignificant increase in days needed to reach full feeds of about 2.6 days compared to the placebo group (p value =0.07). This difference was found to be similar after excluding the IUGR infants in both treatment groups. However, after adjusting for birth weight, the increase in days to reach full feeding was only 1.8 days more for DHA vs placebo supplemented infants[27].

As regard days on omega 3 supplementation in group 1 we found that mean days of omega-3 supplementation was 18.48 ± 2.63 with 48% of patients < 20 days and 52% of patients > 20 days.

Regarding length of NICU stay we found that the mean length of hospital stay in group I was 27.20 ± 7.45 and in group II was 30.16 ± 8.63with statistically insignificant difference in between them (p value =0.200) .

Manley et al. found that mean days in NICU in (high-DHA diet) group was 21.7 while in (standard-DHA diet) group was 21.3 with statistically insignificant difference in between (p value=0.88) [36].**Skouroliakou et al.,** showed that total days of hospitalization in group A was 54.00 ± 24.81 while in group B was 58.94 ± 21.55 with p value =0.559 which were statistically insignificant in both groups [30].

Limitation of this study:

1-Small number of cases enrolled in the study.
2-No short and long term follow up of the cases.
3-Some preterm conditions are so critical to be enrolled to the study.

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Conflicts of interest
There were noconflicts of interest.

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