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Short communication

Association between high titers of glutamic acid decarboxylase antibody and epilepsy in patients with type 1 diabetes mellitus: A cross-sectional study

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ABSTRACT

Purpose: Individuals with type 1 diabetes mellitus (T1D) are at higher risk of epilepsy. T1D is a progressive immune-mediated disease and the etiology of epilepsy remains unknown in most. Glutamic acid decarboxylase (GAD) catalyzes GABA formation. GABA-secreting neurons and pancreatic beta cells are the major cells expressing GAD.

Methods: Cross-sectional study. Patients with T1D from a multiethnic population underwent GADA measurement to investigate possible association between T1D and epilepsy of unknown etiology.

Results: T1D patients were analyzed (n = 375). Overall frequency of epilepsy was 5.9% (n = 22). Frequency of epilepsy of unknown etiology was 3.2% (n = 12). Of these, 8 (2.1%) had idiopathic generalized epilepsy (IGE) and 4 (1.1%) MRI-negative temporal lobe epilepsy (TLE). Patients with T1D and epilepsy of unknown etiology did not show differences in GADA frequency (83.3% vs 50%; p = 0.076); however, their titers were higher (106.9 ± 136.5 IU/mL; median 7; IQR 1.65–256 vs 10.2 ± 14.5 IU/ml; median 4.3; IQR 1.9–8.9; p = 0.019) compared to patients without epilepsy. Moreover, epilepsy of unknown etiology was associated with GADA titers ≥ 100 UI/mL [odds ratio (OR) 4.42, 95% CI 2.36–8.66].

Conclusion: Epilepsy frequency was elevated in patients with T1D and multiethnic background. Presence of epilepsy of unknown etiology was associated with high titers of GADA in this population with long-standing T1D, which has different ethnic and genetic background compared to previous studies. Further prospective studies are required to identify if GADA presence or its persistence are directly responsible for epilepsy in individuals with T1D.

1. Introduction

Type 1 diabetes mellitus (T1D) is characterized by progressive immune-mediated destruction of pancreatic beta-cells [1]. Epilepsy is a frequent disorder; however, its etiology is unknown in most cases [2]. The hypothesis that epilepsy of unknown etiology involves autoimmunity has found increased support [3].

Growing evidence indicates that patients with T1D have higher risk of epilepsy [4]. Glutamic acid decarboxylase antibody (GADA) can play a role in this association. The glutamic acid decarboxylase (GAD) catalyzes gamma-aminobutyric acid (GABA) formation. GABA-secreting neurons and pancreatic beta cells are the major cells expressing GAD. GADA is well-known antibody associated with central nervous system (CNS) disorders [5].

Most studies that have evaluated the link between epilepsy and T1D were performed in Caucasians [6]. It is still not known if ethnicity can influence the risk of epilepsy in T1D, but differences in diabetic complications have been shown in different ethnic groups [7]. Therefore, studies in different ethnic groups are crucial. The Brazilian population is diverse, has multiethnic background and racial admixture [8]. The

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objective of this study was to analyze this association in a multiethnic population by evaluating GADA as possible link.

2. Methods

This was a cross-sectional study. Data were collected from patients with T1D longitudinal follow-up at the *Clementino Fraga Filho* Hospital between August 2015 and March 2018. Ethical approval was taken from ethic committee (47009014.6.0000.5257) and all participants signed informed consent form.

All patients with T1D and epilepsy of unknown etiology were compared to group of T1D without epilepsy matched by age, gender and T1D duration. Individuals were defined as Caucasians or non-Caucasians (mostly African descent) based on their phenotype and family background.

T1D was defined according to American Diabetes Association. Epilepsy was diagnosed following two or more unprovoked euglycemic seizures (blood glucose $\geq$ 70 mg/dL and $\leq$ 200 mg/dL at the time of seizure) with interval of at least 24 h. Blood glucose were measured at seizure or unconsciousness. All subjects met these criteria. Classification of epilepsies and drug resistance were in accordance with International League Against Epilepsy. Positive initial precipitating injury (IPI) was considered: birth injury, traumatic brain injury, febrile convulsion, status epilepticus or meningoencephalitis.

T1D and epilepsy patients underwent electroencephalogram (EEG) and 1.5-Tesla brain magnetic resonance imaging (MRI). GADA $\geq$ 1.0 IU/mL were considered positive.

Patients with abnormal brain MRI, EEG background or neurological exam, identifiable cause for epilepsy and severe episode of hypoglycemia were excluded of the GADA analysis.

For each patient, clinical characteristics of T1D and epilepsy were evaluated. Categorical variables were expressed as proportions; continuous variables as means, medians, standard deviations and interquartile range (IQR). Fisher's exact and the Mann-Whitney tests were used for categorical and continuous data, respectively. McNemar's test was applied to compare proportions of high GADA titers. Significant associations between epilepsy and high levels of GADA were assessed by McNemar's test and their strength by odds ratios with their 95% confidence intervals directly estimated from the 2 × 2 crosstables. P-values < 0.05 were statistically significant.

3. Results

Data from 375 patients were reviewed. Overall, 54.7% were female and 57.9% were non- Caucasians. Their mean age, T1D duration and age at onset were 33.8 ± 9.85 (median 28), 19.6 ± 7.62 (median 18), 11.4 ± 8.38 (median 10) years, respectively.

Epilepsy was confirmed in 22 individuals (5.9%). Their mean age and T1D duration were 30.3 ± 7.21 (median 30.4) and 21.7 ± 7.57 years (median 20) years, respectively. In this group, 50% were male and 54.6% non-Caucasians. Ten patients were excluded from GADA analysis due to: Down syndrome (n = 1), chronic hypoxic-ischemic encephalopathy (n = 3), hereditary spastic paraplegia plus epilepsy (n = 1) and Lennox-Gastaut epileptic encephalopathy (n = 1). The remaining 4 patients had well-documented history of severe hypoglycemia.

Epilepsy had unknown etiology in 12 patients (3.2%). Of these, 8 (2.1%) had idiopathic generalized epilepsy (IGE) and 4 (1.1%) MRI-negative temporal lobe epilepsy (TLE). Among IGE, the phenotype juvenile myoclonic epilepsy (JME) was found in 4 out of 8, generalized tonic-clonic seizure alone (GTCS) in 3 out of 8 and juvenile absence epilepsy (JAE) in 1 out of 8. One patient with IGE had history of febrile convulsion. None of the individuals with TLE had had classical IPI. Two patients fulfilled the criteria for drug resistant epilepsy, both TLE. The onset of T1D preceded that of epilepsy in 10 of the 12 of cases (83.3%), with a median time until the onset of epilepsy of 7.5 years (Table 1).

Table 1
Clinical characteristics of patients with type 1 diabetes mellitus and coexistent epilepsy of unknown etiology.

	Sex	Ethnicity	Type of epilepsy	Age (years)	Age at onset of T1D (years)	Age at onset of epilepsy (years)	T1D preceding epilepsy (years)	Family history of epilepsy	IPI	EEG	AED	Response to AED	Time at GADA analysis (Years since epilepsy onset)	GADA	GADA titers
1	F	N-C	TLE	31	10	22	12	Y	No	Normal	PHT	Adequate	9	Neg	–
2	F	C	TLE	38	11	22	12	N	No	Temporal SW	CBZ CLB	Partial	16	Pos	4
3	M	C	TLE	20	5	15	10	N	No	Temporal SW + focal slowing	LTG CBZ	Partial	5	Pos	303
4	M	C	TLE	26	11	10	–	N	No	Temporal SW	OCX	Adequate	16	Pos	214.4
5	M	N-C	IGE-JME	23	11	17	6	N	No	Normal	VLP	Adequate	6	Neg	–
6	F	N-C	IGE-GTCS alone	36	4	6	2	N	Yes[a]	Generalized SSW	VLP	Adequate	30	Pos	1.9
7	M	C	IGE-JME	38	1	14	13	N	No	PSW	LTG	Adequate	24	Pos	1.4
8	F	N-C	IGE-GTCS alone	31	12	17	5	N	No	Generalized SSW	VLP	Adequate	14	Pos	312.1
9	F	C	IGE-JAE	25	9	2	–	N	No	Generalized SSW 3Hz	LTG	Adequate	23	Pos	7.2
10	M	N-C	IGE-GTCS alone	30	9	18	9	N	No	Generalized SSW	PB	Adequate	12	Pos	6.9
11	F	N-C	IGE-JME	27	9	15	6	N	No	Normal	VLP	Adequate	12	Pos	134.1
12	F	N-C	IGE-JME	20	8	14	6	Y	No	Normal	VLP	Adequate	6	Pos	297.9

Abbreviations: F: female; M: male; C: Caucasian; N-C: non-Caucasian;TLE: temporal lobe epilepsy; IGE: idiopathic generalized epilepsy; JME: juvenile myoclonic epilepsy; GTCS: generalized tonic-clonic seizure; JAE: juvenile absence epilepsy; T1D: type 1 diabetes mellitus; IPI: initial precipitating injury; SW: sharp wave; SSW: spike wave; PSW: polyspike wave; AED: antiepileptic drug; PHT: phenytoin; CBZ: carbamazepine; CLB: clobazam; LTG: lamotrigine, OXC: oxcarbazepine; VLP: valproate; PB: phenobarbital; Neg: negative; Pos: positive; GADA: glutamic acid decarboxylase antibody. a Febrile convulsion.

Table 2
Demographic and clinical characteristics.

	T1D and coexistent epilepsy of unknown etiology n = 12	T1D n = 24	p values
Age, years			
Mean (SD)	28.7 (6.39)	29.8 (6.1)	
Median (IQR)	30 (20-38)	28.5 (25.2-32.2)	0.626
Ethnicity (N-C)	58.3%	45.8%	0.480
Gender (M)	41.7%	37.5%	1.000
Autoimmune disease	33.3%	16.7%	0.397
Age at diagnosis of T1D, years			
Mean (SD)	8.3 (3.28)	9.7 (2.0)	0.341
Median (IQR)	9 (1-11)	9,5 (8.5-11)	
Duration of T1D, years			
Mean (SD)	20.5 (7.81)	20.2 (6.33)	1.000
Median (IQR)	18.5 (12-37)	18 (15.5-24.5)	
Chronic complications of T1D			
Retinopathy	8.3% (1)	20.8% (5)	0.640
Nephropathy	25.0% (3)	20.8% (5)	1.000
Any complication	25% (3)	33.3% (8)	0.715
1 complication	16.7% (2)	16.7% (4)	1.000
2 complications	0.0% (0)	12.4% (3)	0.530
Annual mean HbA1c levels			
N of annual mean	78	861	0.066
Mean (SD)	8.16 (1.86)	8.44 (1.67)	
Median (IQR)	7.70 (6.96-8.95)	8.13 (7.38-9.2)	
Generalized epilepsy, % (N)	66.8% (8)	NA	
Age at diagnosis of epilepsy, years			
Mean (SD)	14.3 (7.57)	NA	
Median (IQR)	14 (6-22)	NA	
Family history of epilepsy in first-degree relatives, % (N)	16.7% (2)	NA	
Classical IPI, % (N)	8.33% (1)	NA	
Drug resistant, % (N)	16.7% (2)	NA	
EEG, % (N)			
Normal	41.7% (5)	NA	
Focal abnormality	25.0% (3)	NA	
Generalized abnormality	33.3% (4)	NA	
GADA			
Positive % (N)	83.3% (10)	50.0% (12)	0.076
Mean titers (SD)	106.9 (136.5)	10.2 (14.5)	0.022
Median (IQR)	7.0 (1.65-256)	4.3 (1.9-8.9)	
GADA ≥ 50 %(N)	41.7% (5)	8.3%(2)	0.18
GADA ≥ 100 %(N)	41.7% (5)	0% (0)	0.016

Abbreviations: SD: standard deviation; IQR: interquartile range; N-C: non-Caucasians; M: male; HbA1c: glycated hemoglobin; IPI: initial precipitating injury; GADA: glutamic acid decarboxylase antibody; NA: not applicable. Fisher's exact test and the Mann-Whitney U test were used for the categorical and continuous data, respectively. McNemar test was applied to compare proportions of high GADA titers.

The frequency of chronic complications and glycemic control were similar between the groups (Table 2).

Patients with T1D and epilepsy of unknown etiology did not show differences in GADA frequency when compared to patients without epilepsy (83.3% vs 50%; p = 0.076) and this finding persisted in subgroups TLE (p = 1.000) or IGE (p = 0.100). However, these patients had higher titer of GADA (106.9 ± 136.5 IU/mL; median 7; IQR 1.65–256 vs 10.2 ± 14.5 IU/ml; median 4.3; IQR 1.9–8.9; p = 0.022). Moreover, GADA ≥ 100 IU/mL (41.7 vs 0; p = 0.016) was more frequent in the epilepsy group and associated with the diagnosis of epilepsy of unknown etiology [GADA ≥ 100 IU/mL odds ratio (OR) 4.42, 95% confidence interval (CI) 2.31–8.47] (Table 2).

4. Discussion

In this study, we evaluated the frequency of epilepsy in a cohort of patients with T1D and multiethnic background, as well as the possible role of GADA in this association. Although the frequency of GADA was not different between the groups, high GADA titers were associated with the diagnosis of epilepsy of unknown etiology.

The epilepsy frequency was elevated and IGE was the most frequent epilepsy phenotype. High rates of epilepsy in T1D had been reported in Caucasians [6], recent studies evaluated East Asian population of Taiwan [9], but information on other ethnic groups such as ours were still lacking. Therefore, it is essential to assess the Brazilian population, which has multiethnic background and it is considered one of the most admixed populations in the world [8].

In this study, IGE was more common than TLE. The most common epilepsy phenotype in T1D is still a matter of debate, some studies showed higher frequency of IGE [10,11] while other of TLE [12]. The reason remains unknown, but contributing factors may include genetic predisposition, effects of hypo/hyperglycemia, cerebrovascular damage, autoimmune features or even an interaction between them. So far, no definitive genetic predisposition has been identified. Promising candidate genes involve those encoding for enzymes of glucose metabolism and proteins of major histocompatibility complex [13].

Hypoglycemia or hyperglycemia might cause abnormalities in microstructural brain integrity, which would affect brain development; however, these findings were not evaluated in association with epilepsy [14]. Our study does not show abnormalities in 1.5 T brain MRI; however, future studies should add advanced neuroimaging. Although this was a cross-sectional study, we did not find differences in difference in HbA1c levels nor chronic complications of T1D between those with epilepsy and others, which implies similar glycemic control.

Autoimmunity have received increased attention in T1D and epilepsy [12]. GADA has emerged as major candidate as it is related to neurological disorders and interfere in GABAergic synapse [3,5]. In this study, all patients who underwent to GADA measurement had MRI-negative epilepsies: TLE (possibly more related to antibody-mediated epilepsy) and IGE (presumably genetic) [2], but no differences in GADA frequency were found, even when patients with TLE or IGE were separately analyzed. A reasonable explanation could be the limited number of participants. However, Schober et al evaluated 45,851 T1D patients and was not capable to identify this link [6].

In this group of patients with long-standing T1D, those with epilepsy of unknown etiology had higher titer of GADA than T1D alone and GADA in high titers were association between both diseases. We measured GADA only once and patients had long duration of disease. Therefore, it is not possible to identify if epilepsy is associated with GADA persistence in high titers or its appearance after T1D diagnosis, as marker of the neurologic disease. On the other hand, according to Nakajima et al, patients with neurologic disorders showed higher serum GADA titers than patients with T1D alone and this persisted over time [5] and Kwan et al found the highest concentration of GADA in patient with JME [15]. The association with high GADA titers does not necessarily imply causality and other factors can be implicated in pathophysiology of this link.

This study has some limitations. Firstly, this was cross-sectional study with limited sample size. As the numeric difference in percentage of GADA was remarkable, it is possible that a multicenter prospective cohort study with a larger sample size would be able to demonstrate difference. However, Schober et al were not able to find this association in larger sample [6]. Another potential limitation was the measurement of only one antibody. Ideally, others autoantibodies and genetic markers should also be investigated. Furthermore, GADA was tested only once and the measurement was performed only in the serum and not in cerebrospinal fluid. Moloney et al were the first to study antibodies other than GADA in this scenario and found no difference; however, GADA was more frequent in T1D patients with epilepsy [13]. Besides,

effects of subclinical hypo/hyperglycemia on the brain were not investigated and GADA was not measured in patients with structural etiology. We cannot exclude that more than one etiology could be responsible for epilepsy and the identification of GADA in structural epilepsy might represent a combined etiology [2]. It is also possible that GADA is an epiphenomenon and represents only a nonfunctional biological marker.

To conclude, the frequency of epilepsy was elevated and IGE the phenotype more common in patients with T1D and multiethnic background. High GADA titers were associated with diagnosis of epilepsy of unknown etiology in this population. Further prospective studies are required to identify if GADA presence or its persistence are directly responsible for epilepsy in individuals with T1D.

Authorship

All authors are eligible for author listing.

Compliance with ethical standards

This study was performed after approval by a local ethics committee and with informed consent from all individual participants.

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Declaration of Competing Interest

None.

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