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Original Article

Efficacy of the Ketogenic Diet for the Treatment of Refractory Childhood Epilepsy: Cerebrospinal Fluid Neurotransmitters and Amino Acid Levels

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ABSTRACT

OBJECTIVE: The mechanisms of the ketogenic diet remain unclear, but several predictors of response have been proposed. We aimed is to study the relationship between the etiology of epilepsy, cerebrospinal fluid neurotransmitters, pterins, and amino acids, and response to a ketogenic diet. **METHODS:** We studied 60 patients who began classic ketogenic diet treatment for refractory epilepsy. In 24 of 60 individuals, we analyzed cerebrospinal fluid neurotransmitters, pterins, and amino acids in baseline conditions. Mean age at epilepsy onset was 24 months, 83.3% were focal epilepsies, and in 51.7% the etiology of the epilepsy was unknown. **RESULTS:** Six months after initiating the ketogenic diet, it was effective (greater than a 50% reduction in seizure frequency) in 31.6% of patients. We did not find a link between rate of efficacy for the ketogenic diet and etiologies of epilepsy, nor did we find a link between the rate of efficacy for the ketogenic diet and cerebrospinal fluid pterins and biogenic amines concentrations. However, we found statistically significant differences for lysine and arginine values in the cerebrospinal fluid between ketogenic diet responders and nonresponders, but not for the other amino acids analyzed. **SIGNIFICANCE:** The values of some amino acids were significantly different in relationship with the ketogenic diet efficacy; however, the epilepsy etiology and the cerebrospinal fluid biogenic amine and pterin values were not.

Keywords: ketogenic diet, refractory epilepsy, children, cerebrospinal fluid, amino acids, neurotransmitters

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Introduction

About 20% to 30% of epilepsies in childhood do not respond to antiepileptic drugs. This implies that there is an inadequate response to a minimum of two first-line antiepileptic drugs despite correct drug dosage and indications. Ketogenic diet has been traditionally used to treat refractory childhood epilepsy. In fact, independent of age, some studies had a 33% positive response, with greater than a 50% reduction in seizure frequency.[1]

How a ketogenic diet has this effect is unknown, although several hypotheses have been suggested.[2,3] It is supposed that the diet's efficacy is a result of multiple aspects: biogenic amine metabolite and amino acid changes in cerebrospinal fluid,[4,5] direct effects of ketone bodies or polyunsaturated fatty acids, potassium channels activation, glutamatergic pathway inhibition, mammalian target of rapamycin pathway regulation, glycolysis inhibition, or adenosine serum levels changes.[2,3] All of these factors may contribute to an improvement in neuronal mitochondrial metabolism.

Biogenic amines' status could be secondarily affected in several neurological disorders.[6,7] A decrease in 5-hydroxyindoleacetic values was found in cerebrospinal fluid from

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epileptic encephalopathies.[8] It is thought that this is due to the wide distribution of serotonergic projections in the central nervous system, and the fact that 5-hydroxytryptophan is implied in neuronal proliferation, differentiation, and synaptogenesis. Serotonin and its metabolites are secondarily affected in these childhood-onset pathologies affecting the developing brain.

Alterations in amino acid homeostasis in the central nervous system are crucial for epileptogenesis.[9] Certain amino acids such as tyrosine are biogenic amine precursors, and other amino acids act as neurotransmitters.[10] An imbalance between excitatory amino acids (glutamate, glycine, aspartic acid) and inhibitory amino acids (gamma-aminobutyric acid, taurine)[11] occurs in some epileptic patients.[12,13]

In pterins, neopterin is a cellular immune marker that is elevated in some inflammatory or infectious diseases[14,15] as well as in epilepsy.[16,17] In refractory epilepsy and status epilepticus, there are some immunological mechanisms that lead to seizure perpetuation.[18,19] Furthermore, biopterin is a metabolite in tetrahydrobiopterin's biosynthetic pathway, an essential cofactor for biogenic amine synthesis.[10]

Because few studies[4,5] have investigated the relationship of neurotransmitters, pterins, and amino acid levels in baseline cerebrospinal fluid with the ketogenic diet outcome, our objective was to study these biomarkers as well as selected neurological factors in conjunction with ketogenic diet response.

Materials and Methods

Patient characteristics

We reviewed the clinical data of all the 60 patients with drug-resistant epilepsy monitored by the Neuropediatric and Pediatric Gastroenterology departments in a hospital of reference between 2006 and 2012. We included in the study all the pediatric patients with drug-resistant epilepsy receiving ketogenic diet. Patients having a GLUT-1 deficiency, pyruvate dehydrogenase deficiency, or another inborn error of metabolism were excluded. All patients kept a seizure diary during the ketogenic diet treatment. We considered epilepsy refractory when a minimum of two different antiepileptic drugs were used. The epilepsies were classified according to etiology and electrophysiological criteria. All of our patients were treated with classic ketogenic diet with medium chain triglyceride oil supplements if needed to achieve ketosis and energy requirements. Forty of 60 patients followed a ketogenic diet in 4:1 ratio, 12 of 60 patients followed a ketogenic diet in 3:1 ratio, and the remaining patients followed a ketogenic diet in 2.5: 1 or 2:1 ratio. The ketogenic diet ratio depended on the minimum ratio to achieve ketosis. The diet was administered by oral meals and ketosis was measured on an outpatient basis by urinary test (urine ketones).

Ketogenic diet efficacy evaluation

Ketogenic diet efficacy was evaluated according to the percentage that seizure frequency was reduced. A seizure frequency reduction of more than 50%[1,20,21] was considered a positive response to treatment. However, to analyze predictive factors, a reduction of the seizure frequency of greater than 90% was used.

If there was not a positive response three months after ketogenic diet initiation or an improvement in any other important aspects (such as the child's attention span or general behavior), the diet was stopped. Nine of 60 patients discontinued ketogenic diet because of difficulty adhering to the diet or intolerance, and these individuals were excluded from statistical analysis. According to previous studies,[22] 2 or 3 weeks after ketogenic diet initiation, ketogenic diet efficacy can be assessed.

In seven patients, ketogenic diet was indicated for treating refractory status epilepticus. Ketogenic diet was used as an adjuvant therapy with antiepileptic drugs. In two patients, the response was not evaluable because they continued the diet less than 3 weeks, but in the other five, ketogenic diet was effective. One of the responders was diagnosed as febrile infection-related epilepsy syndrome. The remaining four patients have different diagnoses: Lennox-Gastaut syndrome, symptomatic focal epilepsy secondary to malformations of cortical development, focal epilepsy of unknown etiology, and undetermined epilepsy syndrome with unknown etiology. In addition, in the group of positive responders, three were nonconvulsive status epilepticus and two were convulsive status epilepticus. The seven patients with status epilepticus were all excluded from the general statistical analysis.

The mean ketogenic diet follow-up period was of 10.9 months (95% confidence interval 10.9 $\pm$ 5.98). Ten patients continued with ketogenic diet for longer than 1 year. In our sample, the most common cause for discontinuing the ketogenic diet was the lack of efficacy (38.5%), followed by difficulties in fulfillment of the dietary treatment (27.3%).

Glucose, lactic acid, neurotransmitters, pterins, and amino acids study in cerebrospinal fluid

Baseline cerebrospinal fluid samples were obtained by lumbar puncture early in the morning in 24 patients, according to previously described protocols, before ketogenic diet was adopted by patients. After lumbar puncture, the cerebrospinal fluid was stored immediately at -80°C until the time of analysis. Glucose, lactic acid, amino acids, pterins (neopterin and biopterin), 5-hydroxytryptophan, and biogenic amine metabolites (3-O-methyldopa, homovanillic acid, 5-hydroxyindoleacetic acid, and 3-methoxy-4-hydroxyphenyl glycol) were analyzed by standard automated procedures, ion-exchange chromatography with ninhydrin detection, and reversed-phase high-performance liquid chromatography with electrochemical and fluorescence detection, as previously reported.[23] Reference values were established in a control population (pediatric patients of different ages who had lumbar punctures to rule out an infectious central nervous system disorder).

Statistical studies

Statistical analysis was performed using SPSS. Odds ratio was calculated to study the association between response to the diet and epilepsy etiologies. Patients were divided in two groups: responders with greater than 90% reduction in seizure frequency, and non-responders with a $<$90% reduction. Odds ratio was performed to assess different associations between the clinical features of the patients and ketogenic diet efficacy.

Because neurotransmitter and pterin values in cerebrospinal fluid are influenced by patient's age, Fisher's exact test was performed and odds ratio was calculated to find any association between positive ketogenic diet response and pterin and neurotransmitter values (normal, high, or low, classified according to patient's age).

Glucose, lactic acid, neurotransmitter metabolites ratio, and amino acids values in cerebrospinal fluid were analyzed by using Kolmogorov-Smirnov test to rule out if they follow or not a Gaussian distribution. Then, cerebrospinal fluid values in responders and nonresponders were compared by using Mann-Whitney U test or Student t test for nonpaired samples. A Bonferroni correction for multiple testing was applied. The level of significance in all statistical tests was set at $P < 0.05$.

The study was approved by the Hospital Ethical Committee and informed consent from parents was obtained. All lumbar punctures were performed to further the clinical evaluation of patients with refractory epilepsy.