1. Title

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

2. Authors

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4. Short title

Omega-3 treatment in Drug-Resistant Epilepsy

5. Key words

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

6. Clinical trial registration

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Summary

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

Methods:

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

Results:

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

Significance:

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

Key words

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

Introduction

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

The omega-3 (n-3) fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, are known to play a pivotal role in maintenance and modulation of neuronal functions[6, 7], neuronal excitability[8], cell signalling[9, 10], inflammatory response[11] and receptor function and release of neurotransmitters[12]. Moreover, studies have shown altered membrane lipid composition in various neuropsychiatric disorders [13]. These findings have led to the postulation that treatment with DHA and EPA could ameliorate seizures in patients with DRE [14, 15].

Ex vivo and animal studies have provided evidence that omega-3 fatty acids have anticonvulsant protection effects in several models tested[16-20]. In contrast, the findings of the clinical investigations have been equivocal, some demonstrated efficacy[14, 21, 22] while others did not [15]. These inconclusive outcomes of omega-3 trials in patients with DRE were attributed to a number of factors; chief among them are the dietary background of the subjects [23], selected dose, duration of the treatment, and the EPA/ DHA ratios [24, 25]. Hence there is a need for more well- designed trials to help elucidate whether or not these fatty acids have the potential to ameliorate seizures in patients with the DRE.

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

Subject and Methods

Study design

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

Patients

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

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

Randomization and blinding

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

Efficacy assessment

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

Safety assessment

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

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

Procedure

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

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

Statistical analysis

In a previous pilot study, it has been reported that treatment with combination of omega-3 and omega-6 fatty acids (Equazen™) resulted in ≥ 80% decrease in mean number of seizures over all subjects treated for four weeks [40]. Another pilot study using EPA only reported a 16% mean reduction in seizure frequency[26]. Based on these pilot studies, it was assumed that treatment with either DHA or EPA would reduce annualized seizure incidence rate by 25% compared to the placebo group. To detect a 25% difference in annualized seizure incidence rate between the treatment groups and placebo groups with 85% power at a 5% significance and superiority margin equal to zero ($\delta = 0$) to test for statistical superiority, twenty five (n=25) patients were required in each arm of the study. The total number of participating patients was increased to (n=99) to compensate for an anticipated 35% loss to follow-up.

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

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

Results

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

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

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

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

Safety and tolerability:

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

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

Discussion

This study demonstrates that the treatment of patients with a history of drug-resistant epilepsy with a high EPA (EPA:DHA; 4.9:1) or a high DHA (EPA:DHA; 1:8.2) treatment in conjunction with a stable regimen of anti-epileptic drugs reduces significantly monthly seizure frequency. Consistent with these findings, efficacy was reported by Schlanger et al[22] and DeGiorgio et al on those who received a low dose of EPA & DHA[21] . In contrast, Bromfield et al [15] and Yuen et al [14] did not find positive outcomes. Differences in duration of the treatment period, dose of EPA and DHA and their ratios and/or dietary and genetic backgrounds of the participants might account for the conflicting findings. In the current study, the participants were treated with either DHA or EPA for 12 months.

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

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

In contrary to the DeGiorgio et al study results [21], the finding of this study does not show similar lack of efficacy of seemingly high DHA or EPA doses. The varied outcomes of these two studies could be a reflection of the EPA and DHA composition used in each study or the relatively short treatment and washout periods of the cross-over study design of DeGiorgio et al study[28]. DHA is not readily released from neuronal membranes in adult mammals even if their diet is limited in the fatty acid [29]. Therefore, one is unsure whether or not the washout period was sufficient to return neuronal DHA level to baseline in the study conducted by DeGiorgio et al. Moreover, the wisdom of using linoleic acid (corn oil), the parent compound of arachidonic acid, which is pro-inflammatory, as a placebo control is questionable.

DHA is the primary structural and functional fatty acid component of the brain cells, particularly the neurons[30]. EPA, which is found in small amounts in all of the brain cells, accounts for only about 0.1% total fatty acids[31]. Perhaps surprisingly, the treatment of various

neuropsychiatric conditions with pure EPA has been shown to be more effective than DHA [32, 33]. These findings have led many to surmise pure EPA would more efficacious in reducing seizures in patients with DRE [14]. In the current study, and in line with previous preclinical studies [34, 35], both the high EPA and high DHA supplements were effective in reducing seizures.

The observed positive therapeutic effect of DHA and EPA on patients with DRE is consistent with animal models treated with EPA and DHA for long duration[16-20]. Interestingly, a time-course study conducted by Taha et al. showed that at least 3 months are required for DHA and EPA to raise seizure threshold[36]. The delayed effect of DHA and EPA was attributed to the slow process of enriching the neuronal tissues with these fatty acids, delay in formation of unestrified EPA and DHA or their bioactive metabolite such as protectins (NPD1)[20].

A dysfunction in the activity of voltage-gated sodium channels is thought to be central to the pathogenesis of epileptic seizures [37]. Indeed, most of the widely used antiepileptic drugs ameliorate seizures by acting on voltage-gated ion channels in patients with the condition[38]. There is evidence that polyunsaturated fatty acids modulate voltage-gated ion channels and neuronal excitation [8, 39]. Xiao et al. made the seminal observation that EPA suppresses voltage-activated Na+ currents in cultured rat myocyte and both EPA and DHA reduce inward calcium current, prolonging the inactivation state[39]. It is hypothesized that these effects are dependent on the presence of two or more double bonds with a cis configuration [40]. If this is the case, both EPA and DHA independently and/or in concert might reduce neuronal excitability and seizure rate.

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

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

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

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

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Authorship and Conflicts of Interest

K.G. and A.D. conceived the idea, designed and initiated the study. K.G, A.D. M.E, A.A and F.I wrote the study protocol. A.D.; F.I. and M.A. developed the study questionnaires, Clinical Report Form (CRF) and coordinated the implementation of the trial. F.I., A.D.,I.G, A.A., M.A.; A.H.; M.S; G.O; and I.E. recruited, followed the patients and collected clinical data. F.I., Q.O, M.S Conducted laboratory analysis and data entry. A.D and M.A. developed the statistical analysis plan. M.A. and A.D. conducted the statistical analysis. A.D and F.I. interpreted the data. A.D wrote the first draft of the manuscript. F.I, M.E, K.G, M.A, AR, AA reviewed the manuscript and provided critical suggestion and comment. None of the authors disclosed a conflict of interest. AD and AR are currently employee of Sancilio Pharmaceuticals Company.

The sponsors had no influence on the design of the study, collection, analysis and interpretation of data, writing of the manuscript or decision to submit for publication.

Ethical Publication Statement

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

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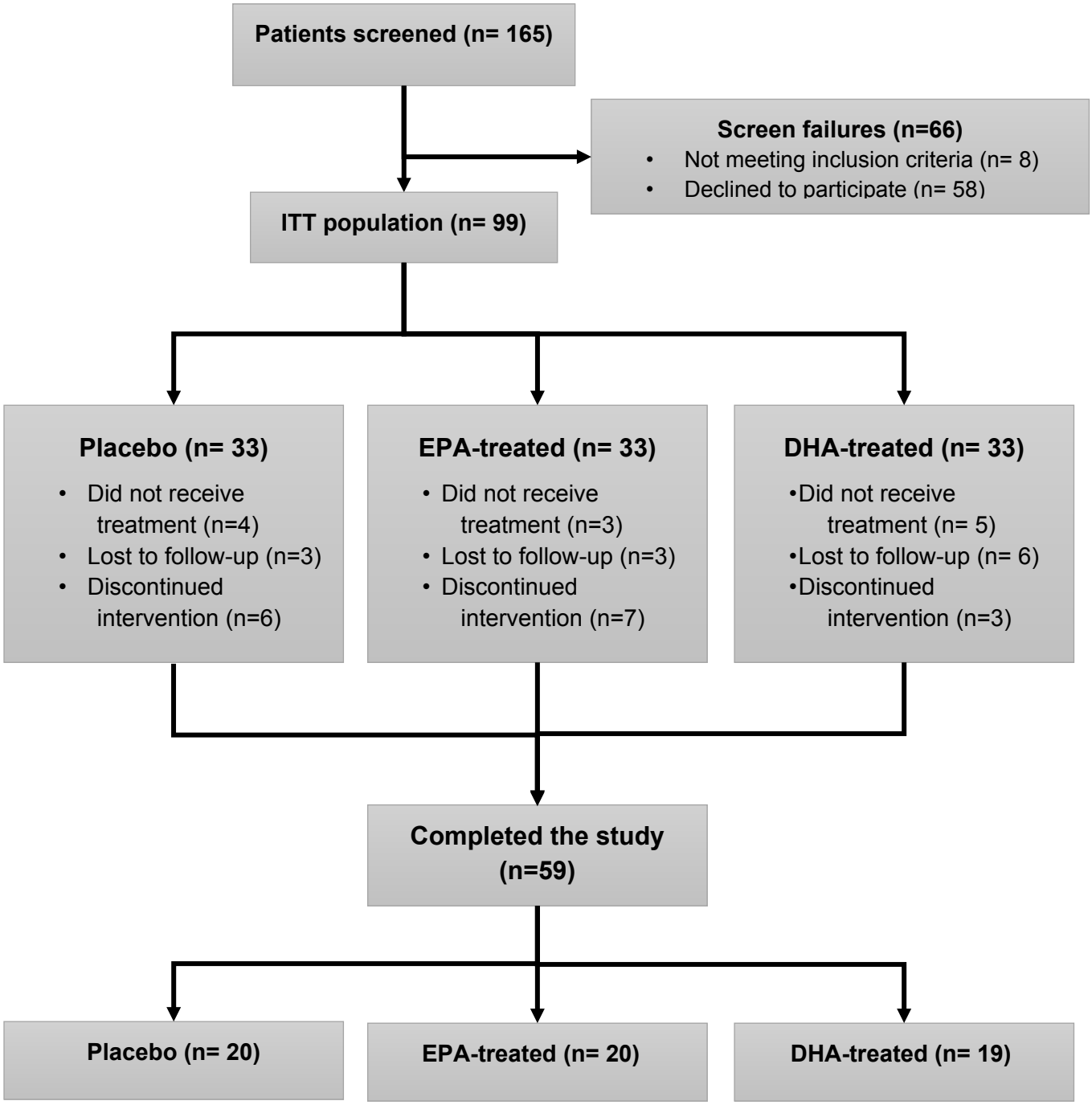
Figure 1. Trial Flowchart of patients' enrollment, randomization assignments and follow-up



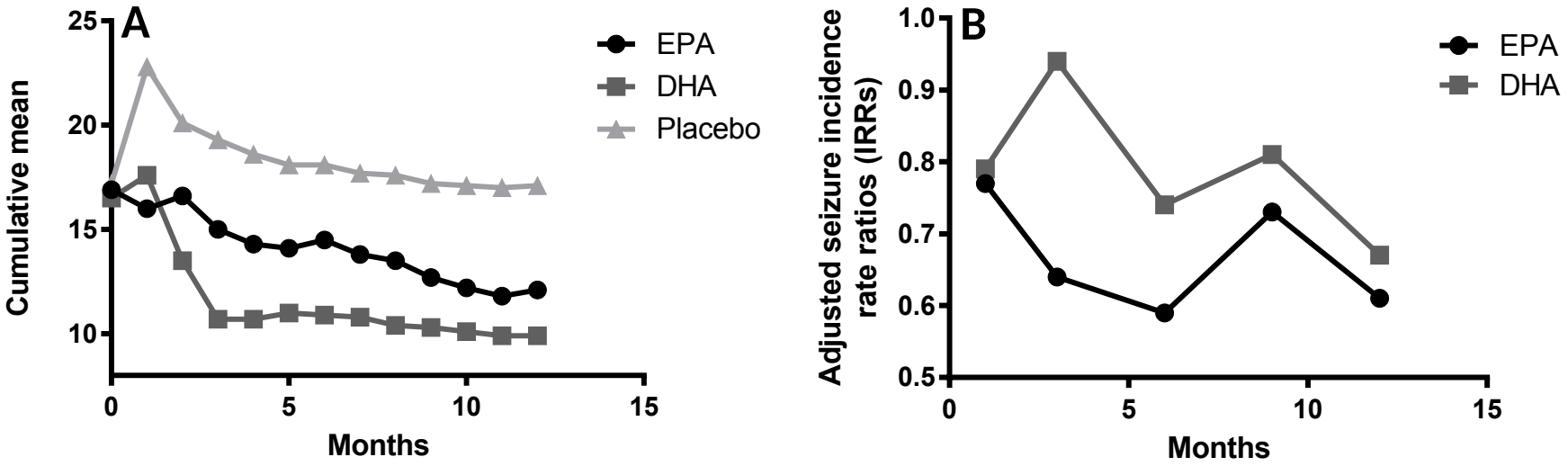


Figure 2: A) Cumulative mean number of seizures in patients with drug resistant epilepsy treated with EPA, DHA or placebo. B) Adjusted seizure incidence rate ratios (IRRs) of the EPA or DHA treated compared with the placebo group.

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).

[1]P-value, DHA-treated versus placebo

[2]P-value, EPA-treated versus placebo

[3]P-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			
	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	p-value 1*	p-value 2**
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 seizer 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)