

Immunoglobulins, Immunoglobulin G Subclasses and Complement C3,C4 Levels in Adult Epileptic Patients on Carbamazepine and Sodium Valproate

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Abstract

Objective: To assess the effects of long-term antiepileptic monotherapy (carbamazepine or sodium valproate) on the levels of some immunological parameters (immunoglobulins IgA, IgG, IgM, immunoglobulin G subclasses IgG1, IgG2, IgG3, IgG4 and complements C3, C4 in adult epileptic patients. Subjects and Methods: Eighty –one adult epileptic patients were included in this study, 43 on carbamazepine monotherapy and 38 on sodium valproate monotherapy, with 50 apparently healthy subjects age and sex matched taken as a control group. From both patients and control group 10 ml venous blood sample were taken and immunoglobulin serum levels (IgA, IgG, IgM), immunoglobulin G subclass (G1, G2, G3, G4) and complement C3,C4 were assessed using kits (Fitzgerald Industries Int. USA). Results: There was a significant reduction in IgM and IgG2 in epileptic patients on carbamazepine and a significant reduction in IgM, IgG2 and IgG4 in epileptic patients on valproate in comparison with controls. Conclusion: Carbamazepine and sodium valproate as long-term antiepileptic monotherapy have some effects on the immune-system parameters in adult epileptics.

المخلص

أهداف الدراسة: بتحديد تأثير العلاج الطويل الأمد بالأدوية المضادة للصرع بشكل احـادي (الكاربامزابين او الصوديوم فالبرويت) على مستوى بعض مفردات الجهاز المناعـي (الكلوبولينات المناعية نوع M, G, A ، الكلوبولينات المناعية نوع G الفرعية G1, G2, G3, G4, G4, G3, G2, G1) والتممات C3, C4) عند البالغين المصابين بالصرع . المشاركون وطرق العمل :- واحد وثمانون من مرضى الصرع البالغين ادخلوا في هذه الدراسة 43 منهم مثبتين على عقار الكاربامزابين كعلاج احادي و 38 مريضا مثبتين على الصوديوم فالبرويت كعلاج احادي بالاضافة الى خمسين شخصا من الاصحاء من اعمار واجناس مقاربة لمجموعة المرضى اتخذت كمجموعة ضبط . لكل من مجموعة المرضى ومجموعة الضبط تم سحب 10 مل من الدم الوريدي وتم قياس مستوى الكلوبولينات المناعية نوع M, G, A ، الكلوبولينات المناعية نوع G الفرعية G1, G2, G3, G4, G4, G3, G2, G1) والتممات C3, C4 باستخدام عدد خاصة للقياس . النتائج : كان هنالك ناقصا معنويا في مستوى كوليولين M, G2 في مرضى الصرع ال مثبتين على عقار الكاربامزابين ونقصانا معنويا في مستوى كوليولين M, G2, G4 في المرضى المثبتين على عقار فالبرويت بالمقارنة مع مجموعة الضبط . الاستنتاج: عقار الكاربامزابين والصوديوم فالبرويت كعلاج احادي طويل الامد يؤثر على بعض مفردات الجهاز المناعي عند البالغين من مرضى الصرع.

Introduction

Epilepsy is a common neurological problem which has occupied clinician for many centuries ⁽¹⁾. Based on the bidirectional interaction between the central nervous system and the immune system, it is attractive to speculate that immune mechanisms may be involved in the pathogenesis of at least some forms of epilepsy or that epileptic seizures may affect the immune system indirectly ⁽²⁾. Another argument for immunological mechanisms being involved is the observation that some patients suffering from some forms of intractable epilepsy e.g West syndrome, Lennox-Gastaut syndrome and Landau-Kleffner syndrome may benefit from treatment with intravenous immunoglobulins ^(3,4). Immunological abnormalities in epileptic patients receiving antiepileptic drugs have been reported ⁽⁵⁾, with conflicting reports been published on serum immunoglobulin concentrations in such patients ⁽⁶⁾. This study aim to assess, serum immunoglobulin levels (IgG, IgM, IgA), immunoglobulin G subclasses (IgG1, IgG2, IgG3, IgG4) and complements C3, C4 in adult epileptic patients on carbamazepine or sodium valproate monotherapy in comparison to healthy controls.

Patients and Methods

The study was conducted from Apr 2005 to Jul 2007 in the Department of pharmacology – College of Medicine – University of Mosul. Patients were referred and selected according to certain criteria by a neurologist. The criteria included, adult epileptic patient, on carbamazepine (CBZ) or valproate (VPA) monotherapy for a duration of treatment not less than one year, patients with any other diseases or medication were excluded. Out of 110 epileptic patients interviewed, only 81 met the criteria and were included in the study. 43 on carbamazepine therapy in a mean daily

dose 581.39 ± 128.05 mg (ranged between 400 and 800 mg/dl). They were 14 females and 29 males with a mean age 26.51 ± 5.57 year (ranged between 17 and 41 years), for a mean duration 5.18 ± 2.37 year (ranged between 1 and 11 years), and 38 patients on VPA therapy in a mean daily dose 568.42 ± 155.77 mg (ranged between 400 and 800 mg/d), they were 10 females and 28 males with a mean age 25.26 ± 4.84 years (ranged between 18 and 36 years), for a mean duration 3.92 ± 2.01 year (ranged between 1 and 8 years). Fifty apparently healthy subjects were taken as a control group. They were 17 females and 33 males with a mean age 25.98 ± 4.96 year (ranged between 18 and 40 years). From the members of both the control and patients groups, 10 ml venous blood samples were taken, and serum levels immunoglobulins (IgG, IgA, IgM), immunoglobulin G subclasses (G1, G2, G3, G4) and complements C3, C4 were assayed using especial kits.

Serum immunoglobulins, immunoglobulin G subclasses and complement assay.

Serum immunoglobulins, immunoglobulin G subclasses and complement C3, C4 were assayed using Radial immunodiffusion method ⁽⁷⁾, using kits from RDI division of Fitzgerald industries Int, USA. Radial immunodiffusion (RID) (Mancini) method is a classical diagnostic method to determine IgG subclasses. This reliable assay is widely used and easy to perform. The RID assay is performed in (ready-for-use) agar plates, containing the specific anti-IgG subclass antibodies. Test samples, standard and control sera are prepared and added to the plates. After 48-64 hours incubation at room temperature the diameters of the immunoprecipitation rings are measured. The IgG subclass concentrations in the test samples may be quantified in two ways: a) Calibration curve method (which was followed in this study): ring diameters and

concentrations of the standards are plotted and the values of the test sample are determined by interpolation.

b) Tabular method: ring diameters of the calibration curve are listed and the values of the test sample are read from a table. It is not necessary to make a calibration curve. The control serum is assayed to check the validity of the calibration curves and also the accuracy of the IgG subclass quantification, when using the table.

The same method was applied to measure serum complement C3,C4 levels. Comparison of **results** between patients on CBZ or VPA monotherapy and controls were done using ANOVA Test (Analysis of Variance), and Duncan's test. All values quoted as the mean \pm SD , P-value of

<0.05 was considered to be statistically significant.

Results

1-By comparing results of epileptic patients on CBZ therapy and controls, there was a significant reduction in the mean serum levels of IgM and IgG2 .While serum levels of total IgG, IgA,IgG subclasses G1, G3, G4 and complements C3,C4 showed insignificant differences (table 1).

2-By comparing results of patients on VPA therapy and controls, there was a significant reduction in the mean serum levels of IgM, IgG2, IgG4. While serum levels of total IgG , IgA, IgG subclasses G1,G3 and complements C3,C4 showed insignificant differences .(Table 1)

Table (1):- comparison between epileptic patients on CBZ , VPA and controls with regard measured parameters :

Measured parameters	Controls (No. =50)	Patients on VPA (No. =38)	Patients on CBZ (No. =43)
Total IgG (mg/dl)	1185.1 \pm 228.4	1106.3 \pm 153.7	1126.2 \pm 170.3
IgG G1 (mg/dl)	814.4 \pm 117.9	799.4 \pm 164.9	790.1 \pm 123.6
IgG G2 (mg/dl)	256.0 \pm 42.5 a	168.4 \pm 35.4 b	199.3 \pm 39.8 b
IgG G3 (mg/dl)	73.8 \pm 10.9	68.2 \pm 12.4	76.6 \pm 9.4
IgG G4 (mg/dl)	58.9 \pm 10.6 a	51.1 \pm 10.3 b	60.8 \pm 9.0
IgA (mg/dl)	306.8 \pm 67.7	288.4 \pm 64.6	293.0 \pm 67.7
IgM (mg/dl)	264.4 \pm 54.9 a	241.3 \pm 42.4 b	239.4 \pm 48.9 b
Complements C3 (mg/dl)	203.0 \pm 26.3	198.7 \pm 24.0	197.4 \pm 21.8
Complements C4 (mg/dl)	32.3 \pm 3.8	32.2 \pm 3.5	32.5 \pm 3.8

Different letters horizontally means significant differences.

Discussion

The results of this study reflect a significant reduction in IgM and IgG2 in adult epileptic patients on CBZ therapy, while the other measurements for immunoglobulins and complement levels were not changed significantly in comparison with the control group. There was also a significant reduction in IgM, IgG2 and IgG4 in adult epileptic patients on VPA therapy while total IgG, IgA and IgG3 with the complements showed insignificant differences from the values in the control group. Gilhus and Lea⁽⁶⁾ by measuring IgG subclass concentrations in the sera of 20 epileptic patients before and 6 weeks after CBZ therapy, concluded that CBZ reduces IgG2 concentration and this reduction was unrelated to serum CBZ concentration, type of epilepsy or age of the patient. In another study Gilhus and Lea⁽⁷⁾ determine serum IgG subclasses in epileptic patients on phenytoin therapy and in untreated epileptic patients as a control. Their results indicated that IgG1 and IgG2 concentration did not differ in phenytoin treated and untreated epileptic patients, while IgG3 and IgG4 were significantly reduced in patients with IgA deficiency. Also with regard phenytoin therapy Basaran et al⁽⁸⁾, studied serum immunoglobulins, complements C3, C4 levels in epileptic patients compared to healthy controls. Their results indicated a significant raise in serum IgM levels, while there was no significant changes in the serum concentrations of IgA, IgG and complements protein. In 1991, Lenti et al⁽⁹⁾, studied the serum immunoglobulin levels in epileptic children treated with CBZ or VPA; untreated patients and healthy controls. They reported that both the treated and untreated patients did not differ significantly from the controls with respect of the mean IgA, IgG and IgM values and that patients on CBZ had insignificantly lower serum levels of IgG than the untreated patients and children on VPA. These results are inconsistent with

the study conducted by Bostantjopoulou et al⁽¹⁰⁾ who reported that epileptic patients on CBZ therapy had increased IgG and IgM levels and decreased C4 complement component, while epileptic patients on VPA had increased IgM levels and decreased C4 complement component and concluded that there is a defective immune mechanism in epileptic patients modified by treatment. Also in contrast to our findings Hemingway et al⁽¹¹⁾, reported that only few subjects on CBZ or VPA monotherapy had any of the values of immunoglobulins outside accepted normal ranges. While in agreement with our results concerning CBZ Basaran et al⁽¹²⁾, reported a significantly lower IgM levels in epileptic patients on CBZ therapy in comparison to epileptic untreated and controls, and concerning VPA, the study of Yosefi-Pou et al⁽¹³⁾ who concluded that IgM values after a year of treatment with VPA were significantly less than before treatment and the controls and the mean concentrations of IgA, IgG, complements C3 and C4 were not significantly different before and after treatment and from the controls. The occurrence of interaction between cells of the nervous system and the immune-system associated with the use of anticonvulsant was suggested, with no elucidation of the pathophysiological process involved⁽¹⁴⁾. The mechanism by which anticonvulsants interfere with the immune-system is not fully clear. The observation of Gilhus and Matre⁽¹⁵⁾, that epileptic patients on CBZ therapy had reduced serum IgA and IgM levels, with no reduction in the number of T or B lymphocytes or in their proliferation ability, might suggest an effect on B-lymphocytes maturation or on immunoglobulin synthesis. Also it has been shown that CBZ in therapeutic concentrations interacts with peripheral benzodiazepine receptors⁽¹⁶⁾, such receptors, which are present in the glial cells and human lymphocytes together with their ligands form the basis of a

neuro-immune network that contribute to the immune –system bidirectional interaction .⁽¹⁷⁾ This study might be one of a few studies that include selected adult epileptic patients on long-term antiepileptic monotherapy (CBZ or VPA), with regard assessment of serum immunoglobulin levels (IgA, IgG, IgM) IgG subclasses (IgG, IgG2, IgG3, IgG4) and complements(C3,C4)in comparison to controls. In conclusion long term therapy with CBZ or VPA in adult epileptic patients, might affect part of the immune system as reflected by the result of this study.

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