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ORIGINAL ARTICLE

Analysis of hematological parameters in patients treated with ketogenic diet due to drug-resistant epilepsy

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Abstract Benefits of the ketogenic diet (KD) in epileptic patients are well known while less is known about the nutritional risks of the diet and its potential impacts on biochemical nutritional status. In this study, we aimed to evaluate the hematological parameters of patients who have drug-resistant epilepsy and are treated with KD. Fifty-three patients with drug-resistant epilepsy (mean age 7.4 ± 4.4 years [2–18], 23 [43.4%] female) were included in the study. Demographic and laboratory data of the patients were retrospectively analyzed at baseline and Month 6 and Month 12 of the treatment. Repeated measures ANOVA (post hoc Bonferroni correction) and Friedman test were used to assess the changes in data during the treatment. Mean hemoglobin levels increased by 0.594 g/dL after 6 months ($p = 0.001$) and by 0.602 g/dL after 12 months of the treatment ($p = 0.002$). Mean hematocrit level was found to be significantly increased at Month 6 and 12 of the treatment compared to baseline [$F(2,94) = 8.9$, $p < 0.0001$]. An increase in MCV levels was determined with the KD treatment [$F(2,94) = 19.7$, $p < 0.0001$]. Mean level of vitamin B12 was found to be significantly increased in Month 12 of treatments compared to Month 6 [$F(1.686,72.479) = 3.472$, $p = 0.035$]. There was no significant effect of KD on other hematological parameters (red blood cell, white blood cell and platelet counts, serum iron, total iron-binding capacity, transferrin saturation, and ferritin and folic acid levels). We can conclude that KD increases levels of hemoglobin, hematocrit, MCV, and serum vitamin B12 in patients with intractable epilepsy. Prospective, multi-center, longitudinal studies are needed to confirm our results.

Keywords Epilepsy · Folic acid · Hematological parameters · Iron · Ketogenic diet · Vitamin B12

Introduction

The ketogenic diet (KD) is a high-fat, low-carbohydrate regimen which was first documented to be used as a potential epilepsy treatment 90 years ago [1]. Diet supplies approximately 70 to 90% of the energy as fat with the remaining as protein and carbohydrate [2]. Ketogenic diet is accepted as a potent antiepileptic treatment for intractable childhood epilepsy, but is still used only in a limited number of patients due to the concerns for its poor tolerability and a significant number of different complications associated with it [3–5].

Benefits of the ketogenic diet are well known, but our knowledge on the nutritional risks of the diet and its potential impact on biochemical nutritional status is limited [6–8]. There is no clinical study that collectively evaluated the hematological parameters of the patients treated with KD in literature. A small number of studies separately reported the effects and side effects of KD on hematological parameters [8–10]. In the literature, study groups consisted of 20–25 cases and KD treatment was administered up to 6 months.

In this study, we aimed to evaluate the complete blood count, iron, folic acid, and vitamin B12 status of patients who were diagnosed with intractable epilepsy and treated with KD at least for 1 year to better understand the effects of KD treatment on hematological parameters.

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Methods

Patients and design

This study was carried out at Dokuz Eylul University Hospital and Center for Ketogenic Mediterranean Diet, Izmir, Turkey. Parent(s) of the patients' gave written informed consent for participation in the study before any trial-related procedures were performed. Patients aged 2 to 18 years who had drug-resistant epilepsy were enrolled. Inclusion criteria were as follows: (a) patient has seizures despite optimal treatment with at least two antiepileptic drugs [11] and (b) strict adherence to KD and attendance to all required clinic visits for follow-up. Exclusion criteria were as follows: (a) use of medications other than antiepileptic drugs such as steroids, multivitamin-mineral supplementations, or fish oils within the last 6 months; (b) previously diagnosed with nutritional anemia and treated with iron, vitamin B12, or folic acid; (c) diagnosis of mitochondrial disease (due to bone marrow abnormalities), chronic diarrhea, obesity, and malnutrition; (d) previously treated with protein restricted diet due to primary disease (aminoacidopathy, etc.); and (e) parents' objection for participation in the study. Finally, a total of 53 patients were enrolled in the study.

Dietary protocol

All children were started on a 3:1 or 4:1 KD ratio [fat/(protein plus carbohydrate)]. Mediterranean KD was prepared with extra virgin olive oil as the principal fat source, a common and locally available food as described before [7]. The ratio of KD was modified as 2:1 or 1:1 according to clinical response and blood ketone level of patients. Blood ketone measurement was used to evaluate ketone levels of the body. Blood ketone levels were targeted to maintain between 4 and 5 mmol/L.

Data collection

Demographic (age, gender, diagnosis), hematological [hemoglobin, hematocrit, mean corpuscular volume (MCV), red blood cell (RBC), white blood cell (WBC), platelets (PLT) count], and biochemical parameters [serum iron, total iron-binding capacity (TIBC), transferrin saturation (TS), ferritin, folic acid, vitamin B12 levels] of patients were retrospectively analyzed at baseline and in the post-treatment period at Month 6 and Month 12. In all patients, fasting blood sample was collected for the analysis. Beckman Coulter LH 780 Hematology Analyzer (Beckman Coulter, CA. USA) was used in the analysis of hematological parameters. Serum vitamin B12, folic acid, and ferritin levels were determined by immunoassay method with Unicel DxI800 (Beckman Coulter, CA, USA). Serum iron was analyzed by spectrophotometric method with AU5800 analyzer (Beckman Coulter, CA, USA).

Statistical analysis

Statistical data analyses were performed with the SPSS computer software (version 24.0; SPSS, Chicago, IL). While categorical data were expressed as number and percentage (%), continuous data were expressed as mean ± standard deviation (minimum-maximum) and median [25th–75th percentile] according to normality of variables. Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted to examine the normality of data. While repeated measures ANOVA (post hoc Bonferroni correction) was used to analyze the changes in normal distributed data (hemoglobin, hematocrit, MCV, WBC, platelets, and TIBC, TS, folic acid, vitamin B12 levels), Friedman test was performed to assess the nonparametric distributed data (RBC, RDW, serum iron, and ferritin levels). A two-tailed p value of < 0.05 was considered significant.

Results

Fifty-three patients were enrolled in the study. Twenty-three (43.4%) were female and 30 (56.6%) were male. Mean age of patients was 7.4 ± 4.4 years (2–18). Baseline characteristics of the patients are listed in Table 1. Etiology could not be determined in 17 patients (32.1%). KD outcomes were as follows: 22 (41.5%) patients became seizure free, 17 (32.1%) had a ≥ 50% decrease in seizures, and 14 (26.4%) patients had a < 50% decrease in seizures during KD treatment. Baseline

Table 1 Baseline characteristics of the patients (n = 53)

Etiology, n (%)	
Idiopathic	17 (32.1)
Perinatal asphyxia	12 (22.6)
Cortical dysplasia	4 (7.5)
Neurocutaneous syndromes	5 (9.4)
GLUT-1 deficiency	1 (1.9)
Other	14 (26.4)
Epilepsy type n (%)	
Generalized	45 (84.9)
Local	8 (15.1)
Electroclinical syndromes (n = 11, 20.8%)	
West syndrome	4
Dravet syndrome	3
Lennox-Gastaut syndrome	2
Ohtahara syndrome	1
Landau-Kleffner syndrome	1

KD ratio was 3:1 in 44 children (83.1%) and 4:1 in 9 children (16.9%). At Month 12 of the treatment, only 1 child (1.8%) was on 4:1 ratio, 20 children (37.7%) on 3:1 ratio, 21 children (39.6%) on 2:1 ratio, and 11 children (20.7%) on 1:1 ketogenic diet ratio. All patients were receiving multiple antiepileptic drug combinations including phenobarbital, carbamazepine, oxcarbazepine, levetiracetam, clobazam, topiramate, benzodiazepines, zonisamide, valproic acid, and primidone at baseline. A median of 3 [2, 3] antiepileptic drugs were prescribed at the beginning of KD. Antiepileptic drugs were decreased or discontinued after 3 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 10 patients (18.9%) were medication free.

In the analysis of complete blood count, repeated measures ANOVA with Sphericity Assumed correction showed that mean hemoglobin level differed significantly among time points [$F(2,94) = 8.18$, $p = 0.01$]. Post hoc test (Bonferroni correction) revealed that mean hemoglobin level increased by 0.594 g/dL after 6 months ($p < 0.001$) and increased by 0.602 g/dL after 12 months ($p = 0.002$). With the same statistical analysis, mean hematocrit level was found to be significantly raised at Months 6 and 12 of the treatment compared to baseline [$F(2,94) = 8.9$, $p < 0.0001$]. Mean hematocrit level increased by 1.681% and by 1.402% after 6 and 12 months of the KD treatment, respectively ($p < 0.0001$, $p = 0.011$). Similarly, an increase in MCV level was determined with the KD treatment [$F(2,94) = 19.7$, $p < 0.0001$]. Mean MCV level increased by 3.62 fL with the 6-month KD treatment ($p < 0.0001$) and 3.85 fL after 12 months of KD treatment ($p < 0.0001$). There was no significant effect of KD on other hematological parameters (WBC [$F(1.746,82.069) = 0.540$, $p = 0.585$]; PLT [$F(2,94) = 0.905$, $p = 0.408$]; RDW $p = 0.702$; RBC $p = 0.934$) (Table 2).

In the analysis of serum vitamin B12, folic acid, and iron status of patient, there was no significant change in mean serum folic acid level during the KD treatment [$F(2,80) = 2.884$, $p = 0.062$]. Mean serum iron and TS and TIBC levels did not differ in the follow-up ($p = 0.821$, [$F(2,78) = 0.707$, $p = 0.496$] and [$F(2,78) = 3.741$, $p = 0.669$]). Although increased mean serum ferritin levels were determined with the KD treatment (Baseline 30.6 ng/mL [14.6–59.8]; Month 6 37.2 ng/mL [19.5–66.3]; Month 12 46.9 ng/mL [15.3–63.7]), these findings were not significant ($p = 0.428$) (Table 2). Mean vitamin B12 level was found to be significantly increased in Month 12 of the treatment compared to Month 6 [$F(1.686,72.479) = 3.472$, $p = 0.035$]. Post hoc test (Bonferroni correction) revealed that mean serum vitamin B12 levels increased by 123.7 pg/mL between 6 months and 12 months ($p = 0.026$), (Table 2).

In two cases, increase of vitamin B12 was remarkable. They were 4 and 9-year-old girls and were receiving treatment for West syndrome and perinatal asphyxia (hypoxic injury sequelae). Vitamin B12 levels of patients increased from 208 and 478 pg/mL to 984 and 2327 pg/mL, respectively. At baseline, patients with West syndrome were treated with levetiracetam, valproic acid, and carbamazepine. At the end of the year, she became seizure free with levetiracetam. A patient treated for hypoxic injury sequelae was treated with valproic acid and topiramate at baseline and became seizure free with levetiracetam at the 12 month of KD. None of them were treated with vitamin B12 supplementation. There were no differences between baseline and 12th month of weight standard deviation score (SDS), height SDS, and body mass index SDS of patients.

In a 6-year-old boy who was treated with levetiracetam, carbamazepine, and topiramate due to hypoxic injury sequelae, hemoglobin level increased from 10.4 to 13.6 g/dL after 12 months of ketogenic diet. He was not received any vitamin/mineral supplementation and was seizure free with levetiracetam at the end of the year.

Discussion

In this study, effects of 1-year ketogenic diet treatment on hematological parameters were retrospectively analyzed. Exclusion of patients who use steroids, multivitamin-mineral supplementations, or fish oils and treated with iron, vitamin B12, or folic acid provided us to eliminate confounding factors and to better evaluate the effect of KD. Generally, this study suggested that hemoglobin, hematocrit, and MCV level elevated with the KD treatment and these changes observed at Month 6 of treatment.

Couch et al. reported that no significant changes in levels of hemoglobin, hematocrit, leukocytes, erythrocytes, and platelets of 21 patients who treated with KD for 6 months [8]. In another study, 14-day calorie-restricted, low-carbohydrate, and high-fat KD was administered to 20 healthy women; no significant differences were determined in WBC, RBC, hemoglobin, and hematocrit levels [9]. In our study, similar to previous studies, we determined no significant changes in WBC, RBC, and PLT of patients treated with KD. However, contrary to these studies, increase in hemoglobin and hematocrit was determined in Month 6 of KD treatment. In the analysis of 317 children with refractory epilepsy for KD treatment in China, thrombocytopenia was reported in 3 patients (0.9%) [12]. We did not observe thrombocytopenia in any patient. To date, there is no study in the literature that evaluates the effect of KD on MCV, RDW, TIBC, and TS levels. For these parameters, except MCV, we did not find any significant changes with the use of KD. We revealed that elevation in MCV levels of patients at Month 6 of treatment that persisted at Month 12.

Liu et al. revealed increased serum ferritin level in patients (14 patients were treated with classic KD and 11 were treated with medium-chain triglyceride diet) after

Table 2 Hematological and biochemical parameters of patients (n = 53) at baseline, Month 6 and 12 of ketogenic diet treatment

Parameters	Baseline	Month 6	Month 12
Hemoglobin (g/dL) mean ± SD (min-max)	12.6 ± 1.2 (10.2–16.3)	13.1 ± 1.2 (9.3–15.8)	13.2 ± 1.0 (10.0–16.0)
Hematocrit (%) mean ± SD (min-max)	38.2 ± 3.3 (31.7–48.2)	39.8 ± 3.4 (31.2–46.6)	39.6 ± 2.96 (32.9–46.2)
MCV (fL) mean ± SD (min-max)	83.0 ± 7.2 (67.4–93.4)	86.6 ± 7.1 (61.5–96.9)	86.9 ± 7.1 (68.8–103.6)
RDW (%) median [25th–75th percentile]	13.8 [12.7–14.4]	13.3 [12.6–14.5]	12.9 [12.4–14.3]
RBC (10^6/μL) median [25th–75th percentile]	4.7 [4.4–4.9]	4.6 [4.4–4.9]	4.5 [4.3–4.9]
WBC (10^3/μL) mean ± SD (min-max)	7.9 ± 2.6 (3.4–15.8)	7.6 ± 2.2 (2.9–12.8)	7.6 ± 2.9 (3.1–15.5)
Platelets (10^3/μL) mean ± SD (min-max)	276 ± 79 (104–463)	264 ± 83 (117–505)	261 ± 74 (123–466)
Folate (ng/mL) mean ± SD (min-max)	17.1 ± 6.9 (4.5–30.0)	20.2 ± 5.3 (7.7–30.0)	17.6 ± 6.6 (3.4–32.0)
B12 (pg/mL) mean ± SD (min-max)	676.5 ± 330.8 (127–1696)	633.8 ± 277.4 (200–1365)	772.4 ± 434.7 (230–2327)
Ferritin (ng/mL) median [25th–75th percentile]	30.6 [14.6–59.8]	37.2 [19.5–66.3]	46.9 [15.3–63.7]
Serum iron (μg/dL) median [25th–75th percentile]	71 [48–96]	70 [50–98]	71 [51–100]
TIBC (μg/dL) mean ± SD (min-max)	325.9 ± 77.7 (172–605)	287.2 ± 63.7 (177–442)	292.5 ± 75.2 (126–469)
TS (%) mean ± SD (min-max)	24.4 ± 13.0 (4.0–61.4)	30.2 ± 18.5 (6.9–88.8)	29.1 ± 15.7 (8.8–66.2)

SD, standard deviation; *min*, minimum; *max*, maximum; *MCV*, mean corpuscular volume; *RBC*, red blood cell; *WBC*, white blood cell; *TIBC*, total iron-binding capacity; *TS*, transferrin saturation

4 months KD treatment in a non-randomized prospective study [10]. However, this finding was not statistically significant. In the same study, although insufficient folate intake with the classic KD was determined, no significant changes in RBC folate levels were detected in both diet groups. In our study, consistent with the study of Liu et al., an increase in serum ferritin levels was observed over time and this change was not significant. We analyzed serum folic acid levels and no significant change was determined. Kang et al. [13] assessed 129 patients who were treated with KD for intractable epilepsy, and they revealed iron deficiency anemia in 2 patients (1.6%) with the KD treatment. In our study, we did not detect iron deficiency anemia in any patient, and no effect of KD on serum iron level was revealed.

There is no study that investigates the vitamin B12 status of patients treated with KD. In our study, we determined that decrease in vitamin B12 level at Month 6 of KD treatment was not significant. Interestingly, at month 12 of treatment, we revealed an elevation in serum vitamin B12 levels of patients and this change was statistically significant compared to Month 6 vitamin B12 level. Volpe et al. reported that children with intractable epilepsy have lower vitamin B12 intake, as well as other vitamins and minerals [14]. Furthermore, Bertoli et al. determined a decreased nutrient intake of iron in children with refractory epilepsy [15]. We speculate that intractable seizures, postictal periods, and side effects of antiepileptic drugs decrease the wake time of children and may lead to a decreased food intake and negatively affect the vitamin and mineral status. Seizure reductions with the KD treatment and decrease in the number of antiepileptic drugs may have a positive effect on wake time and nutrition status of patients. In our patients, elevation of hemoglobin, hematocrit, ferritin, and vitamin B12 levels without any vitamin and mineral supplementation may be the result of well-fed children with well-structured KD. Furthermore, in clinical follow-up, decrease in the ratio of KD [fat/(protein plus carbohydrate)] may have positively affected the hemoglobin levels by providing more protein than patients previously consumed.

This study has several limitations. First, we retrospectively evaluated only the first year of KD treatment. Secondly, we did not investigate the dietary intake of children before and after KD treatment. Thirdly, low-number of patients limited us to conclude the effect of KD on hematological parameters. Lastly, we cannot exclude the hematological effects of antiepileptic drugs in our patient group.

As a result, we can conclude that KD increases the hemoglobin, hematocrit, MCV, and serum vitamin B12 levels of patients with intractable epilepsy. No adverse effect of KD was determined in hematological parameters. Prospective, multi-center, longitudinal studies are needed to confirm our results.

Author contributions All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was designed in compliance with the Declaration of Helsinki, 1964. Informed consent was obtained from the parents of participants during enrollment in the study. The study was initiated after the approval of the Ethics Committee of the Dokuz Eylul University Hospital (Date: 03.03.2016, number: 2016/06-01).

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