Immunological Correlates of Adult Onset Idiopathic Generalized Tonic-clonic Epilepsy before and after Sodium Valproate Treatment

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ABSTRACT

Objective: To investigate possible immunological humoral correlates in newly diagnosed adult-onset generalized tonic-clonic epilepsy among Iranian patients before and after sodium valproate treatment. **Patients and Methods:** 72 adult patients with newly diagnosed idiopathic generalized tonic-clonic epilepsy were recruited. Serum anti-nuclear antibodies (ANA), anti-cardiolipin antibodies (aCL), anti-dsDNA antibodies, total serum immunoglobulins (IgM, IgG, IgA) and C3 and C4 complements were determined before and after 12 months of therapy with sodium valproate. Similar parameters were also measured in 32 age and sex-matched healthy volunteers. **Results:** Patients group had a significantly greater level of IgG class aCL (30.6% versus 12.4%, $P = 0.004$) and anti-dsDNA antibodies (23.9% versus 0%, $P = 0.001$) when compared with healthy volunteers, however, ANA titre was relatively the same in both groups. Sodium valproate significantly decreased anti-dsDNA antibodiles ($P = 0.002$), IgM concentrations ($P = 0.034$), and increased the number of ANA positive patients ($P = 0.002$). **Conclusion:** Changes in serum level of autoantibodies in patients with new onset idiopathic generalized convulsion were found to be high. These abnormalities are associated with both seizure disorders per se and also antiepileptic drugs. We suggest that in epileptic patients with an autoimmune basis, administration of anti-epileptic drugs having modulatory effects on immune system should be considered.

Key words: Adults, Autoantibodies, Epilepsy, Sodium Valproate

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INTRODUCTION

Epilepsy is a common neurological problem which has occupied clinicians for many centuries. However, little is known about the aetiology of the disease. There is strong evidence for polygenic predisposition and, in some cases, genetic factors causing epilepsy (1). Environmental factors like toxins also play an important role in some cases of epilepsy. Immunological mechanisms have also been implicated as aetiology of epilepsy, particularly in intractable childhood epilepsy like West syndrome and Lennox Gastaut syndrome (2).

Certain clinical studies suggested that aberrations of the immunologic system may be associated with untreated epilepsy (3,4) and pharmacologically treated epileptic patients (5-7). For example, IgA deficiency has been detected in up to 25% of patients with epilepsy (8) and serum aCL have been observed in 19-26% of adult patients with both chronic epilepsy and newly diagnosed seizure disorder (9,10). In addition, Angelini and colleagues measured aCL in 27 children with partial epilepsy and found three patients had positive result with no radiological or clinical evidence of anti-phospholipid antibody syndrome (11).

These works in addition to many similar reports indicate possible immunological mechanisms underlying epileptogenesis at least in a subpopulation of patients with epilepsy. Reports that recommend the therapeutic effect of intravenous immunoglobulin (IVIG) in children with intractable epilepsy is supporting this hypothesis, although proper double blind controlled trials are needed to clarify this (4).

The purpose of this study was firstly to determine the prevalence of abnormality of immunological markers, like autoantibodies, immunoglobulin, C3 and C4 complements, in Iranian patients with untreated adult-onset generalized tonic-clonic epilepsy, and secondly to assess if anticonvulsant like sodium valproate affects these abnormalities after 1 year of treatment.

PATIENTS AND METHODS

72 adult patients with untreated newly diagnosed adult onset idiopathic generalized tonic-clonic epilepsy as well as in 32 age and sex matched healthy volunteers were included in this study. Autoantibodies including anti-nuclear antibodies (ANA), anti-cardiolipin antibody (aCL), anti-dsDNA antibodies, total immunoglobulin levels (IgM, IgG, IgA) and complement components C3 and C4 levels were measured in sera of patients before and 12 months after therapy with sodium valproate. The therapeutic does of sodium valproate varied from 600-2400 mg/daily (mean 1200 mg/day). Patients were recruited from Shiraz University Hospitals, and healthy volunteers from hospital and laboratory staffs. No co-morbidity was found in any of the patients.

Inclusion criteria for subject recruitment included, i) age 15-45, ii) recent diagnosis of idiopathic generalized tonic-clonic epilepsy made by a neurologist, iii) minimum of two witnessed attacks, iv) lack of co morbidity, v) not to be on any medication. Exclusion criteria included, i) inability to consent, ii) presence of any identifiable cause for the patient's seizures i.e. metabolic causes, electrolyte disturbances, history of head trauma,

CNS infections, structural brain lesions on MRI scan and history of an collagen vascular diseases.

Five ml of blood was drawn from each subject. The sera were frozen at –20°C for duration of 12-28 months. Total Immunoglobulins and complements were measured by SRID method. IgG class aCL by enzyme-linked immunosorbent assay (ELISA) and results were expressed in GPL unit. ANA were measured by immunofluroscent assay (IFA), and IgG anti-dsDNA antibodies by ELISA (GENESIS, England). None of the laboratory personnel who performed the tests were aware of the hypothesis or the origin of the samples.

Statistical analysis was performed on a computer using SPSS-10 for windows. Levene's test used for equality of variances, t-test for equality of means and Fischer's exact test was used to compare the differences between the study and control groups and also for comparison of before and after treatment with sodium valproate.

RESULTS

Demographic characteristics of our subjects are shown in Table 1. Forty-nine patients had only two witnessed attacks of tonic-clonic convulsion and the rest had more than two attacks. An increase in at least one of the immunological markers studied was detected in 48.3% of the study group. There were no correlation between the number of attacks and prevalence of immunological abnormalities.

Autoantibodies. Low titres (10-20 GPL units) of IgG class of aCL were found y in higher proportion of patients (30.6%, $P<0.004$) than healthy volunteers (12.4%). However moderate titres (20-40 GPL units) in patients were not significantly different from that found in healthy individuals. Patients treated with sodium valproate for 1 year did not show any significant changes in titre of aCL (table 2). However ANA were detected at a value higher than 1/80 in 11.1% of patients (mean 1/210, usually in *speckled* pattern) and in 12.2% of healthy volunteers (mean 1/190, mainly in *rim* pattern). Treatment with sodium valproate increased prevalence of positive ANA (mainly in *speckled* pattern) to 22.2% of patients ($P<0.05$).

Positive anti-dsDNA antibodies (titres >50 IU/ml) were only detected in the patient population (23.9%). Sodium valproate significantly decreased anti-dsDNA antibodies in patient group ($P<0.002$). Only one patient with high ANA titre also had high anti-dsDNA antibodies titre. In this patient, following a year of treatment with sodium

Table 1. Demographic data of patient and control groups

	Male	Female	Age (mean ± SD)
Patients	39	33	15-45 (22.34 ± 7.98)
Healthy volunteers	18	14	16-40 (22.34 ± 5.97)

Table 2. Frequencies of immunological markers studied in healthy volunteers, untreated patients with newly diagnosed adult-onset generalised tonic-clonic epilepsy. Treated patients had 12 months of treatment with sodium valproate with dosage of 600-2400 mg/day. dsDNA antibody titre is expressed in mean ± standard deviation IU/ml.

	Immunological markers in epileptic patients and normal volunteers							
	Healthy Volunteers (n=32)				Patients with Epilepsy			
Factors	Normal		Abnormal		Untreated (N=72)		Valproate-treated (N=72)	
	%	Mean titre	%	Mean titre	Abnl%	Mean titre	Abnl%	Mean titre
IgA	75	2.26 ± 1.15	25	2.31 ± 1.41	18.3	2.23 ± 1.05	22.4	2.14 ± 1.122
IgG	84.9	12.87 ± 6.3	15.1	12.81 ± 4.8	11.2	12.77 ± 6.4	20.3	16.63 ± 6.738
IgM	89.9	2.13 ± 1.72	9.1	1.11 ± 1.13	22.2	2.23 ± 1.82	13.1	2.44 ± 1.624
Low-aCL	87.6	Below 10	12.4	17	30.6*	14	35.1	16
Mod-aCL	96.9	Below 10	3.1	30	2.8	38	2.8	36
ANA	87.8	1/20	12.4	1/190	11.1	1/210	22.1**	1/160
dsDNA	100	19.5	0	0	23.9*	97	10.5**	76

* indicates $p<0.05$ when compared to healthy volunteers and ** $p<0.05$ when compared to untreated patients.
Abbreviations: Abnl = abnormal (in case of Immunoglobulins level, the values referred to below or above normal adult range. In case of autoantibodies it is referred to the % of positive individuals in study groups), Low-aCL = IgG class anticardiolipin antibodies titre 10-20 GPL unit,
Moderate-aCL = IgG class anticardiolipin antibodies titre 20-40 GPL unit, ANA = antinuclear antibody, dsDNA = double strand DNA.

valproate, ANA titre increased but anti-dsDNA titre decreased. Among patients with abnormal aCL (22 cases), 7 had also positive anti-dsDNA antibodies and 5 had high ANA titres.

Immunoglobulins and complements. In the patients group, mean IgM concentrations (mean = 2.21 mg/ml) was higher in comparison to the normal volunteers (mean = 1.11 g/L, P = 0.002). IgM values after a year of treatment with sodium valproate were significantly less than before treatment (P = 0.034) (table 2).

Mean concentrations of IgA, IgG, C3 and C4 complements in the study and control groups were not significantly different before and after treatment. In 3% of the study group, IgA concentration was not measurable.

DISCUSSION

The main findings of our study were a high prevalence of aCL (30.6%) and anti-dsDNA antibodies (23.9%), in adult patients with newly diagnosed idiopathic generalized tonic-clonic convulsion. The use of sodium valproate for one year after first sampling indicated that a significant decrease in anti-dsDNA antibodies was noticeable without significant change on aCL titre. Erikson et al. reported high prevalence of aCL (44%) among

children with symptomatic or cryptogenic generalized epilepsy (12). Peltola et al. found high aCL titres in 8% of adult patients with generalized epilepsy and 14% with localized related epilepsy (10). Results of our study revealed 30.6% low positive and 2.8% moderate positive aCL among adult patients.

The reported data of low IgA concentration in aCL positive patients was not consistent in our investigation (13,14). ANA was reported in 11.1% of our patients and 13.2% of the controls, this is in consistent with the finding of Bardana et al. (4) and in disagreement with finding of Peltola et al. The clinical significance of increased in production of aCL and anti-dsDNA antibodies in epilepsy is unclear. It is postulated that a thrombotic-ischaemic event following injury or trauma within the central nervous system may trigger the humoral arm of the immune system to reactivate directed against the released phospholipids or cardiolipine components within the microenvironment of damaged tissues (15,16). As a result, the pathological antibody may contribute to the disease manifestation (8,17). Data from experimental studies revealed that aCL can disrupt neuronal function by direct action on nerve terminals (18) and reduce GABA receptor mediated chloride currents, suggesting a direct and reversible mechanism through which aCL may induces its effects (19). It is worth mentioning that none of our patients with positive aCL had ischemic lesions in their brain images at the onset of the study. Results of our work clearly indicate that sodium valproate significantly decreased anti-dsDNA antibodies in patient group. The effect of sodium valproate treatment in relation to the inhibition of anti-dsDNA antibody synthesis is particularly interesting. Data of this study indicate that after one year use of sodium valproate a substantial percentage of positive anti-dsDNA antibody cases became negative. As none of the control individuals were found to be positive for antibody to dsDNA, it is conceivable to argue that the presence of this autoantibody might be considered as a marker of disease association. It is not clear weather the anti-dsDNA antibody have a role in the pathophysiology of epilepsy as its involvement in the pathogenesis Systemic Lupus Erythematosus (SLE). An important point to be mention is the mechanism by which sodium valproate induced its effect to inhibit production of anti-dsDNA antibody. By considering the fact that none of those positive patients was receiving any corticosteroid, nor other immunosuppressive agents during the one year study, the role of sodium valproate in this process need a through investigation. To our knowledge, no report on the immunosuppressive activity of sodium valproate has yet been published.

In conclusion, results of this study revealed that a number of idiopathic generalized tonic-clonic epilepsy among Iranian adults was positive for anti-cardiolipin and anti-dsDNA antibodies. Treatment of patients with sodium valportate significantly affect on the production of anti-dsDNA antibody in a way that a significant percentage of positive patients before treatment became negative after one year receiving the sodium valproate.

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