Original article:

Evaluation of Serum Interleukin - 1 beta (IL-1β) in seizure patients

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ABSTRACT

Introduction: Interleukin-1beta (IL-1β) is a pro inflammatory cytokine which activates additional cytokine cascade and enhances susceptibility of seizures. The aim of our study is to compare the serum levels of IL-1β in seizure patients and controls.

Material and methods: The cross sectional study included thirty patients of both genders in the age group 20-40 years diagnosed as Generalised Tonic Clonic seizure patients(GTCS) and thirty age and sex matched control subjects. Serum IL-1β was estimated by ELISA method. Student t test was carried out to compare the means of variables between seizure patients and control subjects.

Result: The mean serum IL-1β levels in seizure patients was 2.15 ± 0.59 pg and controls was 1.81 ± 0.62 pg (p = 0.039).

Conclusion : The mean serum IL-1β levels were significantly higher in seizure patients than controls indicating the antagonists of this cytokine can be used in clinical practice for seizure inhibition in near future.

Keywords: Seizures, Cytokine, Interleukin -1β

Introduction

Cytokines are soluble potent glycoproteins secreted by the glial cells of central nervous system (CNS). They bind to high affinity surface receptors mediating cell to cell sigalling and serve as a biomarker for earlier detection of brain damage to prevent further neurological complications. Abnormalities in the expression of cytokines and immune cells is noted in epilepsy patients and in various animal models[1] .

Interleukin-1beta (IL-1β) is one such pro inflammatory cytokine released from glial cells during seizures.The chronic expression of IL-1β during epileptogenesis contributing to neuronal injury suggests that IL-1β activated pathways play a vital role in the genesis of spontaneous seizures[2]. This cytokine can affect the permeability of blood brain barrier by disruption of tight junctions or nitric oxide production along with activation of metalloproteinases in endothelial cells resulting in chronic hyperexcitability of neurons. It also favours the entry of the cells of adaptive and innate immunity into the brain which perpetuates inflammation. Hence the immune system and its associated inflammatory reactions appear to play a major role in epileptogenesis and aggravate brain damage[3]. L 1β augment nitric oxide formation to raise the seizure susceptibility and also increase the neuronal excitability by directly inhibiting GABA(A)receptors, enhancing NMDA receptor function and inhibiting K efflux[4]. The cytokine increases the phosphorylation of the NR2B subunit of NMDA receptor thereby enhancing Calcium influx into the neurons[5]. Through the activation of sphingomyelinase, IL-1β induces the production of ceramide which in turn activates the Src family tyrosine kinases leading to NR2B phosphorylation. The activation of this pathway underlies the proconvulsant activity of IL-1β[6]. The levels of various cytokines increase transiently in the blood and CSF of patients with epilepsy after different types of seizures[7].

FIGURE 1: Interleukin-1 β in epileptogenesis[8]

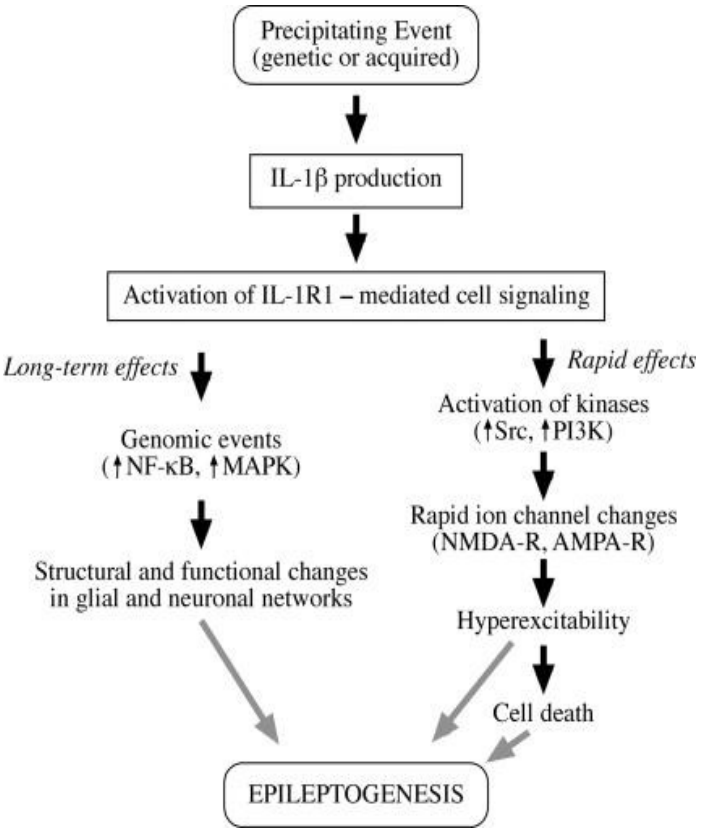

FIGURE 1: IL-1R1 (IL-1Type1 receptor), NF-kB (Nuclear Factor – Kappa B),

MAPK (Mitogen Activated Protein Kinase), PI3K (Phosphoinositol 3 kinase), NMDA-R(N methyl D aspartate receptor)

Aim and objective

To evaluate the serum Interleukin -1β levels in patients with Generalised Tonic Clonic Seizures and compared with age and sex matched controls. The outcome of this study would help in the exploration of possible newer anti-epileptic drugs if the influence of IL-1β on epileptogenesis is established.

Materials and methods

The study was conducted in the Institute of Physiology and Experimental Medicine, Madras Medical College after obtaining approval from the Institutional Ethics Committee, Madras Medical College, Chennai.

Thirty Patients of both sexes in the age group between 20-40 years diagnosed as generalized tonic clonic seizures were included in this cross sectional study. They were selected from the Institute of Neurology, Rajiv Gandhi Government General Hospital, Chennai - 3. The duration of the disease ranges from 1-2 years. Thirty age and sex matched apparently healthy people were selected as controls. With all these criteria, a total of 60 individuals were selected for the study. Informed verbal and written consent was obtained from the participants after explaining the procedure.

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Under strict aseptic precautions, blood samples were collected from the ante cubital vein by means of venepuncture within 6 hours of the previous seizure episode and the separated serum was stored in deep freezer at -20 centigrade. Estimation of serum interleukin 1 beta levels was carried out in the Department of Experimental medicine, The Tamil Nadu Dr. M.G.R. Medical University. Human IL-1β ELISA (Enzyme linked immunosorbent assay) kit is an in vitro enzyme linked immunosorbent assay for the quantitative measurement of IL-1β in serum, plasma and cell culture supernatants. The minimum detectable dose of IL-1β is typically less than 0.3 pg/ml.

This assay employs an antibody specific for human IL-1 beta coated on a 96 well plate. 10 µl of each standard and sample are added into appropriate wells which is covered well and incubated for 2.5 hours at room temperature. After washing away unbound substances with antibody, 100 µl of prepared biotinylated antibody is added to each well and incubated for 1 hour at room temperature. Follwing second wash, 100 µl of prepared streptavidin solution is added to each well and incubate for 45 min at room temperature.Following third wash, 100 µl of TMB ($3,3^1,5,5^1$ tetramethyl benzidine) one step substrate reagent is added to each well and repeat the incubation for 30 min at room temperature in the dark. Finally 50 µl of stop solution (0.2 M sulfuric acid) is added to each well and read at 450 nm immediately in a ELISA reader.

A standard graph is constructed by marking the average values of absorbance of each reference standard in the Y axis versus its corresponding concentration in X axis. The corresponding IL- 1β was obtained by simple interpolation from this standard curve. Average % was obtained by dividing observed value by expected value.

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 21. Student 't' test was carried out to compare the means of variables between GTCS patients and normal subjects.

Observation and Results:

Our study population consists of 30 GTCS patients (15 males and 15 females) and 30 control subjects with same gender distribution in the age group of 20-40 years . The mean age was calculated to be 30.86±2.54 years in the control group and the mean age of GTCS patients was found to be 30.86±2.54 years. The mean serum IL-1β levels in seizure patients was 2.15 ± 0.59 pg and controls was 1.81 ± 0.62 pg . The mean level of IL-1β was significantly higher in GTCS patients when compared with controls.

TABLE 1: Comparison of serum Interleukin-1 beta levels in controls and GTCS patients

GROUPS	IL-1 BETA MEAN±SD in pg	p VALUE
CONTROLS	1.81±0.62	0.039
GTCS PATIENTS	2.15±0.59	

P value< 0.03, significant

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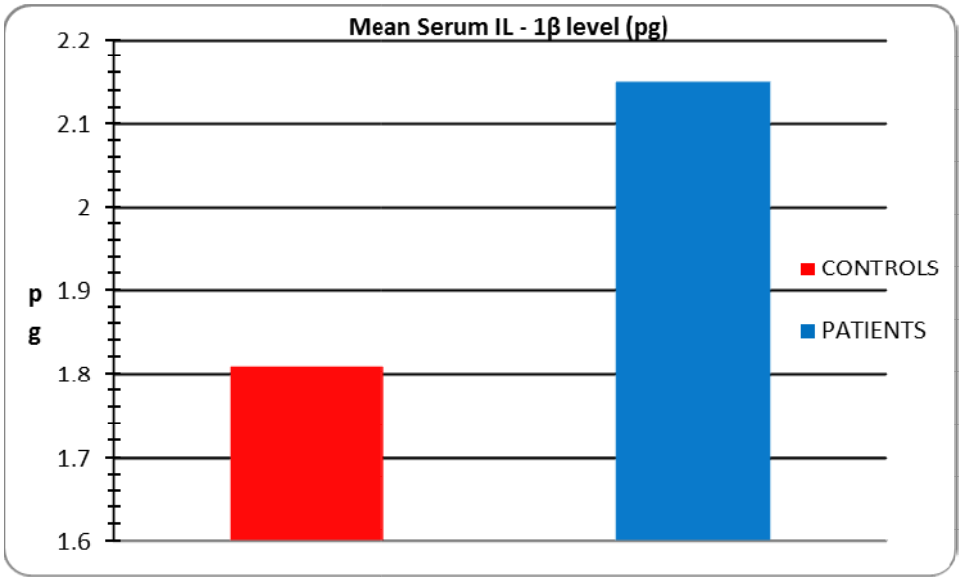
FIGURE 2: Comparison of mean serum IL-1 levels in controls and GTCS patients



Discussion

In the present study, the mean serum level of IL-1 β in GTCS patients is 2.15 ± 0.59 pg which is significantly higher when compared to controls(1.81 ± 0.62 pg) indicating its potential role in seizure development. Interleukin -1β is a pro inflammatory cytokine originating peripherally or in the CNS, can modulate neuronal excitability and play a major role in epileptogenesis. The study is supported by Lehtimaki et al[9] and Sinha et al[10] . They observed an increase in serum cytokine levels including IL-I β within a few hours after the seizure episode followed by a trend towards normalisation by 16 days, thus revealing important relationship between seizure and increased cytokine levels.

Peltola et al[7] suggested that increased levels of cytokines including IL-1 β are found to be higher after more severe seizure (generalised), acknowledging that increased levels of cytokines are related to the seizure activity. Plata –Salaman CR[11] observed abnormalities in the expression of cytokines and immune cells in patients with epilepsy. Kalueff AV et al[12] recognised that the immune system and its associated inflammatory reactions play an important role in the process of epileptogenesis.

Steffensen SC et al[13]implicated cytokines as mediators of spontaneous seizures. Rosenbaum KJ et al[14] acknowledged that seizures themselves can activate the sympathetic nervous system and induce the release of catecholamine which mediates cytokine release from the peripheral blood mononuclear cells. Pacifi et al[15] identified that antiepileptic treatment does not affect IL-1beta production by mononuclear cells along with Hulkkonen et al[16] who acknowledged that antiepileptic drugs do not affect the plasma levels of IL-1β.

Conclusion

The serum Interleukin-1β which is a pro inflammatory cytokine is increased significantly in patients with GTCS suggesting the occurrence of inflammation in seizures. Since IL-1β activated pathways contribute to epileptogenesis, the antagonists of this cytokine can be targeted as novel anticonvulsant drugs in near future.

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