Original Article

Autoimmune encephalitis: Clinical diagnosis versus antibody confirmation

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Abstract

Context: Autoimmune encephalitis is a heterogeneous disorder which is being diagnosed with increasing frequency. The diagnosis of these disorders is based on the detection of autoantibodies and characteristic clinical profiles. **Aims:** We aimed to study the antibody profile in encephalitis patients with suspected autoimmune etiology presenting to a tertiary care center. **Settings and Design:** The subjects were selected by screening all patients with clinical profile suggesting autoimmune encephalitis admitted in the neuromedical intensive care unit (ICU) of a tertiary care center in South India. **Materials and Methods:** Patients who fulfilled modified Zuliani *et al*.'s, criteria for autoimmune encephalitis were identified during the period December 2009–June 2013. Blood samples from these subjects were screened for six neuronal antibodies. **Statistical analysis used:** Chi-square test was applied to compare the antibody positive and negative patients. **Results:** Out of 1,227 patients screened, 39 subjects (14 males: 25 females) were identified with a mean age of 15.95 years and 19 cases were assessed in the acute and 20 in the convalescent phase of the illness. Seizure (87.8 %) was the most common presenting symptom; status epilepticus occurred in 23 (60.5%) patients during the course of the illness. Fourteen (35.9%) patients were N-methyl-D-aspartate receptor (NMDAR) antibody-positive and all were negative for the other antibodies tested. **Conclusions:** One-third of patients presenting with acute noninfective encephalitis would be positive for NMDAR antibodies with the remaining two-thirds with clinically suspected autoimmune encephalitis being antibody-negative. There are few markers in the clinical and investigative profiles to distinguish antibody-positive and -negative patients.

Key Words

Autoimmune, encephalitis, NMDA antibody, seizures

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Introduction

The discovery of autoantibodies specific for the neuronal cell membrane surface or synaptic proteins has led to the emergence of the novel concepts of autoimmune epilepsy[1] and autoimmune encephalitis. The pathogenesis of the unexpected and fulminant disorder characterized by altered sensorium, cognitive and behavioral impairment, focal neurological deficits, epileptic seizures, and status epilepticus had been an enigma.

Previously cited as rare entities, recent literature[2,3] shows that autoimmune factors may account for a majority of the nonviral encephalitides. The antigens that are frequently identified in such cases include the N-methyl D-aspartate receptor (NMDAR);[4] the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR); the γ-aminobutyric acid receptor-B ($GABA_B$ receptor); and proteins that associate with voltage-gated potassium channel (VGKCs) — leucine-rich glioma-inactivated protein 1(LGI1) and contactin-associated protein-like 2 (Caspr2).[5,6] It is often difficult to distinguish these from mimics like obscure central nervous system (CNS) infections, mitochondrial encephalopathy, and occult focal cortical dysplasia by clinical features alone. Failure to identify the etiology as autoimmune may deprive the patient of treatment for a potentially curable entity.

Our objective was to screen a large number of patients admitted to neurology intensive care service with fulminant seizures that eluded specific diagnosis in order to identify and characterize those with autoimmune encephalitis.

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Materials and Methods

Setting of the study

The study was carried out in the Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology,

Trivandrum which is a tertiary neurology referral center in South India.

Patients

All patients admitted in the neuromedical intensive care unit (ICU) of the Department of Neurology, between December 2009 and June 2013, were screened to identify patients with unexplained status epilepticus or encephalitis-like presentation. Children below 2 years of age (in view of the difficulty in obtaining the adequate quantity of blood sample and more heterogeneous etiologies of encephalopathy and seizures), persons with encephalitis due to an infective pathology, and epilepsies related to structural, genetic, or metabolic causes were excluded. We applied the criteria proposed by Zuliani *et al*.,[7] and certain additional criteria[8] to identify possible cases associated with neuronal surface antibodies. The criteria were:

1. Acute or subacute (less than 12 weeks) onset of symptoms;
2. CNS inflammation as evidenced by at least one of the following:
   a. Inflammatory cerebrospinal fluid characteristics (lymphocytic pleocytosis, cerebrospinal fluid (CSF) specific oligoclonal bands, or elevated IgG index);
   b. Magnetic resonance imaging (MRI) characteristics suggesting inflammation (T2 hyperintensities, contrast enhancement, or restricted diffusion);
   c. Inflammatory neuropathology — lymphocytic infiltrates or other signs of immune activation in brain biopsy specimens;
3. Exclusion of other causes of encephalitis or other CNS conditions mimicking encephalitis; and
4. Good response to immunotherapy.

Of the 1,227 patients admitted in the ICU, 47 patients fulfilled the above criteria for possible autoimmune encephalitis presenting seizures. Thirty-nine of these subjects consented for the study and had blood samples drawn either in the acute or convalescent phase. Their demographic and clinical characteristics and details of the treatment and outcome were obtained from the clinical records. The current status of the patients was updated at the time of their review/blood sampling or by telephone contact if the blood sampling was done in the acute phase.

We assayed the autoantibodies of six neuronal proteins, namely NMDAR, AMPA1, and AMPA2 receptors; VGKC complex proteins Lgi1 and Caspr2; and GABAB1 receptor with the Autoimmune Encephalitis Mosaic 1 kit from EUROIMMUN, Luebeck, Germany. The diluted serum samples were applied to the reaction fields of a reagent tray. The biochip slides were then placed in the reagent tray to allow the reaction. After the necessary incubation and rinsing, the slides were further incubated with fluorescein isothiocyanate-conjugated secondary antibody for 30 min for labeling the bound antibodies. The slides were further washed and examined by two independent assessors/observers, who were unaware of clinical details. They classified every sample as positive or negative based on the intensity of surface immunofluorescence in comparison with positive and negative controls provided in the kit [Figure 1]. The fluorescence was read with

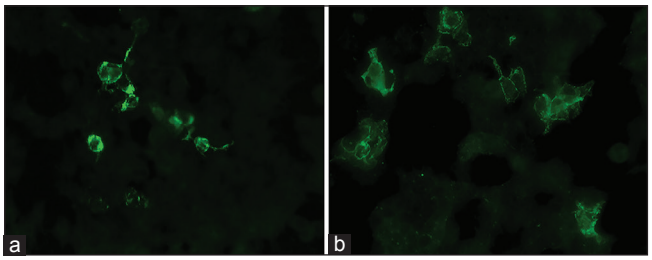

Figure 1: Detection of anti-NMDAR antibodies by indirect immunofluorescence (Autoimmune Encephalitis Mosaic 1 kit from EUROIMMUN). (a and b) Illustrates the positive immunofluorescence staining of NMDA (NR1 subunit)-transfected HEK-293 cells with sera from two patients positive for NMDAR autoantibodies. Both the cases were classified as positive due to the high intensity of surface immunofluorescence. Both patients had excellent response to immunotherapy. NMDAR = N-methyl-D-aspartate receptor, HEK = Human embryonic kidney

EUROIMMUN LED fluorescence microscope at × 20 and × 40 magnification.

Chi-square test was applied to compare the antibody-positive and -negative patients.

Results

Thirty-nine clinically suspected cases (14 males and 25 females) were studied in the age range of 2–55 years (mean age: 15.95years). Nineteen (48.7%) were in the acute stage and 20 (51.3%) in the convalescent stage of disease presentation. Their clinical details are given in Table 1.

Neurological symptoms

Seizure was the most common presenting symptom in these patients (87.18%). Other common symptoms at presentation were behavioral problems in 11 (28.2%), altered sensorium in six (15.38%), and abnormal movements in four (10.26%).

Seizures were present during the course of the acute illness in all 39 patients. The seizures were in the form of generalized tonic-clonic status epilepticus (12.82%), complex partial status epilepticus (43.59%), epilepsia partialis continua (25.6%), or isolated generalized or complex partial seizures (38.46%). During the acute phase of the illness, other neurological manifestations such as altered sensorium, language dysfunction, and behavioral and cognitive disturbances were noted in more than half of the patients. Extrapyramidal signs were seen in 13 patients (33.3 %) and psychiatric manifestations in six (15.38%). Fever was present in 51.3% of the patients during the disease.

The details of their investigations (CSF, MRI, and electroencephalogram (EEG) findings) and treatment are given in Table 1. The patients were negative for viral antigens and antibodies in CSF and blood at the time of the acute presentation (all were tested for herpes simplex virus polymerase chain reaction (PCR) or antibody and other viral markers were tested based on clinical suspicion).

Immunotherapy was administered in 37 of 39 (94.8 %) patients. Immunotherapy comprised of oral steroids, intravenous

Table 1: Demographic, clinical, and laboratory characteristics of antibody positive and negative patients

	Total	NMDA positive	NMDA negative	*P* - value
Patients; *N* (%)	39	14 (35.9%)	25 (64.1%)	
Males; *N* (%)	14 (35.9)	3 (21.4)	11 (78.6)	
Age of onset in years; mean (range)	15.95 (2–55)	14.7 (2–42)	16.75 (2–55)	
Acute phase; *N* (%)	19	9 (47.4)	10 (52.6)	
Clinical and neurological manifestations; *N* (%)				
Fever	20 (51.3)	5 (35.71)	15 (60)	0.14
Altered consciousness	23 (58.9)	9 (64.28)	14 (56)	0.61
Extrapyramidal signs	13 (33.3)	7 (50)	6 (24)	0.59
Language dysfunction	22 (56.4)	10 (71.42)	12 (48)	0.16
Seizures	39 (100)	14 (100)	25 (100)	
Status epilepticus	22 (56.41)	7 (50.0)	15 (60.0)	0.79
Generalized status	5 (12.82)	2 (14.25)	3 (12.0)	0.84
Complex partial status	17 (43.59)	5 (35.71)	12 (48.0)	0.68
Epilepsia partialis continua	10 (25.64)	2 (14.28)	8 (32.0)	0.40
Investigations; *N* (%)				
CSF pleocytosis	13	6 (42.85)	7 (28)	0.345
CSF protein elevated	6	0	6 (100)	0.046
MRI abnormal	22	8 (57.14)	14 (56)	0.945
EEG characteristics; *N* (%)				
Epileptiform abnormalities	28	11 (78.57)	17 (68)	0.482
PLEDs	5	1 (7.14)	4 (16)	0.427
BiPLEDs	3	0	3 (12)	0.177
Treatment; *N* (%)				
AEDs	39 (100)	14 (100)	25 (100)	
IVIG	15 (38.46)	7 (50)	8 (32)	
IVMP	38 (87.17)	13 (92.85)	21 (84)	
Other steroids	28 (71.7)	11 (78.5)	17 (68)	
PE	4 (10.2)	1 (7.14)	3 (12)	

NMDA = N-methyl-D-aspartate, CSF = Cerebrospinal fluid, MRI = Magnetic resonance imaging, EEG = Electroencephalogram, PLEDs = Periodic lateralized epileptiform discharges, AEDs = Antiepileptic drugs, IVIG = Intravenous immunoglobulin, IVMP = Intravenous methylprednisolone, *N* = Number, PE = Plasma exchange

methylprednisolone (IV MP), intravenous immunoglobulin (IVIg), or plasma exchange therapy. Among them, 35 (89.74%) patients received either oral or intravenous steroids, 15 (38.46%) received IVIg, and four (10.2 %) received plasma exchange. In the combination therapy group, steroid and IVIg together were given to 14 patients; steroid and plasma exchange therapy were administered in four patients; and steroid, IVIg, and plasma exchange were given to two patients. All the patients had received one or more antiepileptic drugs (AEDs).

Out of the 37 treated patients, 35 improved and two died in the acute phase. Both the patients who expired were antibody-negative. Two patients who were not given any immunotherapy in the acute phase also improved with symptomatic management alone.

Antibody profile

Out of the 39 cases, 14 (35.9 %) were positive for NMDAR autoantibodies. All samples were negative for VGKC complex proteins LGI1 and Caspr2, AMPA 1 and AMPA 2, and GABARB1 antibodies. The clinical characteristics of the NMDA-positive group are compared with that of NMDA-negative group in Table 1. None of the patients from either group was detected to have any visceral tumor.

The patients were followed up for a period of 1-4 years. There were three deaths; two in the acute phase and one during follow-up. The death in the NMDA positive group occurred after discharge from hospital and was related to aspiration pneumonia and upper gastrointestinal tract bleed. The deaths in the NMDA antibody negative group occurred during acute phase of the illness in hospital.

There were serious sequelae (seizures or cognitive or motor deficits) for 50% in the NMDA positive group and 40% of the NMDA negative group. One or more relapse of the disease was recorded in 28.5% of the NMDA positive patients and 36% of the antibody positive patients.

Discussion

The setting of a busy neurological intensive care service provided us the opportunity to identify a large number of suspected autoimmune encephalitis in a short period. We added a few additional conditions to the criteria put forward by Zuliani *et al*., in order to ensure that the sample is homogenous with respect to clinical profile and to exclude conditions that may mimic autoimmune encephalitis.

Our key observation is that a third of the suspected cases of autoimmune encephalitis were positive for NMDAR antibody.

The prevalence of antibodies to NMDAR in patients with encephalitis varies widely. In a large epidemiological study (The California Encephalitis Project), NMDAR antibodies were positive in half of the children with idiopathic encephalitis and psychiatric symptoms, and the frequency was four times that of encephalitis caused by herpes simplex, varicella-zoster, and West Nile viruses.[2] A smaller number of cases were reported in a prospective study of encephalitis from England,[3] with only 4.5% being NMDAR antibody positive. Our data indicates that NMDAR antibody-associated encephalitis constitutes at least a third of suspected autoimmune encephalitis.

None of the patients in our study had antibodies to VGKC, LGI1, Caspr2, AMPA1, AMPA2, and GABAB1 receptor. Previous studies have shown the presence of autoantibodies to VGKC and glutamic acid decarboxylase (GAD) in patients with epilepsy.[9,10]

The clinical characteristics of NMDA-positive cases in our series were indistinguishable from the NMDA-negative cases. It appears that NMDAR autoantibodies have no strong association with specific clinical characteristics, natural history or outcome. This is in agreement with the other studies where they have compared the different antibody mediated encephalitis. However the individual sample sizes were low in these studies.[3,11]

In this series, five cases were positive for NMDAR antibodies as late as two years from the acute event. These patients did not have any clinical evidence of disease activity. In the absence of any antibody titration and measurement of CSF antibody levels, it is difficult to comment on the relationship between disease activity and the antibody levels. However, other studies have demonstrated persistent serum and CSF antibodies for up to several years after clinical resolution of the illness in antibody-mediated encephalitis.[12,13] The titers show a gradual fall over time and during relapses the antibody levels show increase in titers, which is more concordant with the clinical course in CSF.[14] The persistence of antibodies in the interictal period is attributed to systemic unknown factors which trigger the immune response, but at levels insufficient to cause a clinical event.[13,15]

Twenty five cases (10 in the acute phase of disease and 15 in the chronic phase) in our series were negative for all tested antibodies, although their clinical profile fitted an autoimmune mechanism. The positive response of our autoantibody negative cases to immunotherapy and evidence of CNS inflammation by CSF or MRI characteristics also suggest that these were probable cases of autoimmune encephalitis. These patients were negative for other causes of encephalitis and systemic autoimmune disorders. The negative results in our series can be due to the presence of other autoantibodies which were not tested such as GAD or encephalitis due to other yet to be recognized antibodies. A recent study has shown that analysis of NMDAR antibodies in CSF is more likely to yield positive results as compared to serum (100 vs 85.6%).[14]

This study had highlighted that a third of persons presenting with acute, noninfective encephalitis would be positive for NMDAR antibodies. A clinician needs to maintain a high index of suspicion of autoimmune encephalitis in such instances as there are few clinical or investigational markers that distinguish the antibody-positive cases from negative cases.

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