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Serum Alpha Synuclein and Interleukin 1β levels as Biomarkers in Epilepsy

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Abstract

Epilepsy is a chronic brain disorder accompanied by behavioral and cognitive problems, which include intellectual dysfunctions and attention deficits. The International League against Epilepsy (ILAE) defines epilepsy as a condition in which a patient has "two or more unprovoked seizure attacks occurring at least 1 day apart". Approximately one-third of patients with epilepsy have drug resistant epilepsy, defined as epilepsy that is not controlled by two antiepileptic medications to sustain seizure freedom. The causes of regression may include the etiology of the underlying epilepsy, the spike discharges on electroencephalogram (EEG) outputs, the seizures themselves and a variety of medications, but there is no known biomarker for disease severity or neurocognitive comorbidity. The search for noninvasive biomarkers of neuroinflammation and neurodegeneration has focused on various neurological disorders, including epilepsy. We sought to determine whether α-synuclein and cytokines are correlated with the degree of neuroinflammation and/or neurodegeneration in children with epilepsy and with acquired demyelinating disorders of the central nervous system (CNS), as a prototype of autoimmune neuroinflammatory disorders.

Keywords: Alpha Synuclein, Interleukin 1β, Epilepsy.

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1. Introduction

Identification of biomarkers that can help in guiding the diagnosis and therapy, International League against Epilepsy defines a biomarker for epileptogenesis as an objectively measurable characteristic of a biological process that reliably identifies the development, presence, severity, progression, or localization of an epileptogenic abnormality. In recent years, there have been advances in the development of accessible and cost effective blood-based biomarkers in neurology, and these are increasingly studied in epilepsy [1]. There are advancements regarding human blood biomarkers in epilepsy, for example biochemical markers that have been shown to have higher blood concentrations in study subjects with epilepsy include brain proteins like S100B or neuronal specific enolase, glial fibrillary acidic protein (GFAP), neurofilament light protein (NfL), microtubule- associated protein tau, ubiquitin C-terminal hydrolase 1 (UCHL-1) and matrix metalloproteinase 9 (MMP-9), all of which are abundantly present in neural tissues [1].

And also there are neuroinflammatory proteins like interleukins, and tumor necrosis factor-alpha. Some of the blood biomarkers also seem to reflect seizure duration or frequency, and levels decrease in response to treatment with anti-seizure medication. There was no reliable methods to distinguish non-intractable epilepsy from intractable epilepsy. Therefore, a new clinical diagnostic biomarker would be of considerable practical importance to the clinical identification of the reactivity of patients to anti-seizure medications and the drug-resistant epilepsy [2]. The advances in our understanding of how biochemical markers can reflect brain pathology has given rise to optimism in the epilepsy field, Blood tests capable of identifying epilepsy and seizure burden would be a more scalable option. Recent reviews document inflammation pathways activated in drug resistant epilepsy, and provide proof of concept that anti-inflammatory agents and strategies can reduce seizure occurrence [3].

1.2. Alpha Synuclein

Alpha Synuclein is one of the most abundant proteins within the neurons of the brain. This protein is suggested to participate in synaptic transmission by regulating neurotransmitter release and vesicle recycling,

also in mitochondrial and synaptic dysfunction and neuronal apoptosis and in calcium homeostasis [4]. Alpha-synuclein (α-syn) is a protein belonging to the synuclein family, consisting of three small soluble proteins, namely alpha-, beta-, and gamma. Although α-syn is present in different cell types, including erythrocytes and platelets, it is predominantly expressed in cytoplasm of neuronal cells. Under physiological conditions, it is ubiquitously expressed in pre- synaptic terminal, where it interacts with membranes of synaptic vesicles, contributing to neurotransmission and synaptic homeostasis [5]. The presynaptic location of α-synuclein, its membrane structures, and interaction with various synaptic proteins suggest that it may have a role in synaptic activity. α-synuclein can modulate gene expression by interacting with nuclear proteins such as histones, transcription factors, and various promoters of genes in cell nucleus [6].

α-syn physiologically exists in a dynamic equilibrium between cytosolic monomeric unfolded forms and helically folded tetramers bound to membranes. Under pathological conditions, α-syn monomers could aggregate into insoluble fibrils, known as Lewy Bodies and Lewy Neurites, which represent pathological hallmark of synucleinopathies, such as Parkinson's disease, dementia with lewy bodies, and multiple system atrophy [7]. Synucleins as a family of soluble proteins, and their functions are not yet fully understood. The presynaptic location of α-synuclein, its membrane structures, and interaction with various synaptic proteins suggest that it may have a role in synaptic activity. α-synuclein can modulate gene expression by interacting with nuclear proteins such as histones, transcription factors, and various promoters of the genes in the cell nucleus [6]. Synucleinopathies are heterogeneous disorders characterized by aggregation of α-synuclein in neurons and glia, in form of Lewy bodies. Pathologically, synucleinopathies can be divided into two major disease groups: Lewy body disease and multiple system atrophy.

Alpha synuclein emerges as an important part of Lewy bodies that are pathological filamentous structures which are seen especially in Parkinson's disease. Despite the known role of α-synuclein in neurodegeneration, limited studies exist on the impact of α-synuclein-mediated neurodegeneration in epileptogenesis. However, emerging data highlight the correlation of α-synuclein with epileptic seizures [8]. It is a small, acidic synaptic protein composed of 140 amino acid residues, normally soluble, which is abundantly localized in neuronal synaptic terminals of brain, it is mainly localized in neocortex, striatum, and thalamus hippocampus, thalamus, and cerebellum. It intrinsically disordered protein but adopts partial α-helix upon binding with membranes. The domain structure of α-Syn consists of an amphipathic N-terminal region (1-60), non-amyloid component (61-95) and C-terminal region (96-140) [9]. It is predominantly a neuronal protein, but can also be found in the neuroglial cells. It affects the synaptic transmission via regulation of neurotransmitter release and vesicle recycling. Alpha synuclein regulates the size of distinct pools of synaptic vesicles in the mature neurons and reduces the distal reserve synaptic vesicle pool by half with intact docked vesicle pool.

It controls traficking of the synaptic vesicles in distal reserve pool and regulates the amount of vesicles docked at synapses for neurotransmitter release [8]. In addition, α-synuclein contributes to folding/refolding of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins, which are crucial for release of neurotransmitters, vesicle recycling, and synaptic integrity. Disruption, aggregation, and deposition of α-synuclein has implicated as a common phenomenon in neurodegenerative disorders, known as synucleinopathies [10]. In the extracellular space, α-synuclein can initiate the activation of neighboring astrocytes and microglia, enhancing glial proinflammatory activity. Microglial activation in turn produces proinflammatory cytokines, nitric oxide, and reactive oxygen species, which may be toxic to neurons. α-synuclein can also be transmitted across neurons to initiate aggregation events, leading to compromised viability of inheritor neuron [10]. Although the prognosis of seizure control by the currently available AEDs is good in the majority of patients, one third of them are refractory to the AEDs, denoting an intractable epilepsy. α- synuclein may serve as a biological marker in both epilepsy and intractable epilepsy, since an up regulation in its concentration (serum and CSF) has been observed in epileptic patients when compared to normal controls [8].

Mesial temporal lobe epilepsy, the most common form of epilepsy, is characterized by alpha synuclein abnormal deposits, including neuronal cell loss and reactive gliosis in the hippocampus. Despite the elevation of α-synuclein expression in epilepsy, the precise mechanism underlying contribution of α-synuclein to disease pathogenesis is still elusive. In addition, more investigations are warranted to determine whether inhibition of aggregation of α- synuclein in epilepsy is beneficial. Elucidation of the underlying mechanism behind its secretion and cytotoxicity might open novel perspectives in deciphering α-synuclein-mediated neurodegeneration in epileptogenesis [8]. Seizure's multiplications were observed in patients with gene mutations in α-synuclein. Although rarely, α-synuclein gene multiplication results in its accumulation in the peripheral and central nervous system leading to different disease phenotypes, including myoclonus seizures. In serum, epileptic patients and CSF α-Synuclein concentration increased, Ceftriaxone, β-Lactam antibiotics help in decreasing seizure frequency in epileptic patients by binding to α- synuclein and inhibiting its aggregation [11].

1.3. Interleukin-1β (IL-Iβ)

Among the variety of cytokine classes, interleukins (ILs) are widely studied in epilepsy. The level of these molecules are altered during inflammation, are associated with seizure susceptibility and are possibly involved in epileptogenesis [12]. Pro-inflammatory cytokine interleukin-1β (IL-Iβ), expressed in activated microglia and astrocytes, enhances the release of glutamate from astrocytes and decreases glutamate re-uptake, thereby increasing glutamate availability in neuronal synapses and promoting neuronal hyper-excitability [13]. IL-Iβ typically concentrated in low quantities within the brain, increase after seizures, IL-1b is also found in blood, and CNS lesions of MS patients. Lastly, many questions remain about the pathogenic role of IL-1b in MS and whether auto reactive subsets of human T cells respond to this cytokine or other pro inflammatory cytokines [14]. IL-1β is one of most extensively studied interleukins in epilepsy, and up regulated in tonic–clonic

seizures and temporal lobe epilepsy. IL-1β levels also predictive of seizure recurrence in a study of ischemic stroke patients after a first epileptic seizure. Plasma IL- 1Ra, the antagonist to IL-1 receptor, was also found elevated in febrile seizures, while IL-1β remained unchanged [15].

IL1β is produced and secreted by several cell types. Although the vast majority of studies have focused on its production within cells of the innate immune system, such as monocytes and macrophages, it is mainly produced by activated microglia as well as neurons, astrocytes, and oligodendrocytes in central nervous system [16]. IL-1β is strongly induced in activated microglia and astrocytes during acute phase of status epilepticus and in chronic phase of spontaneous seizures in brain areas involved in seizure generation and propagation and is mainly sustained by astrocytes. IL-1β mainly binds to IL1R1 and activates NF-κB in target cells to induce and amplify inflammatory response and plays an important role in injury and inflammation [15]. Tissue inflammation is a key component of many diseases, including epilepsy. A striking example is Rasmussen's encephalitis—a rare neurological disorder characterized by the unilateral inflammation of the cerebral cortex and drug-resistant epilepsy [17]. Antibody-mediated limbic and extralimbic encephalitis, multisystem inflammatory disorders, West syndrome, and Landau-Kleffner syndrome are all examples of inflammatory conditions linked to recurrent seizures.

Many focal epilepsies, such as mesial temporal lobe epilepsy and cortical epilepsies associated with dysplastic lesions, also exhibit signs of neuroinflammation, as evidenced by proliferation of reactive astrocytes and microglia, increased expression of pro-inflammatory cytokines and chemokines, and blood–brain barrier abnormalities with infiltration of circulating immune cells [18]. The IL1b-producing microglia showed reduced expression of the purinergic receptor P2RY12, which responds to neuronal activation by sensing ATP released by activated neurons and astrocytes. There is increasing evidence from other studies that adenosine and purinergic system play important roles in epileptogenesis, and that pharmacological manipulation of the purinergic pathway may be used to treat epilepsy [19]. Microglia from patients with drug resistant epilepsy showed expression of pro-inflammatory cytokine (*IL1B*, *IL1A*, *TNF*, *CCL2* and *CCL4*) and chemokine genes, Overall, microglial transcriptional similarity, infiltration of pro-inflammatory lymphocytes and direct interaction of microglia with T cells essentially place DRE closer to an immune-mediated disease, with many functional and transcriptional characteristics similar to multiple sclerosis [20].

One hypothesis to explain the effect of Il1b in neuronal excitability is that it directly inhibits K+ efflux and increases Ca2+ influx and NMDA-R function, all of which contribute to seizure susceptibility and seizure-related damage [21]. More recently, several studies have begun to unravel non-immunological roles played by endogenously produced Il1b in the brain; this molecule is crucial to maintain homeostasis and is far from neurotoxic [22]. Furthermore, studies involving IL1B polymorphisms in humans have shown an association between a variant located at the promoter region of IL1B (C-511T), which produces less protein, and increased risk for epilepsy and febrile seizure [23]. These findings support the hypothesis that endogenous Il1b may also have protective roles in the CNS and that insufficient endogenous Il1b predisposes the CNS to hyper excitability. More recently, it has been observed that NMDA-R together with Il1b appear to be necessary to induce the CX3CR1-dependent microglial process convergence —an increase in the interaction between microglia and neurons, especially in the dendrites and axons, that confers neuroprotective effects in chemically induced animal models of epilepsy, thereby decreasing seizure severity and cell death [24].

Seizures and subsequent development of epilepsy after stroke may not only hinder patient's recovery but also increase risk of complications. Interleukin (IL)-1β has been shown to be acutely up regulated after ischemic stroke and play a role in the recurrence of seizures following the first epileptic seizure in patients suffering an ischemic stroke. Meanwhile, variants of the IL-1B gene encoding IL- 1β are involved in the stimulation of febrile seizures [25]. Following an initial insult to the central nervous system, ongoing inflammation can change neuronal plasticity through several transcriptionally mediated effects, which have the potential for aberrant and epileptogenic circuits [26]. It has been demonstrated that IL-1β levels may be significantly different in the cerebrospinal fluid, blood, and central nervous system tissue of the same individual [27], emphasizing the relevance of studying these different biological compartments. In addition, we recognize that upstream and downstream regulatory targets of IL-1β should also be investigated in further studies, including in the pediatric population [28].

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