A Prospective Study of Novel Therapeutic Targets Interleukin 6, Tumor Necrosis Factor α, and Interferon γ as Predictive Biomarkers for the Development of Posttraumatic Epilepsy

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■ BACKGROUND: **Posttraumatic epilepsy (PTE) is a serious and debilitating consequence of traumatic brain injury (TBI). Sometimes, the management of PTE becomes a challenging task on account of its resistance to existing antiepileptic drugs and often contributes to poor functional and psychosocial outcomes after TBI. We investigated the role of inflammatory markers interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and interferon γ (INF-γ) in predicting the development of PTE.**

■ METHODS: **A prospective analysis was performed of 254 patients who were admitted with head injury to our hospital, 35 of whom had posttraumatic epilepsy (32 males and 3 females); 30 adults (28 men, 2 women) with a similar demographic profile were selected randomly as control individuals. Blood levels of TNF-α, IL-6, and INF-γ were evaluated in all participants.**

■ RESULTS: **IL-6 levels were significantly higher in the PTE group (121.36 pg/mL; standard deviation [SD], 89.23) than in the nonseizure group (65.30 pg/mL; SD, 74.75; $P = 0.01$), whereas there was no significant difference between the seizure group (11.42 pg/mL; SD, 7.84) and the nonseizure groups (10.58 pg/mL; SD, 7.84) in terms of TNF-α level ($P = 0.343$). The level of INF-γ in the seizure group tended to be higher (mean, 1.88 pg/mL, SD, 2.13 in seizure group vs. 1.10 pg/mL, SD, 1.45 in the nonseizure group); however, no statistically significant difference was detected among the 2 groups ($P = 0.09$).**

■ CONCULSIONS: **Posttraumatic epilepsy has a strong association with an increased level of IL-6 in the blood. INF-γ may or may not be associated with PTE. However, TNF-α was not associated with PTE.**

INTRODUCTION

Posttraumatic seizures (PTS) are a consequence of physical trauma to the brain. They result from primary insult to neuronal tissue. In 5%–7% of people hospitalized with traumatic brain injury (TBI), ≥1 episode of seizure is reported.[1] Based on time duration between primary head injury and first episode of seizure, PTS is divided into 3 groups: immediate seizures (<24 hours after injury), early seizures (<7 days after injury), and late seizures (8 days after injury).[2]

Although these are the most widely accepted definitions, a few studies[3,4] have narrowed the definition of immediate seizures to those occurring within minutes after injury and late seizures to as late as 1 month after injury. Immediate and early seizures occurring in the first week after TBI are considered to be provoked by head injury. Over a period, cascade of morphologic and biological changes in the injured area leads to hyperexcitability and epileptogenesis. Late unprovoked seizures

Key words
- Cytokines
- Epileptogenesis
- Immunomodulators
- Neuroplasticity
- Seizures

Abbreviations and Acronyms
CI: Confidence interval
CNS: Central nervous system
CSF: Cerebrospinal fluid
GCS: Glasgow Coma Scale
IL-6: Interleukin 6
INF-γ: Interferon γ
NMDA: *N*-methyl-D-aspartate
PTE: Posttraumatic epilepsy
PTS: Posttraumatic seizures
ROC: Receiver operating characteristic
TBI: Traumatic brain injury
TNF-α: Tumor necrosis factor α

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Citation: World Neurosurg. X (2021) 12:100107. https://doi.org/10.1016/j.wnsx.2021.100107

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery-x***

Available online: ***www.sciencedirect.com***

then occur after a variable latency period.[5] Because the risk of recurrence of seizures after a single late PTS is >70%, most consider a single episode sufficient for the diagnosis of posttraumatic epilepsy PTE.[6]

Those seizures that occur spontaneously and are unprovoked ≥1 week after TBI are classified as PTE.[7]

Risk of PTE is high in patients with severe head injury and certain types of injuries increase the risk further.[8] Over time, the risk of PTE decreases gradually, but even 15 years after injury, patients may be at risk of developing PTE.[9]

Thus, PTS may be a risk factor for PTE but every patient with seizure does not develop PTE.

PTE accounts for 20% of symptomatic epilepsy in the general population.[10,11] It constitutes about 5% of all cases of epilepsy and about 20% of acquired cases.[12]

In patients with PTE, the first episode of seizure generally occurs within the first year of injury in approximately 80% of individuals and in >90% by the end of the second year.[13]

The efficacy of available antiepileptic medications in preventing PTE is a matter of debate.[14,15] About 33% of patients developing PTE are resistant to antiepileptic drugs.[16,17] In addition to this finding, the complete pathophysiology of PTE is still under investigation,[18] and hence, the individual risk and prognosis cannot be assessed accurately. There is a need to develop novel therapeutic targets and treatment strategies, based on molecular mechanisms of underlying epileptogenesis after TBI.

There is increasing evidence showing the causal relationship of the immune system with seizures.[19] Both animal models as well as patients with epilepsy have shown the abnormalities in cytokine expression and immune system activation, indicating the possible role of the inflammatory process in pathogenesis of epilepsy. After injury, glial cells are activated and various cytokines produced by them play a major role in epileptogenesis.[20] These cytokines, in turn, through a different mechanism induce changes in neuronal cell membrane, altering their function and connectivity, leading to regional hyperexcitability and subsequent seizure susceptibility.[21,22]

A similar glial cell and cytokine response is also observed after TBI.

Semple et al.[23] in a controlled cortical impact injury model in mice showed that IL-1 contributes to increased evoked seizure susceptibility and that blocking the activity of IL-1 receptors after TBI has potential antiepileptogenic effects but could not prevent the development of PTE. This situation could be attributed to different immunopathologic mechanisms responsible for evoked and spontaneous seizure susceptibility.

Secondary injury to the brain is characterized by a robust immune response, neuronal cell death, and oxidative stress, as well as augmented neurogenesis and neuroplasticity.[24] Various pathologic, cytologic, and biochemical changes occur at an injury site, such as disruption of the blood-brain barrier, edema, infiltration of peripheral leukocytes and lymphocytes, enhanced glial reactivity, and release of proinflammatory and antiinflammatory immunomodulators. All these factors together augment excitatory synaptic activity and also reduce inhibitory synaptic activity, thereby altering seizure susceptibility and paving the way for epileptogenesis.[18,25]

We conducted a prospective analysis of a case series of patients who had PTE assessing the co-relation between cytokines levels in the blood (interleukin 6 [IL-6], tumor necrosis factor α [TNF-α], and interferon γ [INF-γ]) after TBI and development of PTE.

OBJECTIVE

1) To study the age and sex distribution of PTE in our patient population.
2) To analyze the results of cytokine (IL-6, TNF-α, INF-γ) levels in blood samples from our study population in terms of
   a) Serum levels at the time of injury and their association with the development of PTE
   b) Relative sensitivity and specificity of these cytokine markers and their role as a proseizure or seizure-protective mediator.

METHODS

From July 2017 to October 2018, 254 patients with head injury were admitted to our hospital. Our study group consisted of only Indian patients who were 18–75 years old and had moderate to severe, closed head TBI (Glasgow Coma Scale [GCS] score ≤12), positive computed tomography confirming intracranial injury (TBI), and no history of premorbid seizures. Five patients had a GCS score >12, but were included in the moderate TBI category based on positive computed tomography findings. Of 41 patients who had PTE, 6 had premorbid seizures and were excluded from our study group, with 35 patients included in our study with PTE (32 men and 3 women). 30 adults (28 men and 2 women) as control individuals were enrolled. The control group consisted of patients with head injury without PTE, fever, acute illness, or any other immune-mediated disorders.

Patients with PTE were diagnosed based on clinical examination and electroencephalography coupled with neuroimaging findings. There were no statistically significant differences in terms of age, gender, or body mass index between the 2 groups. No patient in the PTE group or control group had undergone any immune-related treatments, such as adrenocorticotropic hormone, glucocorticoid, or immunoglobulin therapies, for at least 6 months before blood sampling. Both the study and control group were on antiepileptic medications. Institutional ethical committee (ethics committee, Postgraduate Institute of Medical Education and Research Dr. Ram Manohar Lohia Hospital, New Delhi, India; registration number ECR/78/Inst/DL/2013/RR-19 issued under New Drugs and Clinical Trials Rules, 2019) approval was obtained and all research was performed in accordance with relevant guidelines issued by Postgraduate Institute of Medical Education and Research Dr. Ram Manohar Lohia Hospital, New Delhi. Informed consent was obtained from all participants and/or their legal guardians. The sample was collected on the day of admission and was subjected to cytokine measurement if the patient developed PTE.

Inclusion Criteria

1) All patients >18 years of age with PTE

2) Patients with closed-type TBI only
3) Patients not on any immune-mediated treatment or immunoglobulin therapies
4) Patients with no history of previous epilepsy or seizures.

Exclusion Criteria

1) Patients with polytrauma with other associated injuries such as fractures and liver injuries
2) Patient with other comorbidities such as liver disease and malignancies
3) Patients with ongoing fever or history of fever in recent past
4) Patients with penetrating head injuries or having preexisting cranial diseases such as primary tumors, brain abscesses, or any other intracranial space-occupying lesions.

DETERMINATION OF SERUM TNF-α, IL-6, AND INF-γ

To analyze the serum cytokine level, about 10 mL of the peripheral venous blood sample was taken from patients on admission and centrifuged at (1000*g*, 10 minutes). Serum obtained after centrifugation was then isolated and stored at −80°C until assay. The samples were subjected to cytokine assessment if the patient developed PTE. Human cytokines (TNF-α, IL-6, and INF-γ) were assayed using Invitrogen (Diaclone, Besançon, France) kits (catalog number 950.030.096, 950.000.096, and 950.090.096 for IL6, INF-γ, and TNF-α, respectively). A PW 40 enzyme-linked immunosorbent assay reader (Bio-Rad, Watford, United Kingdom) was used according to the manufacturer protocol.

Capture antibody,[26] which is highly specific for respective cytokine, is coated to the wells of the microtiter strip plate. Samples in which cytokines have to be measured together with standards to the capture antibodies and biotinylated anticytokine secondary antibody to the analyte is pipetted into the wells during the same incubation period. Cytokine present in the sample is captured by the antibodies immobilized to the well and by the biotinylated antibody. Any excess unbound analyte and secondary antibody are removed. The horseradish peroxidase conjugate solution is then added to the wells and again incubated, followed by removal of excess conjugate by careful washing. After this second wash, a chromogen substrate is then pippeted to the wells, resulting in the progressive development of a blue complex proportional to the amount of bound cytokine. The color development is then stopped by the addition of acid, turning the resultant final product yellow. Intensity of the color produced by the complex is directly proportional to the concentration of cytokine present in the samples and standards. The intensity of the color complex is then measured and the generated optical density values for each standard are plotted against expected concentration, forming a standard curve. This standard curve is then used to accurately determine the concentration of respective cytokine in the samples tested.

RESULTS AND STATISTICAL ANALYSIS

Population Description

A total of 254 adult patients with TBI were admitted to our institute during the study period. Of these patients, 41 had late PTE (16.14%). Six patients had a history of seizure before the injury, so they were excluded from the study group.

Among these 35 patients with PTE, 32 were men and 3 were women. Thirty patients with similar injury and demographic pattern including age and sex distribution were taken as controls (Table 1). The youngest patient was 18 years of age and the oldest was 75 years of age. The most common mechanisms of injury included automobile accidents (59.04%) and falls (19.68%).

The incidence of PTE in our patient population was 14.1%. Of the total male patients admitted during the study period, 18.5% developed PTE, whereas the incidence of PTE among the female population was 8.0% ($P < 0.05$). Of 41 patients who developed PTE, 20 (48.78%) had a history of alcohol abuse, whereas 68 of the remaining 213 patients with head injury (31.9%) were alcoholics ($P < 0.05$). The incidence of PTE was greater in patients with severe head injury (GCS score <8) (51.4%), whereas in others in whom PTE did not occur, only 28.16% had GCS score <8 ($P < 0.05$).

Statistical Analysis

Statistical analyses were performed using the Mann-Whitney U test. A P value <0.05 was considered to indicate statistical significance. After having removed the outliers, the area under the receiver operating characteristic (ROC) curve and 95% confidence interval (CI) were determined.

IL-6

IL-6 levels were significantly higher in the seizure group (mean, 121.36 pg/mL; SD, 89.23) than in the nonseizure group (mean, 65.30 pg/mL; SD, 74.75; $P = 0.01$) (Figure 1, Table 2).

TNF-α

The mean TNF-α level was 11.42 pg/mL (SD, 7.84) in the seizure group and 10.58 pg/mL (SD, 9.50) in the nonseizure group (Figure 2, Table 2). No statistically significant difference was detected between the 3 groups ($P = 0.33$).

INF-γ

Although the level of INF-γ in the seizure group tended to be higher (mean, 1.88 pg/mL, SD, 2.13 in seizure vs. 1.10 pg/mL, SD, 1.45 in the nonseizure group) (Figure 3, Table 2), no statistically significant difference was detected between the 2 groups ($P = 0.09$).

The median value of IL-6, TNF-α, and INF-γ in the seizure group was 98.70, 9.52, 0.89 pg/mL, respectively, whereas in the nonseizure group, it was 30.02, 7.43, and 0.60 pg/mL, respectively (Table 3).

ROC Analysis of IL-6

For IL-6, the graph was above the 45° line and covers 69.6% (95% CI, 55.6–81.7) of the area under the ROC curve (Table 4, Figure 4), indicating that IL-6 is a reliable biomarker for PTE.

IL-6 levels ≥31 pg/mL could identify 80% of the patient population with seizures (sensitivity) and had a specificity of 50% (Tables 5 and 6).

Table 1. Demography

	PTE Group (N = 35), n (%)	Non-PTE Group (N = 30), n (%)
Age		
18–60 years	27 (77.1)	23 (76.6)
>60 years	8 (22.8)	7 (23.3)
Sex		
Male	32 (91.4)	28 (93.3)
Female	3 (8.5)	2 (6.6)
Glasgow Coma Scale Score		
≤8	5 (14.2)	4 (13.3)
9–12	25 (71.4)	22 (73.3)
13–15	5 (14.2)	4 (13.3)
Injury mechanism		
Motor vehicle	26 (74.2)	22 (73.33)
Fall	4 (11.4)	3 (10.0)
Other	5 (14.2)	5 (16.6)
Subdural hematoma	10 (28.5)	9 (30.0)
Contusions		
Single	13 (37.1)	11 (36.6)
Multiple		
With/without haematoma	7 (20.0)	6 (20.0)
Depressed skull fracture	3 (8.5)	2 (6.6)
Diffuse cerebral edema with subarachnoid hemorrhage	2 (5.7)	2 (6.6)
Antiepileptic drug treatment	35 (100)	30 (100)
Operative intervention	20 (57.1)	16 (53.3)

PTE, posttraumatic epilepsy.

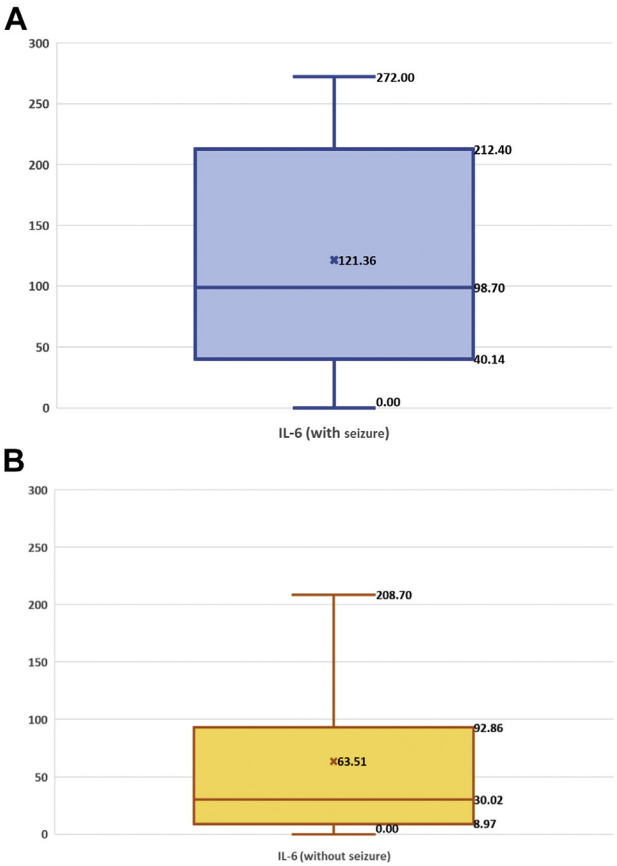

Figure 1. Levels of inflammatory marker interleukin 6 (IL-6) in (**A**) seizure group and (**B**) nonseizure groups. The center lines indicate medians, X indicates mean, boxes indicate 25th–75th percentiles, and whiskers indicate the minimum and maximum. Values are in pg/mL.

Of 43 patients including case and controls who had IL-6 levels in serum >31 pg/mL, 28 actually had seizure. The positive predictive value of IL-6 was 65.1% (95% CI, 49.1–79.0).

Thus, IL-6 level showed significant association with seizures, levels >31 pg/mL had a sensitivity of 80%, and the positive predictive value was 65.1% (95% CI, 49.1–79.0).

TNF-α showed no difference in blood levels in the seizure and nonseizure groups nor was there any statistically significant difference between the 2 groups ($P = 0.343$).

However, in cases of INF-γ, the mean rank was higher in the seizure group but this finding was not statistically significant.

DISCUSSION

Inflammation Contributing to Seizures

The correlation between neuroinflammation and seizure activity is supported by clinical evidence of immune activation occurring in the epileptic foci of brain tissue in patients with epilepsy. After seizures, the glial cells that are present in the vicinity of epileptic foci start producing inflammatory mediators, which promote an inflammatory immune cascade. More recently, it is has become evident that neuroinflammation may also be causal to seizures and epilepsy.

Lloyd et al.[27] showed the directly proportional role of glial cell activation and subsequent production of cytokines with

Table 2. Mean and Standard Deviation of All the Three Mediators in Both the Groups

Variables			N	Mean	Standard Deviation	Standard Error of the Mean
Interleukin 6	Seizure	Yes	35	121.3638	89.23455	15.08339
		No	30	65.3020	74.75392	13.64814
Interferon γ	Seizure	Yes	35	1.8816	2.13890	.36154
		No	30	1.1036	1.45380	.26543
Tumor necrosis factor α	Seizure	Yes	35	11.4237	7.84741	1.32645
		No	30	10.5086	9.50249	1.73491

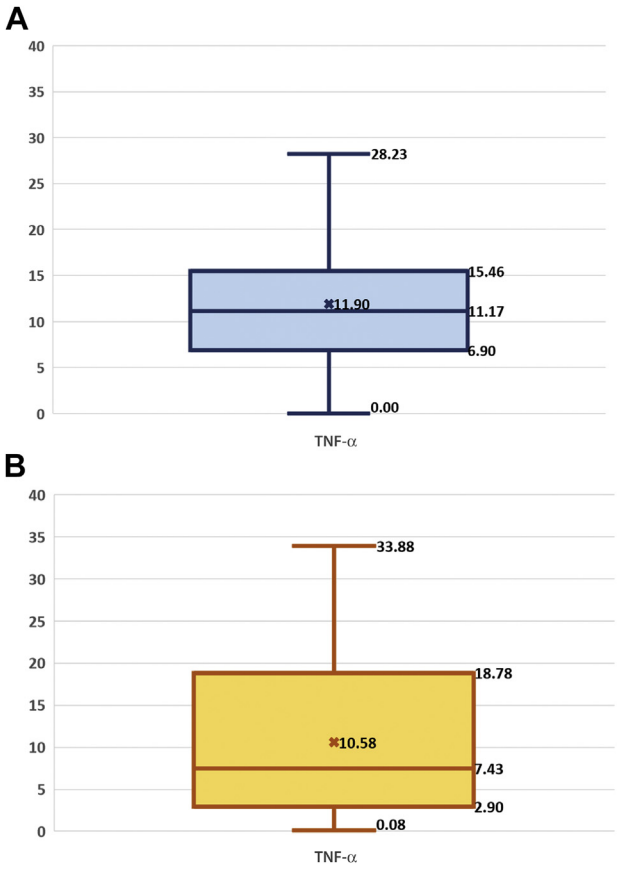

Figure 2. Levels of inflammatory marker tumor necrosis factor α (TNF-α) in (**A**) seizure group and (**B**) nonseizure groups. The center lines indicate medians, X indicates mean, boxes indicate 25th—75th percentiles, and whiskers indicate the minimum and maximum. Values are in pg/mL.

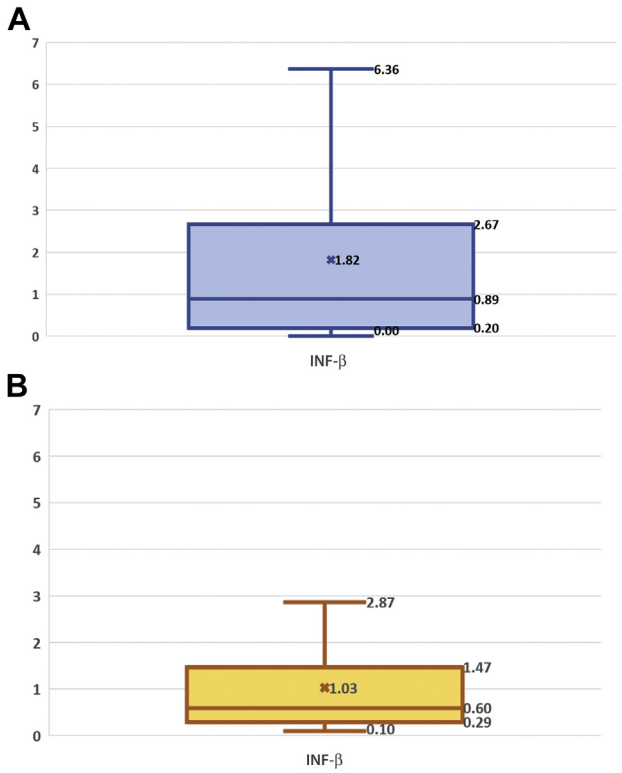

Figure 3. Levels of inflammatory marker interferon γ (IFN- γ) in (**A**) seizure group and (**B**) nonseizure groups. The center lines indicate medians, X indicates mean, boxes indicate 25th—75th percentiles, and whiskers indicate the minimum and maximum. Values are in pg/mL.

epileptogenic events. In their experimental study, minocycline, a tetracycline antibiotic known to inhibit brain infiltration of monocytes and microglia, when applied increases the threshold of electroconvulsive shock.

Frugier et al.[28] performed a postmortem study of brain samples of 21 patients who died as a result of head injuries. In this study, these investigators measured and analyzed the expression of IL-6, TNF-α, and IL-1β messenger RNA levels as an inflammatory response after trauma to the brain and concluded that their levels were significantly increased after injury. The increase in messenger RNAs of these inflammatory mediators was proportionately higher to their respective levels, suggesting an immediate cerebral inflammatory response after brain injury.

An experimental study in mice with closed head injury was performed by Stahel et al.,[29] who found that recruitment of inflammatory cells, disruption of the blood-brain barrier, and neuronal death were higher in mice lacking the genes for tumor necrosis factor (TNF)/lymphotoxin α and IL-6.

The mechanism by which these cytokines causes seizures is not fully understood. The probable causes may be

1) By phosphorylation of receptors present at the neuronal cell membrane, causing receptor modulation, hence themselves acting as neurotransmitters[30]
2) These mediators might damage the GABAergic neurons in the hippocampus, leading to a decrease in synaptic inhibition and hence an increased propensity for seizures[31]

Table 3. Median Values, 25th Percentile, and 75th Percentile of All the Three Mediators in Both the Groups

Variables			N	Median	Percentile 25 (Q1)	Percentile 75 (Q3)
Interleukin 6	Seizure	Yes	35	98.70	40.14	212.40
		No	30	30.02	9.22	92.86
Interferon γ	Seizure	Yes	35	.89	.07	2.69
		No	30	.60	00	1.45
Tumor necrosis factor α	Seizure	Yes	35	9.52	6.39	14.57
		No	30	7.43	2.90	18.72

Q, quartile.

Table 4. Area Under the Curve Using Receiver Operating Characteristic for the Parameters

Parameter	AUC (%)	95% Confidence Interval for AUC	*P* Value
Interleukin 6	68.6	55.6–81.7	0.005*
Interferon γ	62.5	48.9–76.1	0.072
Tumor necrosis factor α	57.1	42.3–71.8	0.348

AUC, area under the curve.
*Significant.

3) These cytokines inhibit the uptake of excitatory neurotransmitter such as glutamate by astrocytes, modulating excitatory neurotransmission in the brain
4) By activation of N-methyl-D-aspartate (NMDA) receptors, which increase in the intracellular calcium. This calcium in turn releases the glutamate, leading to extracellular hyperexcitability and excitotoxicity.

We studied 3 inflammatory mediators (IL-6, TNF-α, and INF-γ) to find their possible role as mediators in PTE. Finding inflammatory markers and targeting antiepileptic therapy against them may have the potential benefit of reducing the incidence, and improving the psychosocial aspect associated with PTE.

IL-6

IL-6 is usually not detectable in the healthy central nervous system (CNS); however, it increases remarkably in cerebrospinal fluid (CSF) after TBI. Various cells are present inside the brain, such as astrocytes and microglia, which express IL-6 in response to injury.[32–35] Various studies have shown that the increased serum level of IL-6 is associated with a wide range of epileptic conditions.[36] IL-6 promotes inflammation by increasing the secretion of various chemokines and adhesion molecules, which in turn causes leukocyte recruitment.[37] It binds with the IL-6 receptor complex consisting of IL-6 receptor and 2 molecules of gp130 on target cells.[38] Samland et al.[39] in their experimental study of transgenic mice found increased production of IL-6 by astrocytes, causing a remarkable increase in sensitivity to glutamatergic agonist–induced seizures. Also, transgenic mice overexpressing IL-6 show spontaneous tonic-clonic seizures.[40]

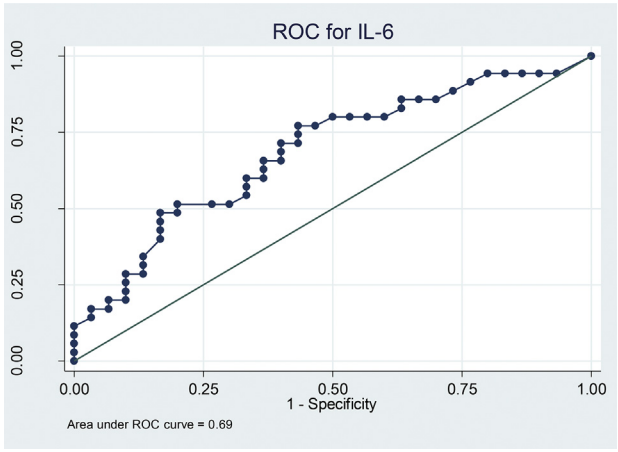

Figure 4. Receiver operating characteristic (ROC) curve for interleukin 6.

Table 5. Diagnostic Accuracy of Interleukin 6 in Predicting Seizure

	Gold Standard (Seizure)		
Interleukin 6	Yes	No	Total
≥31	28	15	43
<31	7	15	22
Total	35	30	65

Cutoff of 31 for interleukin 6 derived from receiver operating characteristic.

However, during recent years, there have been conflicting reports regarding the role of IL-6 in seizure activity and neuroinflammation. It has been reported that IL-6 reduces NMDA-mediated neurotoxicity[41] and promotes neuronal differentiation and survival. Intranasal administration of IL-6 shortened the duration of hyperthermia–induced seizures in developing rats, whereas it had an antagonist effect on adult rats, exacerbating the severity of seizures.[42]

Thus, increased level of IL-6 in plasma and CSF might be associated with increased epileptogenicity, although it is difficult to have conclusive evidence about the expression and function of IL-6 in epileptogenic brain areas.

McClain et al.[43] in 1991 and Kossmann et al.[44] in 1995 reported increased levels of IL-6 in ventricular CSF as well as in plasma for days after TBI. IL-6 was also found to be increased in plasma in temporal lobe epilepsy but not in extratemporal lobe epilepsy during the interictal stage.[45] Chaudhary et al.[46] in their study of patients with subarachnoid hemorrhage concluded that IL-6 had a potential role in epileptogenesis, in which IL-6 was significantly increased in patients who developed seizures.

A meta-analysis of 66 studies including 1934 patients was performed by de Vries et al.,[36] who concluded that among different cytokines IL-1ra, IL-1β, IL-6, IL-10, IFN-γ, and TNF-α were the most extensively investigated proteins for epilepsy and IL-6, IL-17, and CSF IL-1β were found to be increased in human epilepsy.

Table 6. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of Interleukin 6

Parameter	Point Estimate	95% Confidence Interval
Sensitivity	80.0	63.1–91.6
Specificity	50.0	31.3–68.7
Positive predictive value	65.1	49.1–79.0
Negative predictive value	68.2	45.1–86.1

Ishikawa et al.[47] in their studies of 29 children with epilepsy found that IL-6 level was significantly higher in children with daily seizures then in either the intermediate seizure group or the control group. There were no significant differences in plasma pentraxin (PTX3), TNF-α, or IL-1β levels.

In our study also, an increased IL-6 level showed a statistically significant association with PTE and can provide a potential target for antiepileptic medications, preventing incidence of PTE.

TNF-α

TNF-α has a dichotomous role in neuroinflammation. It binds with 2 different receptors in a dose-dependent manner (TNF-R1 [also known as p55] and TNF-R2 [also known as p75]) and produces its effect via the ligand receptor pathway. P55 initiates apoptosis,[48] whereas P75 mediates cell proliferation.[49] Many studies have been reported to support the protective actions of TNF-α by its ability to modulate neurotrophin production and prevent influx of calcium, causing neuronal death. Moreover, TNF-α has been shown to protect cultured neurons against an excitotoxic death induced by the glutamate receptor agonist NMDA.[50] Ballasso et al.[51] in their study of mice found the protective effect of TNF-α to cause a decrease in seizure frequency, and this action was mediated by neuronal p75 receptors. On the contrary, as reported by Probert et al.[52] in their study on transgenic mice, TNF-α has a negative effect in the CNS, causing abnormalities in the structure and function of the nervous system.

Thus, different receptors are responsible for this dual role of TNF-α. Seizure activity is mediated by the p55 pathway, whereas anticonvulsive activity is through the p75 pathway. However, there is no clear-cut evidence to determine the predominance of one pathway over the other. Some studies have indicated that TNF-α acts in a concentration-dependent manner. At low concentration, it had a proconvulsive effect in *Shigella*-mediated seizures; however, it was anticonvulsive at higher concentrations.[53] Grell et al.[54] showed that in vitro picomolar concentration of TNF-α could trigger p55 pathway, whereas activation of p75 required relatively higher concentrations.

After TBI, an increase in TNF levels has been reported in the CSF and serum of patients by Goodman et al.[55] in 1990 and Ross et al.[56] in 1994.

TNF-α has been observed to increase within minutes of TBI in postmortem brain tissue, thus proving its role in cerebral inflammatory cascade, which is initiated acutely after severe neurotrauma.[28]

Stein et al.[57] in their study of 24 patients showed the relationship of serum and cerebrospinal fluid TNF-α with intracranial hypertension and cerebral hypoperfusion after severe TBI. These investigators concluded that high serum TNF-α levels but not CSF levels were associated with increased intracranial pressure and decreased cerebral perfusion pressure.

Thus, as in the studies discussed earlier, in our study also, TNF-α levels were increased in serum samples of both cases and controls but there was no statistically significant association with PTE. However, in animal studies, it has been found to have proconvulsive or anticonvulsive effects in a concentration-dependent manner, with higher concentrations resulting in suppression of frequency and severity of seizures and low concentrations having a proconvulsive effect.

INF-γ

Ryu et al.[58] in their experimental study of status epileptics—induced neuronal damage of the rat hippocampus concluded that infusions of recombinant rat IL-18 or IFN-γ in the ventricles of the brain have a protective effect on hippocampal neurons, whereas neutralization of IL-18, IFN-γ, or their receptors had a contrary effect compared with control specimens. These findings thus suggest that the astroglial-mediated INF-γ pathway activated by IL-18 may play a vital role in reducing neuronal injury.

Li et al.[59] in their IL-4/IFN-γ-pilocarpine—induced epilepsy model in mice showed that intraperitoneal injection of IL-4 and INF-γ, 5 hours before pilocarpine injection treatment, induced M2 microglial polarization and suppressed frequency and severity of spontaneous recurrent seizures. Treated mice also showed improved cognitive performance.

However, in 2007, Getts et al.[60] conducted an experimental study of mice and found that intranasal inoculation with West Nile virus causes limbic seizures in C57BL/6 mice, but not in IFN γ—deficient mice. Thus, in their study, these investigators had a conflicting result, in which INF-γ was proconvulsive, causing limbic seizures, and absence of IUFN-γ during the development of the CNS may cause intracerebral alterations, resulting in an enhanced convulsive effect.

Salim et al.[61] concluded that there is significant co-relation between INF-γ and IL-10 levels and status epilepticus in children.

In our study, there was no statistically significant association of INF-γ levels with PTE, although the mean level of INF-γ was higher in the seizure group. This finding could possibly be attributed to a limited sample size, requiring more extensive study to find its proconvulsive or anticonvulsive effect.

These inflammatory cytokines because of their early and varied role in neuroinflammation and probable role in regulating neuronal excitability, pharmacologic targeting of these cytokines may prove beneficial in reducing the incidence of PTE. In the past decade, various animal models have been studied to find out the relationship of inflammatory mediators and their role in PTE. However, the effect of immune-targeting pharmaceuticals has been minimally explored in patients with PTE. IL-6 and TNF-α may act as potential novel antiepileptic therapeutic targets. Monoclonal anti-TNF antibody such as adalimumab caused reduced seizures in some patients with Rasmussen encephalitis.[62]

Various antiinflammatory nonsteroidal drugs, steroids, cannabinoids, or a ketogenic diet probably have an antiepileptic effect because of their antiinflammatory properties[63]; however, their definite role is yet to be established because of a lack of systematic controlled studies. Drugs such as minocycline, erythropoietin, and progesterone have been investigated as therapeutic options for neuroinflammatory intervention after TBI. They have produced promising results in preclinical trials but have failed to produce an impact in clinical trials.

However, the published data stands as the foundation for future prospective study for elucidating biomarkers for PTE; we are still far from reaching the exponential phase in the development curve. Thus, further exploration using standardized study designs with validation cohorts, and by developing and applying novel analytic methods, is needed for PTE biomarker discovery.

LIMITATIONS

1) Sample size is limited to give conclusive evidence about the possible role of inflammatory mediators in PTE.
2) Our patients with PTE had a follow-up period of 1 year, which encompasses only 80% of patients, so is not representative of the complete PTE group.
3) Although utmost care has been taken to eliminate the factors likely to influence serum levels of inflammatory mediators, the possible impact of subclinical injuries cannot be ruled out completely.
4) Subclinical or nonconvulsive seizures occurring in patients beyond the first week of injury who were intubated and sedated may have been missed.

CONCLUSIONS

1) IL-6 has a strong association with PTE and higher levels are associated with increase seizure susceptibility.
2) INF-γ levels may be associated with PTE and more extensive study is required to prove the possible role of INF-γ in PTE.
3) TNF-α levels fail to show any association with either proseizure or antiseizure activity, although TNF-α has a dose-dependent effect in animal models.

DECLARATION OF COMPETING INTEREST

Conflict of interest statement: The authors declare that the article content was composed in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Ajay Choudhary: Conceptualization, Project administration. **Rahul Varshney:** Writing - original draft, Methodology, Validation, Writing - review & editing. **Ashok Kumar:** Data curation, Investigation. **Kaviraj Kaushik:** Resources, Formal analysis.

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Received 18 February 2021; accepted 24 May 2021

Citation: World Neurosurg. X (2021) 12:100107.
https://doi.org/10.1016/j.wnsx.2021.100107

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery-x***

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