

1 **1. Title**

2 The differential effects of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids on
3 seizure frequency in patients with drug-resistant epilepsy – A Randomized, double-blind,
4 placebo-controlled trial

5 **2. Authors**

6 Fatma A.S. Ibrahim¹, Kebreab Ghebremeskel², Manar E. Abdel-Rahman³, Amar A. M.
7 Ahmed¹, Inaam Gashe¹, Ghada Osman¹, Maha Elseed¹, Ahlam Hamed¹, Adrian L.
8 Rabinowicz^{4,5}, Mohamed A.M. Salih¹, , Mustafa I. Elbashir¹, Ahmed A. Daak^{1,2,4,5}

9 ¹Faculty of Medicine, University of Khartoum, Khartoum, Sudan; ² Lipidomics and
10 Nutrition Research Centre, London Metropolitan University, London, UK; ³ College of
11 Health Sciences, Department of Public Health, Qatar University, Qatar. Sancilio
12 Pharmaceuticals Company, Florida, USA; ⁵Center of Molecular Biology and Biotechnology
13 (CMBB), Florida Atlantic University (FAU), USA

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17 **4. Short title**

18 Omega-3 treatment in Drug-Resistant Epilepsy

19 **5. Key words**

20 Drug resistant epilepsy (DRE), anti-epileptic drugs (AEDs), docosahexaenoic acid (DHA),
21 eicosapentaenoic acid (EPA),

22

1 **6. Clinical trial registration**

2 Controlled Trials Registration Number - ISRCTN80844630).

3

1 **Summary**

2 **Objectives:** The omega-3 (n-3) fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic
3 acid (DHA), are known to play an important role in maintenance and modulation of neuronal
4 functions. There is evidence that omega-3 fatty acids may have anticonvulsant effects. The
5 effect of DHA and EPA on seizure rate in patients with DRE was investigated.

6 **Methods:**

7 A double-blind, randomized, placebo-controlled clinical trial included ninety-nine (n=99) DRE
8 subjects, aged 5-16 (n=85) and 17-45 (n=14). After randomization, subjects were given two,
9 four or six capsules per day of DHA (417.8 mg DHA and 50.8 mg EPA/capsule, n=33), EPA
10 (385.6 mg EPA and 81.2 mg DHA/capsule, n=33) or placebo (high oleic acid sunflower oil,
11 n=33) for one year. The primary endpoint was the effect of treatment on rate of seizure.
12 Random-effects negative binomial regression models were fitted to model the patients' total
13 count of seizures per month. The treatment effects on seizure incidence rate ratio was tested
14 after controlling for the covariate effects of gender, age, rate of seizure per week at enrollment,
15 type of seizure and number of AEDs combinations used at enrollment.

16 **Results:**

17 Fifty-nine subjects (n=59) completed the study (59.6%). The average number of seizures per
18 month were 9.7 ± 1.2 in the EPA group, 11.7 ± 1.5 in the DHA group, and 16.6 ± 1.5 in the
19 placebo group. Age, gender and seizure type adjusted seizure incidence rate ratios (IRRs) of
20 the EPA and DHA groups compared with the placebo were 0.61 (CI= 0.42-0.88, p=0.008, 42%
21 reduction) and 0.67 (CI = 0.46-1.0, p= 0.04, 39% reduction), respectively. There was no
22 difference in IRR between the EPA and DHA groups (p=0.56). Both treatment groups had a
23 significantly higher number of seizure-free days compared to placebo (p<0.05).

1 **Significance:**

2 This study demonstrates that EPA and DHA are effective in reducing seizure frequency in
3 patients with DRE

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5 **Key words**

6 Omega-3 fatty acids, seizure incidence rate, anti-epileptic drugs (AEDs)

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1 **Introduction**

2 Epileptic seizures are characterized by unpredictable abnormal electrical discharge and
3 convulsions¹. Epilepsy, the tendency to have recurrent unprovoked seizures², affects over 50
4 million people worldwide and accounts for about 1% of the global burden of disease³. Anti-
5 epileptic drugs (AEDs) are effective in reducing or eliminating seizures in the majority of
6 patients with the condition. However, one third of the patients continue to have two or more
7 seizures a month in spite of treatment with maximum therapeutic doses⁴. Patients with drug-
8 resistant epilepsy (DRE) are managed with alternative treatments including neurosurgery⁵,
9 neurostimulation, and ketogenic and modified Atkins diets⁵ with variable success. Therefore,
10 there is a need for an effective therapy for patients with DRE.

11 The omega-3 (n-3) fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, are
12 known to play a pivotal role in maintenance and modulation of neuronal functions^{6,7}, neuronal
13 excitability⁸, cell signalling^{9,10}, inflammatory response¹¹ and receptor function and release of
14 neurotransmitters¹². Moreover, studies have shown altered membrane lipid composition in
15 various neuropsychiatric disorders¹³. These findings have led to the postulation that treatment
16 with DHA and EPA could ameliorate seizures in patients with DRE^{14,15}.

17 Ex vivo and animal studies have provided evidence that omega-3 fatty acids have
18 anticonvulsant protection effects in several models tested¹⁶⁻²⁰. In contrast, the findings of the
19 clinical investigations have been equivocal, some demonstrated efficacy^{14,21,22} while others
20 did not¹⁵. These inconclusive outcomes of omega-3 trials in patients with DRE were attributed
21 to a number of factors; chief among them are the dietary background of the subjects²³, selected
22 dose, duration of the treatment, and the EPA/ DHA ratios^{24,25}. Hence there is a need for more
23 well- designed trials to help elucidate whether or not these fatty acids have the potential to
24 ameliorate seizures in patients with the DRE.

1 In this study, we investigated the effect of DHA and EPA on seizure rates in patients with DRE.

2

3 **Subject and Methods**

4 *Study design*

5 This study is a randomized, double-blind, placebo-controlled, parallel-group, clinical trial in
6 patients with DRE. The participants were randomized to receive their respective treatments –
7 EPA, DHA or placebo for one year – while kept on their regular AEDs during the intervention
8 period. The study was conducted at Soba University Hospital Neurology Referral Clinic, Ibn-
9 Arafat Pediatric Teaching Hospital Neurology Referral Clinics, Khartoum, Sudan. Approvals
10 were obtained from the ethics committees of the Federal Ministry of Health of Sudan, the
11 Faculty of Medicine, University of Khartoum, Sudan and London Metropolitan University,
12 UK. Self- or investigator-read and explained written consent was obtained from the adult and
13 parents or guardians of the underage participants. This study was conducted in accordance with
14 the International Conference of Harmonization notes for Guidance on Good Clinical Practice,
15 the principles of the Declaration of Helsinki as revised in 2007, the established methodological
16 procedures according to the revised CONSORT statement. The study is registered with
17 ISRCTN registry (ISRCTN57643242)

18 *Patients*

19 Ninety-nine (n=99) DRE patients, aged 5-16 (n=85) and 17-45 (n=14) who have been in regular
20 follow-up at the aforementioned clinics were enrolled between June 2012 and December 2013.
21 The subjects who consented to participate in the study were initially assessed for eligibility
22 before invitation to attend the screening visit. The inclusion criteria were: 5 to 50 years old
23 male or female patients with focal and generalized seizures, well-documented DRE in

1 accordance with the International League Against Epilepsy (ILAE) classification. DRE is
2 defined as two or more seizures per month for three months prior to the screening visit despite
3 treatment with two or more AEDs at optimal stable dosages for more than one month prior to
4 screening. All patients underwent EEG examination as part of the clinical work-up. The
5 exclusion criteria were: epilepsy due to metabolic causes, trauma or space occupying lesions,
6 quadriplegic, ataxic and dyskinetic cerebral palsy, other chronic condition and regular intake
7 of n-3 fatty acid supplement or inability to swallow capsules.

8 *Randomization and blinding*

9 The subjects, after stratification by age and gender, were randomly assigned to receive coded
10 and indistinguishable high DHA, high EPA or placebo capsules. Randomization was 1:1:1 ratio
11 conducted using a sequence of computer-generated random numbers at the Faculty of Life
12 Sciences, London Metropolitan University (UK). It was performed by a person who had no
13 knowledge about demographic, clinical or laboratory characteristics of the patients, and staff
14 of the referral clinics, investigators and participants were blinded until the biochemical and
15 clinical outcome data were analyzed and the database unlocked.

16 *Efficacy assessment*

17 The primary endpoint was the difference in seizure rate between the treatment groups and
18 placebo at the end of the intervention period. Secondary endpoints were the proportion of
19 subjects with $\geq 25\%$ reduction (response rate) in seizure rate compared to baseline and seizure-
20 free days over the treatment period.

21 *Safety assessment*

22 Adverse events were categorized as serious if they were life threatening, resulted in death,
23 required inpatient hospitalization, gave rise to a significant disability and congenital birth
24 defect. Hematology parameters, liver function and lipid profile were measured at baseline and

1 end of treatment. Past medical history, physical examination, vital signs, and body weight were
2 recorded on a monthly basis by the investigators.

3 *Procedure*

4 Subsequent to randomization, the subjects were given daily, for one year, two (5-10 years old,
5 median weight (mw)=25 kg), four (11-16 years old, mw=37 kg) or six (17-50 years old, mw=51
6 kg) capsules of DHA (417.8 mg DHA and 50.8 mg EPA/capsule, n=33), EPA (385.6 mg EPA
7 and 81.2 mg DHA/capsule, n=33) or placebo (high oleic acid sunflower seed oil, n=33).
8 Omega-3 fatty acid dose was calculated by multiplying the median weight of each age group
9 by 25 mg/kg body weight. Vitamin E (1.5 mg/capsule) was added to the three types of capsules
10 to prevent peroxidation. Both types of capsules were carefully matched in appearance and
11 flavor to prevent treatment unmasking. Enrollment identification number, gender, residence,
12 ethnicity, weight, epilepsy clinical diagnosis, type of epileptic seizure, number of seizures per
13 month, the time when the patient was diagnosed with DRE, MRI findings, EEG findings,
14 aetiology of the epileptic seizures and AEDs in use at enrollment were collected using a
15 structured questionnaire at baseline. At baseline and each monthly follow-up visit, vital signs,
16 history and physical examination were obtained. A monthly patient-recorded daily health diary
17 was given to each patient or their guardian to record seizure frequency on a daily basis, AEDs
18 received, any additional medications, study drug intake, visit to health facilities and
19 hospitalization. Whole blood, about 10 ml, was obtained from the patients at baseline and end
20 of the treatment visit (EOT) to analyze the complete blood count, liver function, glucose and
21 lipid profile. Name and telephone number of the medical doctor in charge was given to the
22 patients and their guardians in case they required advice or care outside normal working hours.
23 During each monthly follow-up visit, the self-recorded health diaries were reviewed by the
24 investigators. The data of each follow-up visit were entered by the same investigator in a paper

1 case report form (CRF) and electronic CRF, EpiData Software a comprehensive tool for
2 validated entry and documentation of data. The EpiData Association, Odense, Denmark, 2003-
3 2005.

4 *Statistical analysis*

5 In a previous pilot study, it has been reported that treatment with combination of omega-3 and
6 omega-6 fatty acids (Equazen™) resulted in $\geq 80\%$ decrease in mean number of seizures over
7 all subjects treated for four weeks [40]. Another pilot study using EPA only reported a 16%
8 mean reduction in seizure frequency²⁶. Based on these pilot studies, it was assumed that
9 treatment with either DHA or EPA would reduce annualized seizure incidence rate by 25%
10 compared to the placebo group. To detect a 25% difference in annualized seizure incidence
11 rate between the treatment groups and placebo groups with 85% power at a 5% significance
12 and superiority margin equal to zero ($\delta = 0$) to test for statistical superiority, twenty five (n=25)
13 patients were required in each arm of the study. The total number of participating patients was
14 increased to (n=99) to compensate for an anticipated 35% loss to follow-up.

15 The data is presented as mean \pm standard deviation (SD), median and percentile or median and
16 inter-quartile range (IQR) as appropriate. The epileptic seizures were summarized as average
17 number of seizures/ month and analyses were undertaken on intention-to-treat basis by
18 including all of the randomized patients (n=99). To account for the repeated measures nature
19 of the data and over-dispersion (variance greater than the mean), random-effects negative
20 binomial regression models were fitted to model the patients' total count of seizures per month.
21 The treatment effects on seizure incidence rate ratio was tested after controlling for the
22 covariate effects of gender, age, rate of seizure per week at enrollment, type of seizure and
23 number of AED combinations used at enrollment. Multiple imputation was used to account for
24 missing data. Statistical differences of the continuous variables were evaluated using ANOVA,

1 and post hoc analysis when significance was indicated. A p-value of 0.05 is considered
2 significant. STATA statistical package (version 14) was used for analyses.

3

4 **Results**

5 One hundred sixty-five patients diagnosed with DRE were screened for eligibility and ninety-
6 nine who fulfilled the inclusion criteria were enrolled and assigned to receive either DHA, EPA
7 or placebo. The number of subjects who received at least one dose of the assigned study
8 medication (safety population) were eighty-seven, of those 29 received placebo, 30 received
9 EPA and 28 received DHA. Fifty-nine patients completed the study (n=59). The CONSORT
10 flow chart of patient enrollment, randomization and patient disposition are shown in Figure 1.

11 At baseline, there was no difference in mean age, weight, gender distribution, type of seizure,
12 number of seizures per week, mean illness duration since epilepsy diagnosis and number and
13 type of AEDs in use (**Table 1**), and clinical laboratory parameters (**Table 2**). The most frequent
14 concomitant AEDs were sodium valproate, Lamotrigine, Carbamazepine and Clonazepam. The
15 number of subjects stopped one or two AEDs post randomization was 9, 8 and 7 patients among
16 those received placebo, EPA or DHA, respectively.

17 The mean number of seizures per month of the EPA-treated, DHA-treated and placebo after
18 12 months of treatment were 9.7 ± 1.2 , 11.7 ± 1.5 and 16.6 ± 1.5 respectively (Table 3, Figure
19 2A). Age and gender, seizure type adjusted seizure incidence rate ratios (IRRs) of the EPA and
20 DHA treated groups compared to placebo, the primary endpoint, were 0.67 (CI= 0.46-0.1,
21 $p=0.04$, 33% reduction) in DHA-treated group and 0.61 (CI = 0.42-0.88, $p= 0.01$, 39%
22 reduction) in EPA-treated group. There was no difference in IRR between the EPA and DHA
23 groups ($p=0.6$) (Table 3, Figure 2B). Responder rates were 10.3% and 3.0% higher in the EPA

1 treated and DHA treated groups compared to the placebo. Both treatment groups had a
2 significantly higher number of seizure-free days compared to placebo ($p<0.05$).

3 **Safety and tolerability:**

4 No treatment-related adverse event occurred during the study period. Unrelated to treatment,
5 there were 9 (30%), 4 (14 .3%) and 15 (51.7%) reported adverse events (AEs) in the EPA,
6 DHA and placebo groups respectively. The most frequent AEs among those who received EPA
7 or DHA were a decreased appetite, fever and rash. None of the treated patients developed
8 epistaxis or mucosal bleeding.

9 There were no differences in levels of hematological and liver function parameters between the
10 EPA or DHA treated group and placebo after one year of intervention (Table 2).

11

12 **Discussion**

13 This study demonstrates that the treatment of patients with a history of drug-resistant epilepsy
14 with a high EPA (EPA:DHA; 4.9:1) or a high DHA (EPA:DHA; 1:8.2) treatment in
15 conjunction with a stable regimen of anti-epileptic drugs reduces significantly monthly seizure
16 frequency. Consistent with these findings, efficacy was reported by Schlanger et al²² and
17 DeGiorgio et al on those who received a low dose of EPA & DHA²¹. In contrast, Bromfield
18 et al¹⁵ and Yuen et al¹⁴ did not find positive outcomes. Differences in duration of the treatment
19 period, dose of EPA and DHA and their ratios and/or dietary and genetic backgrounds of the
20 participants might account for the conflicting findings. In the current study, the participants
21 were treated with either DHA or EPA for 12 months.

22 The dose used for this study was based solely on the previous evidence and observation
23 generated out of the myriad of preclinical and few clinical studies, the US FDA Generally

1 Recognized as Safe (GRAS) omega-3 dose/ day (3 g/day) and the available data on DHA and
2 EPA pharmacokinetics. The DHA and EPA doses investigated in this study are higher than the
3 DHA and EPA combination used by Yuen et al and Bromfield et al and study^{14, 15, 21}. Recently,
4 DeGiorgio et al reported the results of the first well-powered RCT on the effect of low (1080
5 mg EPA+DHA/day) and high doses (2160 mg EPA+DHA/day) in patients with DRE. Low
6 dose omega-3 fatty acid was associated with a 33.6% reduction in seizure frequency compared
7 with the placebo, whereas the high dose was not different than placebo. Interestingly, the
8 reported 33% reduction in seizure rate among those received the low dose is similar to the
9 finding we observed in the DHA group. The lack of efficacy of the high dose of the EPA and
10 DHA combination reported by DeGiorgio et al is intriguing. One possible explanation that
11 high dosages may result in excessive reduction in non-esterified fatty acids such as arachidonic
12 acid^{21, 27}.

13 In contrary to the DeGiorgio et al study results²¹, the finding of this study does not show
14 similar lack of efficacy of seemingly high DHA or EPA doses. The varied outcomes of these
15 two studies could be a reflection of the EPA and DHA composition used in each study or the
16 relatively short treatment and washout periods of the cross-over study design of DeGiorgio et
17 al study²⁸. DHA is not readily released from neuronal membranes in adult mammals even if
18 their diet is limited in the fatty acid²⁹. Therefore, one is unsure whether or not the washout
19 period was sufficient to return neuronal DHA level to baseline in the study conducted by
20 DeGiorgio et al. Moreover, the wisdom of using linoleic acid (corn oil), the parent compound
21 of arachidonic acid, which is pro-inflammatory, as a placebo control is questionable.

22 DHA is the primary structural and functional fatty acid component of the brain cells,
23 particularly the neurons³⁰. EPA, which is found in small amounts in all of the brain cells,
24 accounts for only about 0.1% total fatty acids³¹. Perhaps surprisingly, the treatment of various

1 neuropsychiatric conditions with pure EPA has been shown to be more effective than DHA ³²,
2 ³³. These findings have led many to surmise pure EPA would more efficacious in reducing
3 seizures in patients with DRE ¹⁴. In the current study, and in line with previous preclinical
4 studies ^{34,35}, both the high EPA and high DHA supplements were effective in reducing seizures.
5 The observed positive therapeutic effect of DHA and EPA on patients with DRE is consistent
6 with animal models treated with EPA and DHA for long duration¹⁶⁻²⁰. Interestingly, a time-
7 course study conducted by Taha et al. showed that at least 3 months are required for DHA and
8 EPA to raise seizure threshold³⁶. The delayed effect of DHA and EPA was attributed to the
9 slow process of enriching the neuronal tissues with these fatty acids, delay in formation of
10 unestrified EPA and DHA or their bioactive metabolite such as protectins (NPD1)²⁰.

11 A dysfunction in the activity of voltage-gated sodium channels is thought to be central to the
12 pathogenesis of epileptic seizures ³⁷. Indeed, most of the widely used antiepileptic drugs
13 ameliorate seizures by acting on voltage-gated ion channels in patients with the condition³⁸.
14 There is evidence that polyunsaturated fatty acids modulate voltage-gated ion channels and
15 neuronal excitation ^{8,39}. Xiao et al. made the seminal observation that EPA suppresses voltage-
16 activated Na⁺ currents in cultured rat myocyte and both EPA and DHA reduce inward calcium
17 current, prolonging the inactivation state³⁹. It is hypothesized that these effects are dependent
18 on the presence of two or more double bonds with a cis configuration ⁴⁰. If this is the case, both
19 EPA and DHA independently and/or in concert might reduce neuronal excitability and seizure
20 rate.

21 DHA and EPA were well tolerated with no clinically relevant laboratory changes during the
22 study. Some preclinical studies have suggested that chronic intake of omega-3 fatty acids might
23 result in excessive reactive oxygen species (ROS), which could contribute to brain aging.
24 However, other preclinical and clinical studies found no effect or even reduction in ROS

1 production after chronic intake of high n-3 fatty acids. Further studies are needed to delineate
2 the true long term effect of chronic treatment with high omega-3 fatty acids in patients with
3 DRE.

4 The main limitations of the study include: heterogeneity of the study population with regard to
5 seizure frequency and type; reliance on patients reported seizure frequency data to assess the
6 effects of the interventions; due to the study long duration some patients stopped one or two
7 AED; inability to determine markers of inflammation and oxidative stress because of financial
8 constraints. The study was conducted in Sudanese patients whose traditional diet is low in n-3
9 fatty acids. Therefore the findings may not be extrapolated to other patient populations with
10 high omega-3 intake.

11 In conclusion, this randomized, double-blind, placebo controlled study demonstrates that EPA
12 or DHA is a safe and effective add-on therapy for patients with drug resistant epilepsy.

13

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3 Soba University Hospitals and Ibn-Aoaf Pediatric Teaching Hospital (Sudan), and to Mr Peter
4 Clough, Efamol Limited UK, for his expert advice and support throughout the duration of the
5 study. Special Thanks go to Zawaya Group for sponsoring the study.

6

1 **Authorship and Conflicts of Interest**

2 K.G. and A.D. conceived the idea, designed and initiated the study. K.G, A.D. M.E, A.A and
3 F.I wrote the study protocol. A.D.; F.I. and M.A. developed the study questionnaires, Clinical
4 Report Form (CRF) and coordinated the implementation of the trial. F.I., A.D.,I.G, A.A., M.A.;
5 A.H.; M.S; G.O; and I.E. recruited, followed the patients and collected clinical data. F.I., Q.O,
6 M.S Conducted laboratory analysis and data entry. A.D and M.A. developed the statistical
7 analysis plan. M.A. and A.D. conducted the statistical analysis. A.D and F.I. interpreted the
8 data. A.D wrote the first draft of the manuscript. F.I, M.E, K.G, M.A, AR, AA reviewed the
9 manuscript and provided critical suggestion and comment. None of the authors disclosed a
10 conflict of interest. AD and AR are currently employee of Sancilio Pharmaceuticals Company.
11 The sponsors had no influence on the design of the study, collection, analysis and interpretation
12 of data, writing of the manuscript or decision to submit for publication.

13

1 **Ethical Publication Statement**

2 We confirm that we have read the journal's position on issues involved in ethical publication
3 and affirm that this report is consistent with those guidelines.

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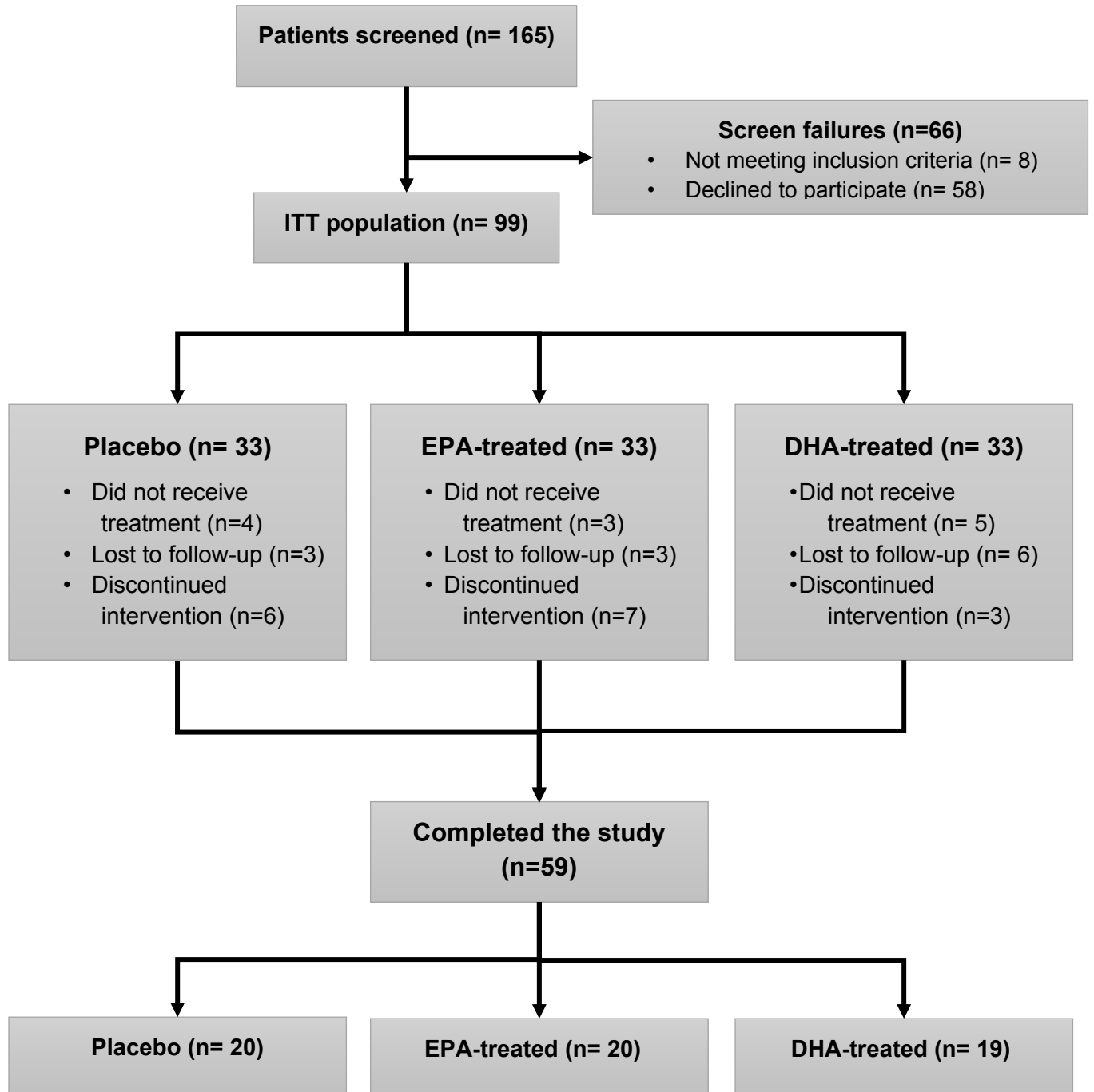
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Figure 1. Trial Flowchart of patients' enrollment, randomization assignments and follow-up



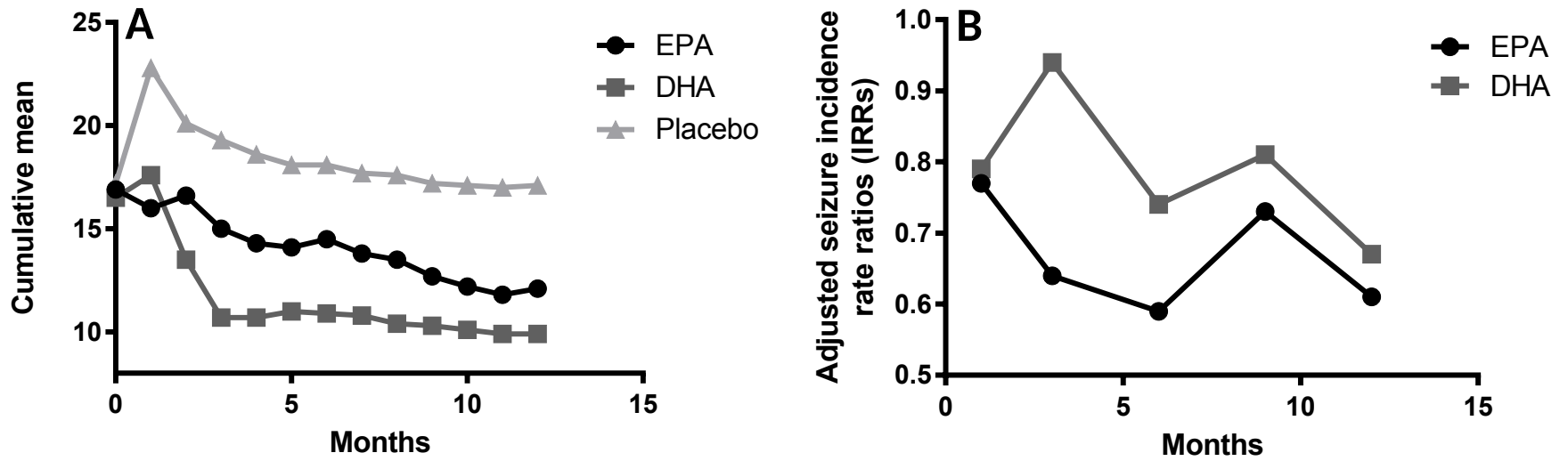


Figure 2: A) Cumulative mean number of seizures in patients with drug resistant epilepsy treated with EPA, DHA or placebo. B) Adjusted seizure incidence rate ratios (IRRs) of the EPA or DHA treated compared with the placebo group.

Table 3 The effect of treatment with DHA or EPA on epileptic seizures in patients with drug resistant epilepsy

	DHA group (n=33)		EPA group (n=33)		Placebo group (n=33)	
	Measure	¹ p-value	Measure	² p-value	Measure	³ p-value
EFFICACY MEASURE						
Average number of seizure/month (mean ± SE) [†]	11.7 ± 1.5	0.012	9.7 ± 1.2	0.001	16.6 ± 1.5	0.419
Seizure incidence rate ratio((IRR (Confidence interval)) ^{††}	0.67 (0.46-1.0)	0.043	0.61 (0.42-0.88)	0.008	Ref=1	0.563
Seizure, percent change from placebo	-30%		-42%		NA	
Response rate ^{†††}	63.4% (n=14)		56.3% (n=16)		53.3% (n=15)	
Mean number of seizure-free days ^{††††}	23.1	<0.001	24.5	<0.001	19.9	0.097

† Statistical significance assessed based on linear regression of total seizure by treatment group with multiple imputations (20 data sets).

†† Seizure incidence rate ratio as analyzed by negative binominal regression model. Statistical significance assessed based on intention-to-treat analysis with multiple imputations (20 data sets).

††† Percentage of patients, with more than 9 month follow up, experiencing ≥50% reduction (response rate) in mean seizure compared with baseline.

†††† Statistical significance assessed based on logistic regression of number of seizure free days by treatment group with multiple imputations (20 data sets).

¹P-value, DHA-treated versus placebo

²P-value, EPA-treated versus placebo

³P-value, DHA-treated versus EPA-treated

Table 1 Demographic and clinical characteristics of the patients

	Placebo Group (n=33)		EPA Treated Group (n=33)		DHA Treated Group (n=33)		Total	
	No.	%	No.	%	No.	%	No.	%
Gender								
Male	19	57.6	22	66.7	22	66.7	63	63.6
Female	14	42.4	11	33.3	11	33.3	36	36.4
Total	33	100	33	100	33	100	99	100
Age (years)								
Adult 18+	3	9.1	6	18.2	5	15.2	14	14.1
Child <18	30	90.9	27	81.8	28	84.8	85	85.9
Total	33	100	33	100	33	100	99	100
Seizure type								
Generalized	21	63.6	23	69.7	23	69.7	67	67.7
Focal	9	27.3	7	21.2	4	12.1	20	20.2
Unknown	3	18.2	3	6.1	6	9.1	11	11.1
Total	33	109.1	33	97	33	100	99	100
Seizures per month at enrollment								
2-4	6	18.2	6	18.2	4	12.1	16	16.2
5-12	8	24.2	10	30.3	11	33.3	29	29.3
13-24	9	27.3	7	21.2	4	12.1	20	20.2
>24	6	18.2	7	21.2	8	24.2	21	21.2
Missing	4	12.1	3	9.1	6	18.2	13	13.1
Total	33	100	33	100	33	100	99	100
Drugs used at enrolment								
Na valproate	26	78.8	23	69.7	27	81.8	76	76.8
Carbamezapin	9	27.3	8	24.2	10	30.3	27	27.3
Lamotrigine	18	54.5	18	54.5	7	21.2	43	43.4
Toprimate	1	3	4	12.1	4	12.1	9	9.1
Clonazepam	13	39.4	15	45.5	16	48.5	44	44.4
Phenobarbitone	1	3	4	12.1	2	6.1	7	7.1
Phenytoin	0	0	0	0	1	3	1	1
Levetiracetam (Keppra)	1	3	1	3	0	0	2	2
others	0	0	1	3	1	3	2	2

Number of drug combinations used at enrollment

2	20	60.6	16	48.5	21	63.6	57	57.6
3	10	30.3	14	42.4	8	24.2	32	32.3
≥4	3	9.1	3	9.1	4	12.1	10	10.1
Total	33	100	33	100	33	100	99	100

Table 2: Hematological, lipid, glucose and liver enzyme profile of the patients with drug resistant epilepsy treated with DHA, EPA or placebo

	Placebo group		DHA-treated group		EPA-treated group		p-value 1*	p-value 2**
	Baseline (n=29) Mean ± SE	One year (n=29) Mean ± SE	Baseline (n=28) Mean ± SE	One year (n=28) Mean ± SE	Baseline (n=30) Mean ± SE	One year (n=30) Mean ± SE		
Hematological								
White Blood Cells (WBCs)	6.2 ± 0.4	6.0 ± 0.4	6.0 ± 0.4	5.8 ± 0.5	6.3 ± 0.4	6.0 ± 0.5	0.323	0.949
Red Blood Cells (RBCs)	4.4 ± 0.1	4.2 ± 0.2	4.3 ± 0.1	4.3 ± 0.2	4.5 ± 0.1	4.5 ± 0.2	0.817	0.424
Haemoglobin (HGB)	11.2 ± 0.2	11.7 ± 0.4	11.1 ± 0.3	11.9 ± 0.5	11.5 ± 0.3	12.3 ± 0.5	0.016	0.740
Haematocrit (HCT)	37.1 ± 1.4	34.9 ± 1.5	36.3 ± 1.4	36.0 ± 1.5	36.7 ± 0.9	37.8 ± 1.5	0.683	0.444
Mean Corpuscular Volume (MCV)	80.8 ± 2.3	83.3 ± 1.2	82.6 ± 1.0	82.9 ± 1.0	81.3 ± 1.1	82.8 ± 1.1	0.071	0.229
Mean Corpuscular Haemoglobin (MCH)	26.2 ± 0.5	30.7 ± 3.0	26.1 ± 0.4	29.0 ± 2.2	25.7 ± 0.6	27.6 ± 2.1	0.039	0.623
Mean Corpuscular Hb Concentration (MCHC)	31.3 ± 0.3	30.4 ± 1.5	31.7 ± 0.3	31.1 ± 1.1	31.1 ± 0.3	31.8 ± 1.0	0.703	0.786
Platelet (PLT)	308.0 ± 17.5	287.7 ± 36.4	275.4 ± 18.3	258.6 ± 32.1	269.4 ± 15.6	291.7 ± 34.7	0.832	0.930
Lipid & Glucose								
Triglyceride mg/dl	72.6 ± 5.9	86.7 ± 13.7	71.3 ± 4.9	88.4 ± 14.8	70.2 ± 4.4	68.5 ± 11.4	0.24	0.876
Cholesterol mg/dl	135.6 ± 7.4	149.4 ± 9.9	124.2 ± 5.6	143.5 ± 8.1	128.0 ± 5.0	144.3 ± 7.2	0.001	0.562
HDL mg/dl	27.4 ± 1.7	38.2 ± 3.7	26.6 ± 2.3	35.1 ± 3.8	27.6 ± 1.9	39.4 ± 2.5	<0.001	0.640
LDL mg/dl	70.9 ± 5.0	80.9 ± 5.8	65.0 ± 3.8	76.4 ± 5.6	65.9 ± 4.2	79.8 ± 5.3	<0.002	0.849
Glucose mg/dl	75.9 ± 2.2	80.4 ± 3.8	70.8 ± 2.8	82.4 ± 4.7	73.6 ± 2.5	80.7 ± 4.1	0.006	0.237
Liver Enzymes								
ALP	138.8 ± 15.7	139.4 ± 22.4	123.7 ± 17.1	134.3 ± 23.1	109.7 ± 14.9	133.3 ± 21.5	0.399	0.737
AST	8.9 ± 1.1	12.5 ± 1.7	8.3 ± 1.1	13.3 ± 1.7	9.1 ± 1.1	12.9 ± 1.5	<0.001	0.541
ALT	5.2 ± 0.6	7.9 ± 1.6	4.7 ± 0.7	7.1 ± 1.4	4.9 ± 0.7	7.6 ± 1.3	0.01	0.823

*P-value1: comparison of the combined group between baseline and after 1 year

**p-value2: p-value for interaction between group and time (baseline & one year) using mixed effect models adjusting for covariates (gender, age continuous, baseline seizure rate per week, number of drug combinations used previously before enrollment and number of drug combinations used previously at enrollment) using xtmixed after multiple imputations (20 datasets)