Evaluation of Glutamic Acid Decarboxylase Antibody Levels in Patients with Juvenile Myoclonic Epilepsy and Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis

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ABSTRACT

Introduction: Several clinical studies have been conducted to investigate the role of autoantibodies and immunological mechanisms in the etiology of treatment-resistant epilepsy in recent years. Some immunological treatments have been suggested as a result of these studies. In this study, we aimed to investigate the role of autoimmunity in partial and idiopathic generalized epilepsy and determine the relationship between drug resistance and autoimmune antibodies.

Methods: Twenty-eight patients (24 treatment-responsive and 4 treatment-resistant) with juvenile myoclonic epilepsy (JME), 26 patients with mesial temporal lobe epilepsy with hippocampal sclerosis (MTLEHS) resistant to antiepileptic drug treatment, and 26 age-matched healthy control subjects were included in a two-year cross-sectional study. Glutamic acid decarboxylase antibody (GADA) levels were measured with a radioimmunoassay method in the serum of the included subjects.

Results: High GADA titers were detected in 2 patients with JME (7.1%), 1 patient with MTLEHS (3.8%), and 1 healthy subject (3.8%). There was no statistically significant difference among the groups regarding the serum GADA level. Although a limited number of drug-resistant patients with JME our study did not show relationships among anti-GADAs, both epileptic syndromes and drug resistance.

Conclusion: Because we did not determine any significant relationship between GADA levels and JME or MTLEHS, we do not recommend analysis of serum GADA levels in routine examinations where there is no evidence to suggest risk factors for autoimmunity.

Keywords: Epilepsy, glutamic acid decarboxylase antibody, mesial temporal lobe epilepsy, hippocampal sclerosis, juvenile myoclonic epilepsy

INTRODUCTION

Epilepsy is one of the most common neurological diseases, and despite abundant antiepileptic drug (AED) treatment options, seizures are still poorly controlled in one-third of the patients. There are several clinical studies that show that some immunological mechanisms and antibodies may play an important role in treatment-resistant epileptic seizures (1,2,3). As a result of these studies, the benefits of immunological treatments have been stated, and this has opened an exciting new era in treatment for patients with treatment-resistant epilepsy.

Several autoantibodies have recently been identified in patients with refractory epilepsy, such as voltage-gated potassium channel antibodies, anti-cardiolipin antibodies, and glutamate receptor type 3 antibodies (1,2,3). Although there is increasing evidence for the relationship between epilepsy and high levels of glutamic acid decarboxylase antibody (GADA), the clinical significance is still uncertain (4,5).

We aimed to investigate the role of GADA in patient groups with two different epileptic syndromes and its association with treatment responses.

METHODS

GADA levels were analyzed in the serum of epileptic patients who had been admitted to the Epilepsy Center at Bakırköy Psychiatry, Neurology, Neurosurgery Research and Training Hospital, from June 2010 to June 2012. The study groups consisted of 26 patients with treatment-resistant mesial temporal lobe epilepsy with hippocampal sclerosis (MTLEHS) (11 male, 15 female) and 28 patients with juvenile myoclonic epilepsy (JME) (6 male, 22 female). Also, 26 healthy volunteers without any history of neurological or endocrinological diseases (10 male, 16 female) were included in the study as the control group. Informed consent was obtained from all participants. The study was approved by the ethical committee of Bakırköy Psychiatry, Neurology, Neurosurgery Research and Training Hospital. Patients who had neurological symptoms such as ataxia, dysmetria, dysdiadochokinesia, rigidity, encephalopathy, and cognitive and/or psychiatric manifestations that are indicative for GADA-associated neurological syndromes were excluded.

The type of epilepsy was determined according to the International Classification of Epilepsies and Epileptic Syndromes (6). Cranial magnetic resonance imaging (MRI) scans were carried out in all patients. Age, duration and type of epilepsy, frequency of seizures, treatment regimens, and personal history of autoimmune disorders were recorded. Treatment-resistant epilepsy was defined as one or more seizures per month despite adequate and well-tolerated treatment with two or more drugs (7,8). As a characteristic feature of the syndrome, all patients with
MTLEHS were drug “resistant” and patients with JME had “mostly” good response to treatment (four JME patients were resistant to drug therapy).

Serum carbamazepine and valproic acid levels were measured and serum concentrations of these AEDs were within the normal range. GADA levels were measured in serum by radioimmunoassay (Cent AK anti-GAD, Berlin; cut-off point for positivity: 1.0 U/mL).

Samples were tested twice and the mean values were used for data analysis. The blood glucose level was measured to discover undiagnosed diabetes mellitus (DM). B2-glycoprotein I antibodies, antinuclear antibodies, anti-gastric parietal cell antibodies, anti-Langerhans cell antibodies, anti-thyroperoxidase antibodies, anti-thyroglobulin antibodies, anti-GM1 antibodies, and anti-cardiolipin antibodies were measured in patients with increased levels of GADA.

Statistical Analysis

GADA levels were compared between groups using the chi-squared test. Fisher’s exact test and chi-squared tests were used for comparing the frequencies, mean values, and standard deviations of the variables. The Kruskal-Wallis test was used to compare the three groups for nonparametric variables. P<0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, New York, USA) version 21.0 for Windows.

RESULTS

The mean age of the MTLEHS patients was 31.9±6.6 years (18–42 years) and the JME patients was 25.3±7.5 years (16–40 years). The mean age of the control group was 28.7±7.3 years (17–39 years).

Descriptive characteristics of epileptic patients and control subjects are summarized in Table 1. Characteristics of seizures are summarized in Table 2.

In the MTLEHS group, 16 patients (61.5%) were receiving three or more AEDs, 10 patients (38.5%) were receiving two AEDs. 15 patients (53.6%) were treated with valproic acid as a monotherapy, 7 patients (25%) were receiving an AED other than valproic acid as a monotherapy, 6 patients (21.4%) were under multiple therapies in the JME group. 26 (100%) patients in MTLEHS group and 4 patients (14.3%) in the JME group had treatment-resistant epilepsy.

Cranial MRI images of the MTLEHS patients were concordant with hippocampal sclerosis and were found in the left side in 11 patients (42.3%), in the right side in 9 patients (34.6%), and bilaterally in 6 patients (23.1%).

The interictal EEGs of the patients with MTLEHS (n: 26) were concordant with focal abnormalities such as disorganization and neuronal hyper excitability. In the JME group, the EEG showed primary generalized discharges in 18 patients (64.3%) and was normal in 10 patients (35.7%).

Titration values of GADA in the patients and control groups are shown in Table 3.

There was no significant difference in plasma levels of GADA among MTLEHS, JME, and control groups (p>0.05). No subjects in this study had a personal and family history for insulin-dependent diabetes mellitus (DMII) or other autoimmune diseases. No significant relationship was found between plasma GADA level and duration or frequency of seizures.

Serum GADA levels were above the normal range in two JME patients (7.6%) with 2.7 U/mL and 1.2 U/mL, in one MTLEHS patient (3.8%) with 1.3 U/mL, and in one control case (3.8%) with 1.06 U/mL. We measured b2-glycoprotein I antibody, anti-antinuclear antibody, anti-gastric parietal cell, anti-Langerhans cell antibody, anti-thyroperoxidase, anti-thyroglobulin, GM1, and anti-cardiolipin antibody levels in the 4 patients with increased levels of GADA. We detected anti-Langerhans cell antibodies in 1 JME patient and anti-thyroglobulin antibodies in 1 MTLEHS patient.

The JME patients with high GADA levels were not treatment-resistant, and anti-Langerhans cell antibodies were positive in one of them. The MTLEHS patient with high GADA levels, in whom anti-thyroglobulin antibodies were present, had undergone epilepsy surgery for seizure control. He had been seizure-free for two years after the surgery.

DISCUSSION

Several clinical studies have recently been performed to investigate the role of autoantibodies and immunological mechanisms in the etiology of epilepsy.
treatment-resistant epilepsy. Some immunological treatments were suggested as a result of these studies.

In our present study, we have found high serum GADA levels in 2 of the 28 JME patients, in 1 of the 26 MTLEHS patients, and in 1 of the 26 healthy control subjects.

We consider that this study is important as it consists of both partial epileptic and idiopathic generalized epileptic syndromes. As a characteristic feature of these syndromes, all patients with MTLEHS were drug resistant and “most of” the patients with JME had a good response to treatment. Although the number of drug-resistant patients with JME our study was limited, it did not show relationships among anti-GAD antibodies in terms of both epileptic syndromes and drug resistance.

Autoimmune pathogenesis may be considered with the detection of neural autoantibodies, inflammatory cerebrospinal fluid (CSF) findings such as leukocytosis or oligoclonal immunoglobulin bands, or MRI findings that suggest inflammation.

As it was an invasive method, lumbar puncture was not performed. Although we did not investigate the CSF of the participants, demonstration of an increased intrathecal synthesis of GADA is important to confirm the correlation between high GADA levels and neurological disorder. Because GADA immunity may also be related to endocrinological diseases such as DM or Hashimoto’s thyroiditis, they may justify the presence of higher GADA levels.

The presence of GADA in patients with epilepsy is supported by few clinical studies and patients in these studies often had serological (anti-thyroid antibodies, anti-GM1 antibodies, antinuclear antibodies) or clinical evidence of other autoimmune diseases that may be related to high GADA levels.4,5,10,11 There is still a necessity to study evaluating the relationship between GADA and its clinical significance.

Limatainen et al.5 identified temporal lobe epilepsy patients associated with high serum GADA level; these patients had several autoantibodies present in the serum, which was suggestive of immunological origins for their epileptic syndrome. Based on the findings, they suggested the need to study the effectiveness of immunosuppressive therapy in epilepsy patients with high GADA levels.

Quek et al.11 described 32 patients with clinical, serological, and imaging findings who had refractory epilepsy of presumed autoimmune basis. Intractable and frequent recurrent seizures were the early and predominant clinical manifestation in these patients. Remarkable improvement in seizure control with immunotherapy was achieved in 81% of the patients and a seizure-free status was achieved in 67% of the patients who were predominantly AED-resistant. Quek et al.11 investigated both serum and CSF of the patients and investigated not only GADA, but also other antibodies such as voltage-gated potassium channel antibodies. In our study, patients in the MTLEHS group were drug-resistant, as it was expected, but we did not search for other neural autoantibodies including voltage-gated potassium channel complexes, collapsin response-mediator proteins and Ma2, N-methyl-D-aspartate receptors, and ganglionic acetylcholine receptors.

The study by Quek et al. supported the indicated relationship between neurologic autoimmunity and epilepsy, and the potential benefit of immunotherapy in improving seizure control.11 They mentioned that clinicians should suspect the autoimmune origin of epilepsy if they see multifocal epileptic origin, high frequency and drug resistance of a seizure, cranial imaging or CSF findings that are suggestive for inflammation, a recent history of neoplasia in the patient, and personal or family history for autoimmunity.11 They attracted attention to normal findings that did not exclude an autoimmune etiology even though they reported normal MRI and CSF findings in half of the patients.

There are some questions that still require answers such as the natural history of the autoimmune epilepsy, the selection criteria for patients with epilepsy most likely to benefit from an autoimmune evaluation, the timing for immunotherapy trials, and optimal duration of long-term immunotherapy maintenance.

Prevalence studies in the literature show that 1.4–5.4% of the epilepsy patients had GADA positivity in low titers, and our findings were concordant with these studies. But there are some conflicting results in terms of GADA that did not report significant differences in the prevalence of GADA between groups of young patients with epilepsy and healthy subjects,5,15 or between patients with treatment-responsive and treatment-unresponsive epilepsy.14 Kwan et al.14 found similar GADA titers between seizure-free patients and those with refractory epilepsy; they also found no relationship among GADA titers and frequency and duration of seizures.

Aykutlu et al.17 investigated GADA in the serum of 96 consecutive JME patients and 25 age-matched controls, and found high GADA levels in four patients (5.8%) who were drug-responsive. They reported no relationship between GADA titers and the “therapy resistant” course in JME, similar to our study.17

The immunological mechanism may develop after the long-term use of AEDs,5,11,17. High GADA levels in our JME patients may also be associated with long-term valproic acid treatment. Although the number of treatment-resistant patients was low in our study, we do not think that high GADA values in low titers have a role in JME patients.

Altndag et al.18 investigated GM1 antibodies and GADA in JME patients and the JME patients showed relatively, but not significantly, increased antinuclear/nucleolar antibody incidence as compared to the controls.18

Falip et al.19 detected high GADA levels in 5 of 42 MTLEHS patients who also had clinical and laboratory findings for other immunological diseases such as DM, thyroiditis, psoriasis, and arthritis.19. As our patients had no autoimmune disease history, the association of a concomitant clinical and serological finding (such as antithyroid, anti-GM1, or antinuclear antibodies) with high serum GADA levels could not be investigated. We measured the levels of b2-glycoprotein 1, anti-antinuclear, anti-gastric parietal cell, anti-Langerhans cell, anti-thyroidperoxidase, anti-thyroglobulin, GM1, and anti-cardiolipin antibodies in 4 patients with increased levels of GADA. We detected anti-Langerhans cell antibodies in 1 JME patient and anti-thyroglobulin antibody in 1 MTLEHS patient, but there was no evidence of DM or thyroid disease.

### Table 3. Serum GADA levels in the MTLEHS, JME, and control groups

<table>
<thead>
<tr>
<th></th>
<th>MTLEHS (n=26)</th>
<th>JME (n=28)</th>
<th>Control (n=26)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-GAD (U/mL)</td>
<td>0.79±0.08</td>
<td>0.9±0.38</td>
<td>0.8±0.07</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(0.65–0.99)</td>
<td>(0.70–2.70)</td>
<td>(0.54–1.06)</td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant, *Kruskal-Wallis test significant at p<0.05.
Rantala et al. (20) measured GADA levels in an unselected population of 114 children with different types of epilepsy; none of the children were positive for GADA. Therefore, they reported that GADA testing cannot be recommended in unselected cases of childhood epilepsy. Our findings with JME syndrome support their conclusion. We believe that GADA positivity in low levels does not play a role in treatment-resistance in this idiopathic generalized epileptic syndrome. The identification of GADA within the subgroup of patients with cryptogenic temporal lobe epilepsy deserves particular attention and further investigation (15).

In conclusion, we did not observe increased serum GADA levels in MTLEHS and JME patients, similar to the results of previous studies (14,15,16,17,18). Routine investigation of GADA levels cannot be recommended if there is no evidence suggesting autoimmunity of the diseases. In our study, we did not encounter GADA autoimmunity in JME and MTLEHS. This may be related to the small number of patients or the fact that other autoantibodies were not investigated.

The clinical significance of GADA positivity in epilepsy remains unclear; and further studies with new-onset epileptic patients are needed to clarify the significance of GADA positivity in the etiology of epilepsy.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bakirköy Psychiatric Training and Research Hospital.

Informed Consent: Written informed consent was obtained from patients and control subjects who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES