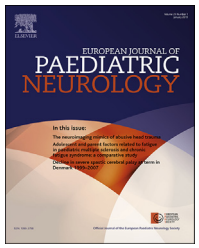
Official Journal of the European Paediatric Neurology Society



Original article

Efficacy and tolerability of olive oil-based ketogenic diet in children with drug-resistant epilepsy: A single center experience from Turkey

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ARTICLE INFO

Article history:
Received 28 May 2018
Received in revised form
20 October 2018
Accepted 13 November 2018

Keywords:
Olive oil-based ketogenic diet
Efficacy
Tolerability
Complications

ABSTRACT

Purpose: Ketogenic diet (KD) is an effective non-pharmacological treatment for drug-resistant epilepsy. The aim of this study was to investigate the efficacy, tolerability and complications of olive oil-based KD in epileptic children.

Method: In this single-center, prospective study, patients were followed up at 1, 3, 6 and 12 months after KD initiation. Initially, blood ketone levels were measured daily, and as needed thereafter to maintain the levels between 4 and 5 mmol/L. Patient demographics, seizure frequency, serum biochemistry, abdominal ultrasonography and adverse effects were recorded. Efficacy of KD was defined as ≥50% seizure reduction.

Results: A total of 389 patients with drug-resistant epilepsy receiving KD from 2012 to 2016 were included. One hundred patients (25.7%) stopped the diet for different reasons in the first year, and 369, 314, 225 and 160 patients have been receiving KD treatment for 1, 3, 6 and 12 months, respectively. At 1, 3, 6 and 12th months, 65.8% (243/369), 74.7% (235/314), 70.6% (159/225) and 83.1% (133/160) of the patients were responders, respectively. None of the children had an increased seizure-frequency. Hyperlipidemia (50.8%), selenium deficiency (26.9%), constipation (26.2%), sleep disturbances (20.0%), nephrolithiasis (3.0%), hyperuricemia (3.0) and hepatic side effects (2.6%) were the most common complications of KD. Previous adrenocorticotropic hormone (ACTH) use due to epileptic encephalopathy and presence of constipation at baseline or during KD treatment were found the predictors of treatment efficacy.

Conclusion: KD is an effective and well-tolerated treatment option for patients with drug-resistant epilepsy. Previous history of ACTH use and constipation during KD treatment are important factors that affect the efficacy of KD treatment.

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https://doi.org/10.1016/j.ejpn.2018.11.007

1. Introduction

Ketogenic diet (KD) is a dietary treatment modality for the patients with drug-resistant seizures.[1] Ketogenic diet stimulates fatty acid oxidation and the generation of ketone bodies in the hepatocyte mitochondria, and subsequently, these ketone bodies cross the blood brain barrier and exhibit anticonvulsant properties.[2]

Various KD versions such as long-chain triglyceride-based classic KD, medium-chain triglyceride-based KD, modified Atkins diet and low-glycemic index containing KD are used for the treatment of intractable epilepsy in most countries. Majority of studies reported at least 50% reduction in seizure frequency in more than half of the patients treated with KD[3–6] However, this therapy is not free of side effects, and may cause gastrointestinal intolerance, constipation, hyperlipidemia, nephrolithiasis, hypoalbuminemia, electrolyte imbalance, hepatotoxicity, cardiac insufficiency and micronutrient deficiencies.[4,7–10] Olive oil has been used for centuries in the diet of Aegean-Mediterranean regions in Turkey. It is the main fat source of the Mediterranean diet and is rich in monounsaturated fatty acids as well as numerous antioxidant molecules including polyphenols. It has beneficial effects on inflammation, cardiovascular diseases and oxidant status of the body.[11–13] Due to familiarity of the caregivers with olive oil-based recipes and better tolerability by the patients, we have been employing olive oil-based KD in our center. The aim of this study was to determine the efficacy and side effects of olive oil-based KD in children with drug-resistant epilepsy.

2. Methods

2.1. Study population

This is a single-center, prospective study conducted in a total of 389 patients with drug-resistant epilepsy receiving KD. The study period lasted from June 2012 to January 2016. Children aged 6 months to 18 years were enrolled in the study. Drug-resistant epilepsy was defined by inadequate control of seizures despite optimal treatment with at least two antiepileptic drugs.[14] Children with known or suspected inborn errors of metabolism in which KD is contraindicated (pyruvate carboxylase deficiency, primary carnitine deficiency, fatty acid oxidation deficiency, ketolysis deficiency, familial hypercholesterolemia etc.), systemic conditions (liver disease or previous liver transplantation, cardiomyopathy, recurrent respiratory infections, immunodeficiency, chronic diarrhea, severe gastroesophageal reflux etc.) or surgically remediable causes of epilepsy were excluded.

All of the study patients received baseline metabolic screening including blood ammonia and lactate levels, urinary organic acids, blood free- and acyl-carnitine profile, plasma amino acids and serum biotinidase activity to assess possible contraindications or metabolic disorders that need a different treatment.[15,16] Family diagnosis of porphyria was also interrogated during the initial visit.

2.2. Dietary protocol and blood ketone level measurements

Ketogenic diet was started to all patients at outpatient clinic of Neurology Department. Non-fasting gradual initiation protocol with a lipid:nonlipid ratio between 2.5:1 and 4:1 (fat/protein plus carbohydrate) was used in the present study. The initial calorie need was calculated individually for each patient according to the energy requirement for ideal body weight calculated by baseline height (for children under 2 years-old) and for ideal body mass index (for children after 2 years-old), and the physical activity level of each patient. In general, energy and protein intakes ranged between 60 and 80 kcal/kg/day and 1–1.5 g/kg/day, respectively. The planned total amount of calories was divided into 3 or 4 daily portions. The ratio of the diet was modified as needed to maintain blood ketosis, and to avoid acidosis, hyperketosis and hypoglycemia.

Blood beta-hydroxybutyrate measurement was used to evaluate the ketone status of the body. Ketone level was measured with FreeStyle Optium Neo Blood Glucose and Ketone Monitoring System (Abbott Diabetes Care Inc, UK) in whole blood samples obtained from the fingertip. Daily ketone level measurements were usually performed in the morning and in the evening. Ketone levels were measured daily in the first week, two days in a week for three weeks, one day in a week for two months, and fortnightly after three months to maintain the levels between 4 and 5 mmol/L. Blood ketones reached the target levels within 4–7 days (mean 6 days) in all patients. Blood ketone and glucose levels, seizure frequency and any adverse events were reported daily by parents to the physician with e-mail and telephone, in addition to regular clinic visits. Ketogenic diet ratio was adjusted according to the blood ketone concentrations and degree of seizure control. The caloric intake was adjusted to maintain an ideal body weight for the height and ideal body mass index based on the patient's weight gain or loss during KD.

The recipes were planned considering the preferences of the child and the family. The menus were prepared according to traditional Turkish food to increase patient compliance and palatability. Dietary composition showed a great variation among the patients. A Mediterranean style KD was prepared to particularly contain extra virgin olive oil as the principal source of monounsaturated fats. Mean olive oil amount was 80–85 per cent of the total fat content of a meal. Other fat sources were cream (especially only for ice-cream), meats and butter (to be used in soups). Palm oil, sunflower oil, hazelnut oil, coconut oil or corn oil were not used. Green vegetables and avocado were employed abundantly in patients' daily menus to prevent and treat constipation. To increase palatability of fatty foods and the variety of the diet; milk, yogurt, cheese and locally available food were frequently used. Stevia powder was used for sweetening the foods. Different flours (almond, walnut, hazelnut and coconut flours) were used for making bread. For example, almound flour (160 g), psyllium husk powder (25 g), egg whites (110 g), salt, baking powder and water were used to make a bread with ketogenic ratio 1.88:1 (energy content: 1140 Kcal, protein content: 43.5 g, fat content 102.4 g and carbohydrate content 11.04 g). Sugar-free multivitamin and mineral supplements were initiated in all

patients. Twenty-eight patients (7.2%), who had nasogastric tube or gastrostomy, or were under the age of one-year and had feeding problems, received a commercial KD formula (KetoCal 4:1, Nutricia) and purified ketogenic soups.

Since most of the liquid medications including antiepileptics, antibiotics and antipyretics contain different amounts of carbohydrate[17]; such medications were modified by switching these drugs to tablet or capsule forms as much as possible, or by continuing these drugs and calculating their carbohydrate content.

2.3. Follow-up investigations

Caregivers received extensive training during the first three days. The training entailed the rationale of KD, ways to prepare the menus at home, and possible side effects. Patients' age, gender, seizure etiology, number of anticonvulsants and seizure frequency per week were recorded at the initial visit as well as each follow-up visit. Patients were monitored through follow-up clinic visits which were conducted at the first month and then every 3 months. Body weight and length, clinical findings (KD ratio, medications, seizure frequency, bowel movements, vomiting, sleeping patterns, food refusal, hunger, weight status, etc.), full blood count, serum biochemistry (glucose, electrolytes, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, albumin, blood urea nitrogen, creatinine, uric acid, calcium, phosphorus, magnesium, zinc, 25-hydroxy vitamin D, vitamin B12, folic acid and selenium levels), free- and acylcarnitine levels, blood antiepileptic drug levels, abdominal ultrasonography and echocardiographic findings, and adverse effects were checked at baseline and at 1, 3, 6 and 12 months of therapy.[6,8–10] The body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). A BMI-SDS was calculated and recorded. Dyslipidemia was accepted as total cholesterol >200 mg/dL, triglycerides >130 mg/dL, LDL-cholesterol >130 mg/dL, and HDL-cholesterol <35 mg/dL at each visit.[8,18] Reference ranges for laboratory parameters were determined as shown: glucose: 60–100 mg/dL; total protein: 6–8 g/dL; albumin: 3.2–4.5 g/dL; uric acid: 2–5 mg/dL; selenium: 48–142 μg/L; vitamin B12: 200–1900 pg/mL; folic acid: 3–17.5 ng/mL; 25-hydroxy vitamin D: 20–42 ng/mL; zinc: 65–140 μg/dL, magnesium: 0.6–1.1 mmol/L.[9,19]

Between clinic visits, the families were asked to keep seizure calendars and to send e-mails to physicians about their follow-up on blood ketone and glucose levels as well as any side effects. Side effects and reason for stopping the diet before one year of treatment were also assessed.

2.4. Etiology, seizure efficacy

For the classification of epilepsy, the International League Against Epilepsy (ILAE) Commission Report and the 2010 Revised Report of ILAE were used.[20,21] Seizure type was classified as generalized, focal and unknown; etiology was classified as genetic, structural-metabolic and unknown; and electroclinical syndromes were described as electro-clinical syndromes and non-syndromic epilepsy.[21] We included seizures evolving to bilateral convulsive seizures involving tonic, clonic, or tonic-clonic seizures under the 'generalized' category due to difficulty of caregivers to reliably differentiate these seizure types from generalized seizures. Seizure reduction was classified into 5 categories: 1) no change in seizure frequency, 2) <50% seizure reduction, 3) 50–90% seizure reduction, 4) 90–99% seizure reduction, and 5) seizure-free status.[7] Patients who were seizure-free or had >50% seizure reduction were accepted as KD responders.

2.5. Ethical approval

The study protocol was designed in compliance with the 1964 Declaration of Helsinki. Informed consent was obtained from parents on enrollment in the study. The study was initiated and data collection was started after the approval of the Ethics Committee of the Dr. Behcet Uz Children Hospital (Number of ethical approval: 2014/03–08).

2.6. Statistical analyses

Statistical analysis was performed using the Statistical Package for Social Sciences version 20.0. Continuous and categorical variables were reported as mean ± standard deviation or median [25–75 percentiles], and numbers (%), respectively. Categorical data were analyzed using Pearson's chi-square test. Comparisons of mean values were analyzed using a two-tailed *t*-test. The significance level for all tests was $p = 0.05$.

Univariate repeated measures ANOVA with a Greenhouse-Geisser correction was made to analyze the anthropometric changes of the patients with time (prior to, and at 1, 3, 6 and 12 months after KD-therapy). To identify the source of significant differences among means, Bonferroni test was used for post-doc analysis. The significance level for post-hoc multiple comparisons was 0.005.

3. Results

3.1. Patient demographics and follow-up characteristics

After the first visit and baseline training, 87 parents (18.2%) found KD too restrictive and stated being unable to spare time for preparation of the meals and therefore did not initiate the diet (Fig. 1). Out of 476 patients, 389 started KD (mean age: 6.9 ± 4.1 years; 202 boys, 51.9%). One hundred patients (25.7%) stopped the diet for different reasons in the first year (Fig. 1). Patients were on the KD for a median of 15 months [3–24 months]. Maximum duration of follow-up on KD was at least 36 months (9 patients, 2.3%).

Table 1 shows the baseline characteristics of the patients. Median age of seizure onset was 4 months. The median age at diet inception was 4.0 years (Table 1). History of previous ACTH (adrenocorticotropic hormone) use for epileptic encephalopathy between 0 and 5 years old was recorded in 46 patients (11.8%). Multivitamin-mineral combinations were the most commonly used supplements (338 patients, 87.1%). Other supplements the patients received included fish oil (108 patients, 28.1%), potassium citrate (31 patients, 8.1%), iron (11 patients, 2.8%) and zinc (5 patients, 1.2%).

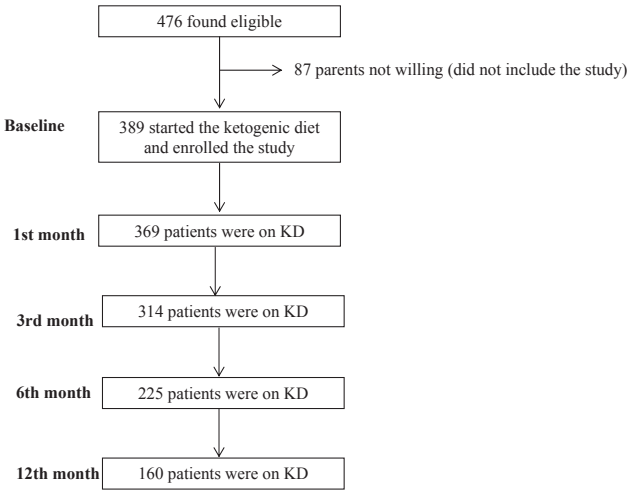

Fig. 1 – Flow of patients in the study.

Table 1 – Baseline characteristics of the patients (n = 389).

Characteristics	Median [Interquartile range]
Age at epilepsy onset (years)	0.4 [0–4]
Age at KD start (years)	4.0 [2–7]
Characteristics	n (%)
Gender (M/F)	202 (51.9)/187 (48.1)
Epilepsy type	
Generalized	345 (88.8)
Local	44 (11.2)
Etiology	
Genetic (n = 103, 26.7%)	
Tuberous sclerosis complex	38 (9.8)
Dravet syndrome	13 (3.3)
Doose syndrome	8 (2.0)
Other	44 (11.6)
Structural/metabolic (n = 160, 40.9%)	
Hypoxic-ischemic encephalopathy	76 (19.6)
Cortical malformation	39 (10.0)
Encephalitis	20 (5.1)
Intracranial hemorrhage	5 (1.3)
GLUT-1 deficiency	13 (3.3)
Non-ketotic hyperglycinemia	2 (0.5)
Neurotransmitter defect	1 (0.2)
Mitochondrial respiratory chain defect	1 (0.2)
Other	3 (0.7)
Unknown	126 (32.4)
Electroclinical syndromes (n = 52, 13.3%)	
West syndrome	22
Dravet syndrome	13
Lennox-Gastaut syndrome	7
Ohtahara syndrome	6
Landau-Kleffner syndrome	2
Malignant migratory epilepsy	2
Mental retardation	361 (93.0)
Autism	60 (15.6)
Motor dysfunction	323 (83.0)
Vagus nerve stimulation	9 (2.3)
Previous epilepsy surgery	7 (1.7)
Method of feeding	
Orally	371 (95.5)
Gastrostomy	11 (2.8)
Nasogastric tube	7 (1.7)

3.2. *Efficacy on seizures*

One, three, six and 12 months after diet initiation, 94.8%, 80.7%, 57.8% and 41.1% children remained on the diet, respectively (Table 2). At 1, 3, 6 and 12 months, 65.8%, 74.7%, 70.6% and 83.1% of the patients were responders, respectively. None of the children had an increased seizure-frequency. Mean seizure-free response was achieved at 101 ± 108 days (median 30 [15–90] days, minimum 3 days - maximum 330 days) in the seizure-free group. The number of seizures decreased from 3 months of treatment to 12 months among the 26 non-responders at 3 months (26/314, 8.2%). On the other hand, 10 patients (10/160, 6.2%) who initially responded to therapy during the first three months subsequently had an increased number of seizures and they were classified as non-responders at 12 months.

All patients were receiving multiple antiepileptic drug combinations including phenobarbital, valproic acid, carbamazepine, oxcarbazepine, levetiracetam, topiramate, benzodiazepines, clobazam, primidone and zonisamide at admission. A median of $3^{2,3}$ antiepileptic drugs were prescribed at the beginning of KD. Antiepileptic drugs were decreased or discontinued after three months of therapy to achieve the minimum amount of AED use as seizure control improved. At 12 months of therapy, patients had a median of $1^{1,2}$ antiepileptic drug and 31 patients (19.4%) were medication-free (Table 2).

Body mass indexes (BMI-SDS scores) of the patients at baseline, 1st, 3rd, 6th and 12th months of the KD were 16.5 ± 3.2 (−0.39 ± 1.7), 16.4 ± 3.1 (−0.42 ± 1.6), 16.2 ± 2.8 (−0.42 ± 1.4), 16.2 ± 2.4 (−0.43 ± 1.5) and 15.9 ± 2.1 (−0.44 ± 1.4) ($p = 0.679$). Two hundred and four parents (52.4%) reported feeding and swallowing problems of their children at baseline. Out of the 160 patients who remained on the diet at 12 months, 33 patients (20.6%) had swallowing problems. Ninety eight (61.2%) of these 160 children exhibited positive effects on development and behavior as reported by the parents, including 15 patients who was non-responders. Drooling was another comorbidity in 90 patients (23.3%) at baseline. At 12 months of therapy, drooling was reported in 9 (5.6%) patients.

3.3. *Withdrawal before 12 months and mortality*

One hundred patients (25.7%) discontinued the diet for different reasons or due to death in the first year (Table 3). There were 48 girls (48.0%) in this group. There was no difference between this group and the remaining patients regarding gender, age, etiology and the number of medications ($p = 0.543$, $p = 0.103$, $p = 0.863$, $p = 0.643$, respectively). In this group, there were 22 patients with perinatal asphyxia, 15 with neurocutaneous syndromes, 6 with cortical dysplasia, 6 with the sequelae of Herpes encephalitis, 5 with West syndrome, 4 with Dravet syndrome, 3 with Doose syndrome, 3 with Down syndrome, 2 with frontal lobe epilepsy, 2 with glucose transporter-1 deficiency, 1 with neuronal ceroid lipofuscinosis, 1 with non-ketotic hyperglycinemia and 1 with Lenoux-Gastaut Syndrome. A specific diagnosis could not to be defined in 29 patients included in this group, and they constituted the group of idiopathic epilepsy. Although KD treatment reduced the seizure frequency more than 50% in 75

Table 2 – Ketogenic diet ratio, antiepileptic medications and response rates of the patients at baseline and during ketogenic diet treatment.

	Baseline (n = 389)	At 1 month (n = 369)	At 3 months (n = 314)	At 6 months (n = 225)	At 12 months (n = 160)
KD ratio (n, %)					
≤2.5:1	10 (2.5)	180 (48.7)	165 (52.5)	126 (56.2)	92 (57.4)
3:1	338 (86.9)	161 (43.8)	135 (43.1)	95 (42.5)	66 (41.3)
4:1	41 (10.6)	28 (7.5)	14 (4.4)	4 (1.3)	2 (1.3)
Seizure outcomes (n, %)	–				
Seizure-free		131 (35.6)	125 (39.8)	86 (38.3)	69 (43.1)
90–99% reduction		66 (17.8)	59 (18.7)	42 (18.6)	38 (23.7)
50–90% reduction		46 (12.4)	51 (16.2)	31 (13.7)	26 (16.3)
<50% reduction		78 (21.3)	63 (20.2)	52 (23.2)	20 (12.5)
No change		48 (12.9)	16 (5.1)	14 (6.2)	7 (4.4)
Efficacy (n, %)	–				
Responder		243 (65.8)	235 (74.7)	159 (70.6)	133 (83.1)
Non-responder		126 (34.2)	79 (25.3)	66 (29.4)	27 (16.9)
Number of antiepileptic drugs (median, [Interquartile range])	3 [2–3]	2 [1–3]	2 [1–3]	2 [1–3]	1 [1–2]
Number of medication-free patients (n, %)	0	0	15 (5.0)	28 (12.5)	31 (19.4)

Table 3 – Reasons for discontinuing the diet before 12 months (n = 100).

Reasons	n
Compliance problems:	**48**
Caregiver's non-compliance	26
Child's refusal to eat fatty food	15
Child's refusal to eat due to autism and mental retardation	6
Child's refusal to eat due to bad taste	1
Lack of efficacy	**18**
Death	**15**
Systemic diseases other than epilepsy	**9**
Increased infections	**4**
Restriction of social life	**2**
Low income of the family	**2**
Side effects	**1**
Surgery	**1**
Total number of patients who discontinued the diet before 12 months n (% of total started on the diet)	**100 (25.7%)**

(75.0%) of these patients, they discontinued the diet due to different reasons (Table 3). Nearly half of the patients (48.0%) who stopped the diet did so due to compliance problems (Table 3). Especially, two patients with glucose transporter-1 deficiency stopped the diet due to pulmonary infections and low income of the family, respectively, although they became seizure-free. Thirty two (32.0%) patients stopped the diet between the 1st-3rd months, 16 (16.0%) patients between the 4th-6th months and 52 (52.0%) patients between the 6th-12th months of the diet. In this group, 15 patients who had started the diet died during KD treatment. Four patients (26.7%) died between the 1st-3rd months, 3 (20.0%) patients between the 4th-6th months and 8 (53.3%) patients between the 6th-12th months of the diet. These deaths were considered as not related to KD, but related to the underlying severe medical problems (five patients, who have swallowing problems and which their families refused to gastrostomy insertion, died due to pulmonary aspiration during feeding; and remaining 10 immobile patients died due to severe respiratory infections). Autopsy was not performed for any of these patients.

3.4. *Side effects, dietary interventions and medications for complications*

Most of the patients developed side effects related to KD treatment (Table 4). Most of the side effects were treated with dietary interventions. Hyperlipidemia was the most common new-onset adverse effect, noted in half of the patients as we described these patients before[8] (Table 4). If a patient was diagnosed with hyperlipidemia, the diet was modified by reducing dietary fats by 20–25% without affecting blood ketone levels and by eliminating egg yolk and saturated fat sources (cream, butter, fatty meats) from the diet.[8] We gave atorvastatin (10 mg/day, PO) to block endogenous cholesterol biosynthesis to three patients (9, 10 and 12 years-old) who had total cholesterol and triglyceride levels above 300 and 400 mg/dL, respectively (305 and 542 mg/dL; 346 and 430 mg/dl; 380 and 405 mg/dL, respectively), even after these dietary interventions. LDL-cholesterol could not be calculated for these patients. After three months of statin therapy, total cholesterol and triglyceride levels decreased to 250–300 mg/dL. Selenium deficiency was seen in 26.9% of the patients during KD

Table 4 – Side effects of ketogenic diet (n = 389).

Side effects	Number of patients n (%)
Hyperlipidemia	198 (50.8)
Selenium deficiency	105 (26.9)
Constipation	102 (26.2)
Sleep disturbances	78 (20.0)
Renal stone	12 (3.0)
Hyperuricemia	12 (3.0)
Hepatic side effects (elevated liver enzymes, fatty liver)	10 (2.6)
Hypoproteinemia	10 (2.6)
Hypoglycemia	6 (1.5)

treatment. Oral selenium supplement (2 μg/kg/day) was initiated in all patients diagnosed with selenium deficiency as previously described and published.[9] Constipation (26.2%) was also common, and attempts to resolve this side effect included increasing dietary fibers (especially okra, chard, dill and leek and avocado). Sleep disturbances occurred in one-fifth of the patients. Sleep problems were more common after the time points of antiepileptic medication cessation. Hyperuricemia was detected in 12 (3.0%) patients, all whom were treated by increasing water consumption with close monitoring. Allopurinol treatment was not initiated in any of the patients. Hypoproteinemia occurred in 10 (2.6%) patients and immediately ameliorated upon increasing the protein content of the diet. Hypoalbuminemia related findings such as edema or cardiac insufficiency were not detected in any patient. Asymptomatic hypoglycemia (blood glucose levels between 45 and 60 mg/dL) was seen 6 (1.5%) patients (2-10 years-old) and immediately treated with fruit juice consumption. None of the patients experienced acidosis, hypocalcemia, electrolyte imbalance, encephalopathy, protein-losing enteropathy, cardiac dysfunction or an increased rate of infections.

3.5. *Possible factors which affect the response rate to KD treatment*

Gender, age group (<5 years old *vs.* ≥5 years old), etiology, presence of autism, presence of constipation, previous ACTH use due to epileptic encephalopathy were screened as possible factors influencing the efficacy of KD treatment at 12 months (160 patients). The number of seizures decreased from 3 months of treatment to 12 months among the 26 non-responders at 3 months (26/314, 8.2%). On the other hand, 10 patients (10/160, 6.2%) who initially responded to therapy during the first three months subsequently had an increased number of seizures and they were classified as non-responders at 12 months.

Previous ACTH use due to epileptic encephalopathy and presence of constipation at baseline or during KD treatment were found to reduce treatment efficacy in our patients (Table 5). Clinically, when constipation occurred in patients, decreasing blood ketone levels and increased numbers of seizures were observed (data not shown).

4. Discussion

In this study, we evaluated the effect of olive oil-based KD therapy in children with drug-resistant epilepsy. Mean response rate of the patients was 83.1% at 12 months of treatment. An important finding of this study is that 8.2% of the non-responders at 3 months of therapy became responders at 12 months. Other important findings are the effects of constipation and history of previous ACTH use on response rates. Low rates of hypoproteinemia and the absence of acidosis in our series are among other important findings. This is the first study evaluating the efficacy and tolerability of KD in Turkish children with refractory epilepsy.

The response rate at 12 months in our patients (83.1%) is comparable to the previous studies on KD in multidrug-refractory epilepsy. Response rates at 12 months were reported to be between 29.3 and 88% in different studies using different KD modalities in epileptic patients.[3,4,6,7,22] This high response rate observed in our patients at 12 months of treatment may be related to the high compliance to diet due to the more preferable taste of menus prepared with olive oil, vegetables and meat. We specifically used different menus which

Table 5 – Possible factors which affect the response rate of ketogenic diet (n = 160) (n,%).

Factor	Responder[b] (n = 133)	Non-responder[c] (n = 27)	*p* value
Gender			0.138
Male	65 (78.3)	18 (21.7)	
Female	68 (88.3)	9 (11.7)	
Age group			0.898
<5 years	60 (83.3)	12 (16.7)	
≥5 years	73 (83.0)	15 (17.0)	
Autism			0.771
Yes	20 (80.0)	5 (20.0)	
No	113 (83.7)	22 (16.3)	
Etiology			0.091
Genetic	34 (82.9)	7 (17.1)	
Structural/metabolic	61 (89.7)	7 (10.3)	
Unknown	38 (74.5)	13 (25.5)	
Electroclinical syndromes			0.357
Yes	16 (76.2)	5 (23.8)	
No	117 (84.2)	22 (15.8)	
Previous ACTH[a] use due to epileptic encephalopathy			0.000
Yes	9 (47.4)	10 (52.6)	
No	124 (87.9)	17 (12.1)	
Constipation			0.005
Yes	68 (75.6)	22 (24.4)	
No	65 (92.9)	5 (7.1)	

[a] ACTH: Adrenocorticotropic hormone.
[b] Including 26 patients who initially non-responders and became responders at 12th months.
[c] Including 10 patients who initially responders and became non-responders at 12th months.

included locally accessible and palatable Mediterranean recipes in order to increase patients' compliance to the diet. We usually use olive-oils from North Aegean area which have lower phenol content, lower acidity and have a softer taste, and they can consumpt easily. A high level of compliance to the diet resulted in a faster and more reliable reduction in the ketogenic ratio. Lower KD ratio translates into higher compliance to these menus with relatively lower fat content. Another possible explanation of the high response rate seen among our cases is the close monitoring of blood ketone and glucose levels. Daily consultations were performed to adjust dietary recommendations according to blood ketone levels. Parents were specifically asked to measure blood ketone and glucose levels and report the Results to physician via e-mail, particularly if their children experienced vomiting, constipation, sleep disturbances, body weight changes or febrile disease. We measured blood ketone levels instead of urinary ketones for different purposes. First of all, urinary ketone levels have been shown to exhibit wide variations throughout the day.[23] Secondly, van Delft et al. showed a better correlation between blood beta-hydroxybutyrate with seizure reduction compared to ketones in urine.[24] Finally, it may not always be easy to collect urine samples, especially from disabled children.

Another important finding of this study is that 26 patients (8.2%) who were non-responders at 3 months of treatment became responders at 12 months. Although the International Ketogenic Diet Study Group recommends discontinuing KD if it is not efficient on seizures at 3 months of therapy,[15] the parents in our study were offered to continue KD to improve children's motor functions, language and social developments even if the therapy was ineffective on seizures at three months of therapy. Nearly one tenth of these patients became responders at 12 months. In addition to possibly additive antiepileptic effects of the diet in upcoming months, KD had a positive effect on development and behavior in epileptic children, as reported by most of the parents (61.2%) in the present study. Due to these two indications, we recommend parents to continue their children's dietary treatment for at least six months to decide whether KD effective is on seizure control.

In this study, side effects of KD were similar to those observed in previous studies.[3–6] Hyperlipidemia was the most frequently seen side effect of this diet with a high level of fat content although we used olive oil as the main fat source of the diet.[8,18,25] We tried some diet modifications to reduce the high lipid levels. On the other hand, we gave atorvastatin to three patients with hyperlipidemia, and after three months of treatment their lipid levels decreased to acceptable levels for this high-fat diet. With the normalization of serum lipids and lowered dietary ratio at the time, statins were discontinued within six months in all patients. Selenium deficiency was frequently seen during KD treatment as we stated before.[9] We used oral selenium tablets for the patients who had selenium deficiency during the diet. Constipation was another commonly seen side effect of KD. Since we detected the relation between the presence of constipation and efficacy of KD on seizures, we strived to solve the constipation problem of our patients as described above. Low rates of hypoproteinemia and hypoglycemia, and the absence of acidosis in our series were the result of immediate dietary modifications.

In our study, lower response rates were seen in patients who had received previous ACTH treatment for epileptic encephalopathy or West Syndrome (response rates of 47.4 vs 87.9% in those with and without previous ACTH use, respectively). Previous studies investigating the efficacy of KD have conflicting Results regarding the response rate among patients previously treated with ACTH. Hong et al. gave KD treatment to 104 infants for infantile spasms, 71% of whom had received previous treatment with ACTH or vigabatrin. They found an overall response rate of 77% after 1–2 years of KD.[26] On the other hand, they did not compare the children with or without previous ACTH treatment in terms of response rates. Hirano et al. gave KD to six patients who had ACTH-resistant infantile spasms. Five of the patients responded to KD with reduced or completely resolved spasms after three months of therapy.[27] Aizaki et al. described a patient with cardio-facio-cutaneous syndrome who received KD after ACTH treatment due to intractable spasms. Although transient seizure control was achieved by KD and antiepileptics, intractable spasms were reported to recur after one month of diet.[28] Similarly, Moseley et al. presented six epileptic patients with *CDKL5* mutations four of whom were previously treated with ACTH. While these patients had a short "honeymoon" period with transient seizure control after KD treatment, refractory seizures manifested again in all children.[29] Pires et al. started KD in 17 patients with infantile spasms refractory to first-line treatment. They added new antiepileptic drugs during the first month of KD treatment in 11 of the patients (65%) and the overall response rate at three months was 76% with this combination therapy.[30] Finally, Saito et al. described four patients with Aicardi syndrome who were previously treated with ACTH and then switched to KD treatment.[31] They found that both of the therapies had limited therapeutic effects in these patients.[31] Our study included more patients than all of these studies and moreover, we evaluated follow-up results of a longer period in our patients to assess the effect of previous ACTH use on the response rates with KD treatment in this study. We conclude that previous ACTH use which is an indicator of severity of the epileptic encephalopathy of patients, is a good predictor for KD response rate in pediatric age group.

Constipation was found to be another factor which influences the response rate in KD treatment. In this study, constipation occurred in 26.2% of our patients during KD treatment. In different studies, constipation was often reported at a rate of 65–75% with different dietary durations.[3,5] The relatively lower rates of constipation in our study is associated with the fact that at least fifty percent of daily menus consisted of leafy vegetables, and additionally, a daily fruit snack containing avocado was given to all patients to avoid constipation. In the literature, we did not encounter any study which evaluated the effect of constipation on KD response rates. At the present time, the exact mechanisms of how constipation reduces blood ketone levels and the response rate of a diet remain unknown. One of the possible explanations may be the blood sugar levels maintained at a certain level due to decelerated absorption of nutrients in intestinal lumen as a result of the decelerated intestinal passage and the resulting decrease in production of ketone bodies. Further studies are warranted to explain this mechanism.

Since traditional Turkish cuisine's main fat source is olive oil, we used olive oil to prepare our menus. Our caregivers were asked to prepare same traditional foods (bread, meatball, cacik, vegetable dishes prepared with olive oil) both for their epileptic children and also for the rest of the family. The differences between the patients' menus compare to family's foods were patients' foods contained more olive-oil and yogurt, and some other low carbohydrate products like stevia powder and psyllium husk powder. In addition to familiarity of the caregivers with olive oil based recipes and better tolerability by the patients, we used olive oil because of its proven anti-inflammatory, antioxidant and neuroprotective effects which have been shown in numerous different animal models.[32–37]

Our study has some limitations. Since the study population is heterogeneous and patient number is small in groups with specific diagnosis, we could not compare the different etiologic groups (GLUT-1 deficiency, cortical malformation, tuberous sclerosis, Dravet syndrome etc) in terms of response rates. The relatively short follow-up period is another limitation of our study. It would have been useful to have further data on efficacy and side effects after 24–36 months of treatment.

In conclusion, olive oil-based KD is a palatable and effective option for the non-pharmacological treatment of multidrug-resistant epilepsy. Measurement of blood ketone levels instead of urine ketones is a more reliable method to monitor the ketone status of the body and also provides the convenience to manage the diet. Lower response rate is seen in patients with history of epileptic encephalopathy who received previous ACTH treatment. Presence of constipation reduce the effectiveness of KD. Since 8.2% of the patients who were not responsive at three months of KD exhibited response at later periods, KD should be continued at least 6 months to see the exact response rate with this therapeutic option.

Declaration of interest

None.

Funding

This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

Acknowledgements

We thank all the patients and parents who contributed to this study.

REFERENCES

1. Branco AF, Ferreira A, Simões RF, Magalhães-Novais S, Zehowski C, Cope E, et al. Ketogenic diets: from cancer to mitochondrial diseases and beyond. *Eur J Clin Invest* 2016;**46**:285–98.
2. Vidali S, Aminzadeh S, Lambert B, Rutherford T, Sperl W, Kofler B, et al. Mitochondria: the ketogenic diet-A metabolism-based therapy. *Int J Biochem Cell Biol* 2015;**63**:55–9.
3. Wibisono C, Rowe N, Beavis E, Kepreotes H, Mackie FE, Lawson JA, et al. Ten-year single-center experience of the ketogenic diet: factors influencing efficacy, tolerability, and compliance. *J Pediatr* 2015;**166**:1030–6.
4. Hallböök T, Sjölander A, Åmark P, Miranda M, Bjurulf B, Dahlin M. Effectiveness of the ketogenic diet used to treat resistant childhood epilepsy in Scandinavia. *Eur J Paediatr Neurol* 2015;**19**:29–36.
5. Sharma S, Gulati S, Kalra V, Agarwala A, Kabra M. Seizure control and biochemical profile on the ketogenic diet in young children with refractory epilepsy-Indian experience. *Seizure* 2009;**18**:446–9.
6. Kang HC, Chung DE, Kim DW, Kim HD. Early- and late-onset complications of the ketogenic diet for intractable epilepsy. *Epilepsia* 2004;**45**:1116–23.
7. Suo C, Liao J, Lu X, Hu Y, Chen L, Cao D, et al. Efficacy and safety of the ketogenic diet in Chinese children. *Seizure* 2013;**22**:174–8.
8. Guzel O, Yılmaz U, Uysal U, Arslan N. The effect of olive oil-based ketogenic diet on serum lipid levels in epileptic children. *Neurol Sci* 2016;**37**:465–70.
9. Arslan N, Kose E, Guzel O. The effect of ketogenic diet on serum selenium levels in patients with intractable epilepsy. *Biol Trace Elem Res* 2017;**178**:1–6.
10. Arslan N, Guzel O, Kose E, Yılmaz U, Kuyum P, Aksoy B, et al. Is ketogenic diet treatment hepatotoxic for children with intractable epilepsy? *Seizure* 2016;**43**:32–8.
11. Martín-Peláez S, Castañer O, Solà R, Motilva MJ, Castell M, Pérez-Cano FJ, et al. Influence of phenol-enriched olive oils on human intestinal immune function. *Nutrients* 2016;**8**:213. https://doi.org/10.3390/nu8040213.
12. Valderas-Martinez P, Chiva-Blanch G, Casas R, Arranz S, Martínez-Huélamo M, Urpi-Sarda M, et al. Tomato sauce enriched with olive oil exerts greater effects on cardiovascular disease risk factors than raw tomato and tomato sauce: a randomized trial. *Nutrients* 2016;**8**:170. https://doi.org/10.3390/nu8030170.
13. Schwingshackl L, Christoph M, Hoffmann G. Effects of olive oil on markers of inflammation and endothelial function-a systematic review and meta-analysis. *Nutrients* 2015;**7**:7651–75.
14. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. *Epilepsia* 2010;**51**:1069–77.
15. Kossoff EH, Zupec-Kania BA, Auvin S, Ballaban-Gil KR, Christina Bergqvist AG, Blackford R, et al. Optimal clinical management of children receiving dietary therapies for epilepsy: updated recommendations of the International Ketogenic Diet Study Group. *Epilepsia Open* 2018;**3**:175–92.
16. van der Louw E, van den Hurk D, Neal E, Leiendecker B, Fitzsimmon G, Dority L, et al. Ketogenic diet guidelines for infants with refractory epilepsy. *Eur J Paediatr Neurol* 2016;**20**:798–809.
17. Misiewicz Runyon A, So TY. The use of ketogenic diet in pediatric patients with epilepsy. *ISRN Pediatr* 2012;**2012**:263139. https://doi.org/10.5402/2012/263139.
18. Nizamuddin J, Turner Z, Rubenstein JE, Pyzik PL, Kossoff EH. Management and risk factors for dyslipidemia with the ketogenic diet. *J Child Neurol* 2008;**23**:758–61.
19. Roberts WL, McMillan GA, Burtis CA, Bruns DE. Reference information for the clinical laboratory. In: Burtis CA,

Ashwood ER, Burns DE, editors. *Tietz textbook of clinical chemistry and molecular diagnostics*. 4th ed. St. Louis, Mo: Elsevier Saunders; 2006. p. 2251–318.
20. Engel J, International League Against Epilepsy (ILAE). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* 2001;**42**:796–803.
21. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia* 2010;**51**:676–85.
22. Vehmeijer FO, van der Louw EJ, Arts WF, Catsman-Berrevoets CE, Neuteboom RF. Can we predict efficacy of the ketogenic diet in children with refractory epilepsy? *Eur J Paediatr Neurol* 2015;**19**:701–5.
23. Urbain P, Bertz H. Monitoring for compliance with a ketogenic diet: what is the best time of day to test for urinary ketosis? *Nutr Metab (Lond)* 2016;**13**:77. https://doi.org/10.1186/s12986-016-0136-4.
24. van Delft R, Lambrechts D, Verschuure P, Hulsman J, Majoie M. Blood beta-hydroxybutyrate correlates better with seizure reduction due to ketogenic diet than do ketones in the urine. *Seizure* 2010;**19**:36–9.
25. Azevedo de Lima P, Baldini Prudêncio M, Murakami DK, Murakami DK, Pereira de Brito Sampaio L, Figueiredo Neto AM, et al. Effect of classic ketogenic diet treatment on lipoprotein subfractions in children and adolescents with refractory epilepsy. *Nutrition* 2017;**33**:271–7.
26. Hong AM, Turner Z, Hamdy RF, Kossoff EH. Infantile spasms treated with the ketogenic diet: prospective single-center experience in 104 consecutive infants. *Epilepsia* 2010;**51**:1403–7.
27. Hirano Y, Oguni H, Shiota M, Nishikawa A, Osawa M. Ketogenic diet therapy can improve ACTH-resistant West syndrome in Japan. *Brain Dev* 2015;**37**:18–22.
28. Aizaki K, Sugai K, Saito Y, Nakagawa E, Sasaki M, Aoki Y, et al. Cardio-facio-cutaneous syndrome with infantile spasms and delayed myelination. *Brain Dev* 2011;**33**:166–9.
29. Moseley BD, Dhamija R, Wirrell EC, Nickels KC. Historic, clinical, and prognostic features of epileptic encephalopathies caused by CDKL5 mutations. *Pediatr Neurol* 2012;**46**:101–5.
30. Pires ME, Ilea A, Bourel E, Bellavoine V, Merdariu D, Berquin P, et al. Ketogenic diet for infantile spasms refractory to first-line treatments: an open prospective study. *Epilepsy Res* 2013;**105**:189–94.
31. Saito Y, Sugai K, Nakagawa E, Sakuma H, Komaki H, Sasaki M, et al. Treatment of epilepsy in severely disabled children with bilateral brain malformations. *J Neurol Sci* 2009;**277**:37–49.
32. Batarseh YS, Mohamed LA, Al Rihani SB, Mousa YM, Siddique AB, El Sayed KA, et al. Oleocanthal ameliorates amyloid-β oligomers' toxicity on astrocytes and neuronal cells: in vitro studies. *Neuroscience* 2017;**352**:204–15.
33. Amel N, Wafa T, Samia D, Yousra B, Issam C, Cheraif I, Attia N, et al. Extra virgin olive oil modulates brain docosahexaenoic acid level and oxidative damage caused by 2,4-dichlorophenoxyacetic acid in rats. *J Food Sci Technol* 2016;**53**:1454–64.
34. Luccarini I, Pantano D, Nardiello P, Cavone L, Lapucci A, Miceli C, et al. The polyphenol oleuropein aglycone modulates the PARP1-SIRT1 interplay: an in vitro and in vivo study. *J Alzheimers Dis* 2016;**54**:737–50.
35. Carito V, Ceccanti M, Tarani L, Ferraguti G, Chaldakov GN, Fiore M. Neurotrophins' modulation by olive polyphenols. *Curr Med Chem* 2016;**23**:3189–97.
36. Luceri C, Bigagli E, Pitozzi V, Giovannelli L. A nutrigenomics approach for the study of anti-aging interventions: olive oil phenols and the modulation of gene and microRNA expression profiles in mouse brain. *Eur J Nutr* 2017;**56**:865–77.
37. Pase CS, Teixeira AM, Roversi K, Dias VT, Calabrese F, Molteni R, et al. Olive oil-enriched diet reduces brain oxidative damages and ameliorates neurotrophic factor gene expression in different life stages of rats. *J Nutr Biochem* 2015;**26**:1200–7.