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The Correlation of Interleukin-1β and Interleukin-6 among Febrile Status Epilepticus Patients

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Abstract

Background: Febrile seizures (FS) are generally defined as seizures occurring in children typically 6 months to 5 years of age in association with a fever greater than 38°C (100.4°F), who do not have evidence of an intracranial cause (e.g., infection, head trauma, and epilepsy), another definable cause of seizure (e.g., electrolyte imbalance, hypoglycemia, drug use, or drug withdrawal), or a history of an afebrile seizure. Elevations in both proinflammatory and anti-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IL-10 in peripheral blood after FS in children with FS but also after an afebrile seizure (AFS) in children with epilepsy. Moreover, IL-1β was significantly increased and was correlated with other proinflammatory cytokines after FS attack compared to febrile children without a seizure attack, implicating the cytokine network in the generation of FS ***(Choi et al., 2011)***. IL-1β has also been found to be increased in the CSF and epileptogenic brain lesions of children with epilepsy, suggesting that this cytokine is important in epilepsy initiation and progression

Keywords: Interleukin-1β, Interleukin-6, Status Epilepticus

Introduction

Febrile seizures are seizures triggered by a fever of at least 100.4° F (about 38° C).

- Most febrile seizures are harmless and caused by fever from a minor infection.
- Less often, a febrile seizure is the first sign of a previously unrecognized neurologic disorder.
- Doctors sometimes do blood tests and a spinal tap to check for serious disorders that can cause seizures.
- Children generally need drugs to end the seizure only if it lasts 5 minutes or more to minimize the risk of a long-lasting seizure or status epilepticus.
- Most children do not need to take drugs to prevent febrile seizures. **(1)**

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Febrile seizures are the most common seizure disorder in childhood. Since early in the 20th century, people have debated about whether these children would benefit from daily anticonvulsant therapy. Epidemiologic studies have led to the division of febrile seizures into 3 groups, as follows: simple febrile seizures, complex febrile seizures, and symptomatic febrile seizures **(2).**

Pro-inflammatory cytokines as a key factor of host response to infection induct fever, leukocytosis, and acute phase protein synthesis. Among these cytokines, interleukin-6 is a cell signaling molecule that has been associated with many diseases, including inflammatory, neurological, vascular, and malignant processes Because of potential immune-modulatory effects of IL-6 and its importance as a major pro-inflammatory cytokine, IL-6 gene polymorphisms might affect individual susceptibility to febrile seizures **(3).**

It was found that the mean serum levels of IL-6 measured in Febrile patients were found significantly higher compared with the control group (P=0.024). These results coincide with the results of many researchers who found that levels of IL-6 were higher in patients with refractory epilepsy compared with healthy controls**(4).**

A previous study suggested that elevation in IL-6 is not fully explained by postictal increase but may reflect a chronic inflammatory system activation resulting from recurrent seizures, as IL-6 level was elevated in patients with frequently occurring seizures. This indicates that poorly controlled seizures may cause inflammatory system activation with potential neurotoxic effects. They also found that the levels of IL-6 were significantly elevated in patients with severe to profound intellectual disability but not in mild to moderate level disability. They also noted that the frequency of seizures is considerably higher in-patient groups with most severe intellectual disability.

A study found that most of the patients with increased levels of IL-6 had frequent seizures, and the chronic overproduction of cytokines may be because of an active epileptic process in the brain, a finding that is not found in the present study, as there was no significant relationship between the frequency of seizures and IL-6 level. They suggested that chronic stimulation of IL-6 may lead to structural changes that may predispose to refractory seizures **(5).**

It was found slightly increased IL-6 levels in patients with chronic epilepsy when compared with healthy controls. They suggested that cytokine levels might be disturbed in chronic epileptic state during the interictal period. However, an effect resulting from recurring seizures cannot be excluded in explaining these slight differences between patient basal levels and healthy controls **(6).**

It is postulated that only single seizures cause activation of cytokine cascade and associated inflammatory signals. In the case of recurrent seizures, these signals may result in structural changes in the nervous tissue with subsequent generation of drug refractory epilepsy.

it was found that the IL-6 level was significantly higher in patients with daily generalized motor seizures than in patients with intermittent seizures or the control group**(6).**

it was found that the mean serum level of IL-6 was found to be higher in patients with focal epilepsy complex partial seizures (CPS) compared with those with generalized tonic clonic fits and focal fits with secondary generalization, but the difference was not statistically significant (*P*=0.08). These results agreed with the results of many publications**(7).**

All patients with increased levels of IL-6 had TLE, which is known to be the most common epilepsy type refractory to drugs. Furthermore, concentrations of IL-6 were higher in patients with TLE compared with patients with extra-TLE. This may be because medial temporal lobe structures have been shown to be extremely vulnerable during global ischemia and excessive excitatory activity. The abundance of projections from the medial temporal lobe to other areas in the brain (including the hypothalamus) may explain the activation of cytokine cascade specifically in TLE. An older finding of increased secretion of prolactin in TLE may support this hypothesis **(5).**

IL-6 level is increased in patients with TLE without hippocampal sclerosis, and this increase is not found in patients with hippocampal sclerosis. This may reflect neuroprotective effect of IL-6 against development of hippocampal sclerosis **(6).**

it was found that the mean value of IL-6 level was higher in patients with abnormal EEG finding than the mean value of IL-6 level in patients with normal EEG finding; however, no statistically significant difference was found (*P*=0.34). Also, there was a higher level of IL-6 in patients with generalized epileptogenic dysfunction than patients with focal abnormality and patients with focal abnormality with secondary generalization; however, no statistically significant difference was found (*P*=0.15) **(5).**

It was found that there was no statistically significant association between history of status epilepticus or duration of fit and serum level of IL-6. it was showed that elevated IL-6 expression after pilocarpine-induced status epilepticus in the cells presumed to be glial and perivascular. there was no statistically significant association between family history of epilepsy and serum level of IL-6 **(7).**

Higher IL-6 level in patients with a family history of epilepsy. In the present study, there was no statistically significant association between duration of fit and serum level of IL-6 **(8).**

There is statistically significant positive correlation between IL-6 level and seizure duration. Moreover, they found that there is a correlation between levels of IL-6 and the severity of seizures. It might be because seizures cause changes in cerebral tissue microenvironment causing the glial cell release of cytokines. So, increased cytokine levels have been found to be higher after more sever seizures (longer duration)**(7).**

The activated glia and elevated cytokines contribute to seizure-related hippocampal pathology, such as neuronal death, reactive gliosis, and mossy fiber sprouting. Accordingly, there may be a postulated role for anti-inflammatory therapy targeting activated astrocytes and microglia as a novel therapeutic strategy to prevent or limit epileptogenesis and cell injury associated with seizures in the vulnerable developing nervous system **(8).**

It was found a significant positive association between febrile seizure and the interleukin-6 G allele at the −174 position (OR: 1.84; P <0.01) and the −174 GG or −597 GG genotypes (OR: 4.17, and 1.96; P <0.01; respectively), thus revealing that patients were more susceptible to febrile seizure. Furthermore, we detected a significant negative association between febrile seizure and the C allele at the −174 position (OR: 0.54; P <0.01) indicating that this association could represent a protective effect against febrile seizures. These results were concordant with those of Nur et al. who reported that the presence of the G allele or the GG genotype at −174 and the GG genotype at −572 positions of the interleukin-6 promoter regions constituted risk factors for developing febrile seizures in Turkish children. These findings support the hypothesis that a positive genotype predisposes individual to febrile seizure. However, we did not find a significant association between febrile seizure and the genotype or allele frequencies for the −572 position of the IL-6 promoter regions as they did **(9).**

IL6 is a pleiotropic cytokine secreted by a variety of cells such as T-lymphocytes, macrophages, endothelial cells and epithelial cells. The systemic concentration of IL6 is mainly regulated at the level of expression, as IL6 is rapidly cleared from the plasma with a short plasma half-life of 20–60 min **(10).**

As expected, it was found that mean systemic IL6 levels in patients with febrile seizure was markedly elevated compared to the control group. However, patients with complex febrile seizure had serum IL6 levels similar to those with simple febrile seizure. Many other studies reported the same findings, together with our results, may support the pro-convulsant action of IL-6 in febrile seizures. Virta et al. found that the plasma interleukin-6 levels and interleukin-1 receptor antagonist were significantly higher in patients with febrile seizures, compared with febrile control subjects and CSF IL-6 levels were detectable in all studied patients with FS **(11).**

Febrile seizures (FSs) are the most common convulsions in childhood. Studies have demonstrated a significant relationship between a history of prolonged FSs during early childhood and temporal sclerosis, which is responsible for intractable mesial temporal lobe epilepsy. It has been shown that interleukin-1β (IL-1β) is intrinsically involved in the febrile response in children and in the generation of FSs. We summarize the gene polymorphisms, changes of IL-1β levels and the putative role of IL-1β in the generation of FSs. IL-1β could play a role either in enhancing or in reducing neural excitability. If the enhancing and reducing effects are balanced, an FS does not occur. When the enhancing effect plays the leading role, an FS is generated. A mild imbalance can cause simple FSs while a severe imbalance can cause complex FSs and febrile status epilepticus. Therefore, anti-IL-1β therapy may help to treat FSs **(9).**

Some clinical studies over the years have attempted to treat FS by treating fever. For instance there have been some attempts to control FS with the use of antipyretics. The main outcome of these studies was that the use of antipyretics was not effective in preventing FS recurrence. Since antipyretics block the action of COX-2 and thus the production of PGE2. the results of these studies indicate that the genesis of FS may involve alternate pathways outside the traditional febrogenic pathway involving COX-2 and PGE2. Thus, the alternative was to examine the effect of cytokine induction and how this may factor into the production of seizure **(12).**

As stated in the introduction, there is evidence from clinical literature that IL-1β may play a role in the genesis of FS. To further explore this, we designed a series of experiments to determine: 1) whether levels of IL-1β and its naturally occurring antagonist IL-1ra are changed as a result of FS, 2) whether the onset of FS was associated with changes in IL-1β levels, and 3) if there was a causal link between changing levels of IL-1β and IL-1ra and the genesis of FS. First using our model, we measured the amount of IL-1β and IL-1ra in several brain regions (hypothalamus, hippocampus and cortex) 3 hrs after KA treatment (2 hrs after seizure onset, approximately 1 hr after seizure cessation) in animals treated with LPS and KA that had FS and those that did not have FS. Ultimately, we found that there were increased amounts of IL-1β found in the hypothalamus and the hippocampus but not the cortex of animals with FS compared to those without FS. However, this was not accompanied by any changes in the levels of IL-1ra within the same brain regions. This suggests that there may be an imbalance of the ratio of IL-1β:IL-1ra which may play a role in FS **(13).**

At this time point there were two likely sources of IL-1β: 1) the LPS induced production of IL-1β, and 2) seizure induced production of IL-1β. In order to determine if IL-1β was associated with the genesis of FS we used a separate group of animals and measured brain levels of IL-1β at the onset of FS. We found that there was a significant increase of

IL-1β in the hippocampus of animals with FS compared to those equally treated, which did not have FS. However, levels of IL-1β were unchanged in the hypothalamus and cortex. This would suggest that a significant increase in hippocampal IL-1β may be a factor in FS. Having established a clear association between altered levels of IL-1β and the presence of a FS we wanted to test for a causal relation between the IL-1β/IL-1ra system and FS. To do so we gave exogenous IL-1β and IL-1ra intracerebroventricularly to animals 30 min after KA treatment. What we found was that IL-1β dose dependently increased the number of animals that went on to develop FS, and that IL-1ra had the opposite effect. This established a more causal role of the IL-1β/IL-1ra system in the genesis of FS **(12).**

Another recent report has also found that IL-1β may be of significant importance in the pathogenesis of FS. A study conducted by Dubé *et al* using IL-1RI knockout mice, in a different model of FS, found that both the receptor and IL-1β played a role in the genesis of FS. They showed that animals that lacked IL-1RI had a greater resistance to FS. They also showed that exogenous IL-1β given to wild type control animals reduced their FS threshold. In addition, they were able to show that IL-1β alone was capable of producing seizures. Their results complement ours in clearly showing that the IL-1β/IL-1RI signalling system is of significant importance in the genesis of FS. Thus, the role of IL-1β in the initial seizure is substantial and it may have a further role in any sequelae that occur as a result of a complex FS or febrile SE **(14).**

The mechanism by which IL-1β influences FS may involve both excitatory (glutamatergic) and inhibitory (GABAergic) systems. In terms of excitatory neurotransmission, IL-1β has been shown to influence seizures through alterations in NMDA receptor phosphorylation. A study by Viviani *et al* showed that cultured hippocampal cells exposed to IL-1β had increased Ca^{+2} influx when exposed to NMDA. This study further showed that the increased influx of Ca^{+2} was due to phosphorylation of the NR2A/B subunit of the NMDA receptor, which was mediated by Src kinases. This alteration in Ca^{+2} permeability could lead to increased excitability and thus could be a putative means by which IL-1β can contribute to FS. In addition, there is also evidence that IL-1β can influence GABAergic inhibition. A study by Wang *et al* showed that IL-1β could decrease $GABA_A$ receptor mediated currents in cultured hippocampal neurons. The percent of $GABA_A$ receptor mediated current inhibition was also dose dependant and blocked by IL-1ra. In addition, there is also evidence that IL-1β can reduce synaptic inhibition in hippocampal CA3 pyramidal cells *in-vitro*. Taken in combination, the notion that IL-1β may concurrently increase excitation and reduce inhibition provides a possible mechanism by which it may promote the genesis of FS. Of course, IL-1β may have a

number of other actions that cause increased excitability, including interactions with peptidergic systems in the brain **(12).**

The role of the immune system can explain some aspects of FS pathogenesis. The immune system influences the nervous system via cytokines produced by macrophages and lymphocytes. Cartmell et al. demonstrated that interleukin receptors, e.g., IL-1, TNF, IL-6 are found in numerous brain sites, particularly in the hippocampus, already in the early stage of development. Hippocampal sites containing cytokine receptors are involved in temperature regulation. IL-1β directly modulates ion channels, enhancing NMDA and AMPA and reducing the effectiveness of GABAA, which can change the excitability of neurons and may promote FS generation. So in an integrative way, IL-1 β can both enhance and reduce neural excitability (neurotoxic and neuroprotective effects). If the enhancing and reducing effects are balanced, an FS does not occur. Moreover, a mild imbalance could cause a simple FS while a severe imbalance could cause complex FSs and febrile SE. In addition, the interaction of IL-1 β, IL-1ra and the other inflammatory cytokines is also of great significance in the generation of FS. Helminen and Vesikari were the first to show increased IL-1β production in peripheral blood mononuclear cells from FS patients after stimulation with lipopolysaccharide (LPS) **(14).**

Leukocytes from children with FSs have an exaggerated IL-1 β response to stimulation with double- stranded RNA. Another study showed that the IL-1ra/IL-1β ratio is significantly higher in FS patients than control children. Since the production of ILra is stimulated by IL-1 β, the IL-ra increase is much later than that of IL-1 β. An association between polymorphism of the IL-1β (-511) promoter and susceptibility to febrile seizures has been reported. Virta et al. have demonstrated a significant allelic association between the IL-1β (-511) allele 2 d febrile seizures **(12).**

In addition, Kanemoto et al. have reported a significant association between theIL-1β (-511) allele 2 and prolonged febrile seizures. It was there were highly significant increase of serum levels of IL-1β and IL-1Ra within 24 hours from convulsion in the children with febrile convulsions than in febrile children without seizures **(14).**

Inflammatory cytokines especially IL1-β plays an important role in the genesis of FSs which may cause subsequent epilepsy. Reducing the excitability effect of IL1β and the level of IL-6, may have an antagonistic effect on the FS and a novel therapeutic strategy to prevent or limit febrile seizures effect on the vulnerable immature brain of children **(12).**

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