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Investigation of Correlation between Resistance to Diazepam and Expression of Inflammatory Markers in The Peripheral Blood of Patients with Status Epilepticus

Corry Novita Mahama[1*], Melva Louisa[2], Fitri Octaviana[3], Dwi Anita Suryandari[4], Astri Budikayanti[3], Heri Wibowo[5]

[1]Doctoral Program in Biomedical Sciences, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [2]Department of Pharmacology and Therapeutics, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [3]Department of Neurology, Cipto Mangunkusumo Central Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [4]Department of Medical Biology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia, [5]Department of Parasitology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia

Correspondence: *corry.mahama@gmail.com*; Tel.: + 62 431 7285741

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Abstract

Objective. This study investigated several inflammatory markers' gene and protein expression in status epilepticus (SE) and their correlation with diazepam resistance. **Materials and Methods.** Peripheral blood samples were collected from 18 adult patients with SE in Cipto Mangunkusumo Central Hospital, consisting of 12 diazepam-responsive and six diazepam-resistant samples, within 72 hours of the onset of the seizure. We collected baseline demographic and clinical data from each subject. Peripheral blood mononuclear cells (PBMCs) were isolated, cultured, stimulated with lipopolysaccharide (LPS) 1 mg/ml, and harvested for RNA isolation. The RNA was used to determine the expression of Human Mobility Group Box 1 (HMGB1), Interleukin-6 (IL-6), IL-10, Toll-like Receptor 4 (TLR4), and Glial fibrillary acidic protein (GFAP). In addition, we performed serum protein assay of HMGB1, IL-6, IL-10, TLR4, and GFAP to compare with gene expression. **Results.** We found a significant difference between the responsive and resistant groups for serum HMGB1 and IL-6 concentration. The mRNA expression of HMGB1 and IL-6 was significantly higher in LPS-stimulated samples in the responsive but not in the resistant groups. The ratio of IL-6 to IL-10 showed a significant difference between LPS and control in the responsive group. Diazepam response was significantly correlated with seizure duration and serum protein concentration of HMGB1. **Conclusion.** HMGB1 was highly expressed in the resistant group and strongly correlated with diazepam response, and there was a significant increase in HMGB1 mRNA expression in response to LPS stimulation. These findings suggest that targeting HMGB1 may be a promising therapeutic strategy and that HMGB1 levels could be a valuable biomarker for predicting diazepam resistance in SE.

Key Words: Diazepam Resistance ▪ HMGB1 ▪ GFAP ▪ Interleukin ▪ Status Epilepticus ▪ TLR4.

Introduction

Status epilepticus (SE) is a seizure that lasts more than 5 minutes or a series of seizures without recovery to baseline (1). SE is a life-threatening neurological and medical emergency that can lead to long-term consequences such as neuronal injury, epilepsy, drug resistance, and death (1-3). Worldwide, the incidence of SE ranges from 8 to 41 per 100,000 people per year (3-5), with mortality reaching up to 30% in adults and 39% in refractory SE (6). Current treatment protocols for SE suggest a timely progression of treatment, with the conceptual definition of SE by the ILAE task force of 5 minutes that indicates the time to start the SE emergency treatment with benzodiazepines (BZD) (e.g., diazepam) as the preferred initial treatment (7, 8). Studies have shown that 30-40% of cases of status epilepticus are not effectively treated by benzodiazepines (9). Escalation of pharmacoresistance, particularly to benzodiazepines, with prolonged seizure duration has been demonstrated in several studies (10). Time-dependent pharmacoresistance

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is a major issue in SE. The anticonvulsant efficacy of BZD may decrease up to 20-fold after 30 minutes of seizure activity (11-13). In addition, 30 minutes of convulsive SE may also indicate irreversible brain damage, as shown experimentally (14).

Recent studies show that inflammatory reactions can cause seizures, and pro-inflammatory pathways may contribute to the development of SE. Hence, it might be feasible for researchers to regulate epileptic seizures by altering inflammatory signals in the brain (1). Higher levels of High-mobility group box 1 (HMGB1) protein and specific pro-inflammatory cytokines have been identified in the serum of children with febrile convulsions and cerebrospinal fluid of patients with refractory SE, in comparison to patients affected by other inflammatory diseases (15-19). Several inflammatory biomarkers have been associated with SE, including HMGB1 (2, 20-22), GFAP (23, 24) and other pro-inflammatory cytokines such as IL-6 and IL-1β (22, 25, 26).

HMGB1, a non-histone DNA binding protein, has been identified as a significant cytokine when released into the extracellular environment. The pro-inflammatory properties of HMGB1 are activated by binding to receptors such as toll-like receptor 4 (TLR 4), thereby mediating nuclear factor-κB (NF-κB) or other pathways (27). In both animal models and human patients, HMGB1 is actively expressed in epileptic tissues (2, 28). TLR4 belongs to the TLR family and is expressed in microglia, oligodendrocytes, and astrocytes within the central nervous system (29). Dysregulation of TLR 4 is implicated in the pathogenesis of several neurological diseases (29–33), including status epilepticus(7, 25). Additionally, there are also unattached forms of TLR4 identified as soluble TLR4 (sTLR4) (34, 35). These unattached protein complexes are considered to have the same structural characteristics as their membrane-bound counterparts. However, they do not participate in the TLR pathway. Rather, they mitigate inflammatory responses by competing with TLRs for ligands (35). HMGB1 and TLR4 can quickly trigger the activation of pro-inflammatory signaling pathways, disrupt the blood-brain barrier (BBB), and increase the severity of seizures (7, 21, 28, 36). Multiple studies in animal models of epilepsy have shown that activating the HMGB1/TLR4 signaling pathway leads to a marked increase in the frequency of seizures (7, 27, 37, 38). The impairment of the blood-brain barrier could result in the release of serum proteins into the brain, resulting in inflammation and glial activation (39-41). In cases of SE, the elevated expression of GFAP, a marker of glial cells have been observed. Glial fibrillary acidic protein (GFAP) is an intermediate filament found only in white matter astrocytes of the CNS. In the CNS, GFAP is crucial for the structural organization of astrocytes. The communication between astrocytes and certain types of neurons, such as Purkinje cells, is imperative in upholding the integrity of the BBB. GFAP could be a promising blood biomarker since there is no evidence of extracerebral GFAP production (39).

In response to infection, tissue injury, trauma, as well as seizures the innate immune system is rapidly activated and triggers an inflammatory response. Lipopolysaccharide (LPS), known to bind to and stimulate cell surface receptors known as TLR4, are a well-studied example of this signal (42–44). LPS-induced inflammatory response in peripheral blood mononuclear cells (PBMC) cultures can serve as a model to investigate immune response in SE patients, which can provide insights into the role of inflammation in the development and progression of the condition (45). In this study, the researchers examined the expression of several genes, including HMGB1, TLR4, IL-6, IL-10, and GFAP, after stimulating with LPS, using qPCR analysis on mRNA isolated from PBMC of patients with SE. The aim of this study was to identify the expression and the correlation between the gene expression and protein concentration of several inflammatory markers with resistance to diazepam. No evidence currently supports a direct link between inflammation and resistance to diazepam, hence the need for this study.

Methods

Patient Characteristics and Clinical Data

Peripheral blood samples were collected from 18 adult patients with Status Epilepticus in Cipto Mangunkusumo Central Hospital Jakarta,

consisting of 12 diazepam-responsive (DRV) and 6 diazepam-resistant (DRT) subjects. We took the samples within 72 hours of the onset of the seizure. The clinical data collected were age, gender, previous history of epilepsy, etiology of seizure, and seizure duration and frequency.

PBMC Isolation, Storage, Thawing, and Culture

PBMCs were isolated from freshly heparinized peripheral blood of patients with SE using the density gradient separation medium Ficoll Paque™ Premium (Cytiva) and centrifugation. After cell counting, the PBMCs were stored at -80 °C for 24 h and then transferred to liquid nitrogen until used for culture. After the thawing protocol at 37 °C, PBMCs were cultured in a complete medium composed of RPMI-1640 with L-glutamine, FBS, 100 U/ml Penicillin + Streptomycin, and Gentamycin, at a concentration of 10^6 cells per well. The cell culture was then incubated in a 5% CO2 humidified incubator for 4 hours at 37°C. After this incubation, LPS from Pseudomonas aeruginosa (L7018, Sigma) was added in a final concentration of 1 μg/ml. There was an unstimulated well (control) in the same cell culture plate for each stimulated well. The cells were harvested at 24 hours after stimulation. The cell culture supernatant was taken from each well, and the harvested cells were kept at –80 °C until use.

RNA Extraction and cDNA Synthesis

The Quick-RNA™ Miniprep Plus kit (R1057, Zymo Research) was used to extract total RNA from PBMC according to the manufacturer's protocol. The Nano spectrophotometer (BioDrop) was used to measure the RNA's quantity and quality. The purified RNA was stored at –80 °C for subsequent cDNA synthesis. The isolated RNA was used to synthesize cDNA according to the manufacturer's protocol by utilizing the ReverTra Ace™ qPCR RT Master Mix (FSQ-301, Toyobo), and continued with the PCR reaction.

Determination of HMGB1, IL-6, IL-10, TLR4, and GFAP expression levels in PBMC by Quantitative real-time PCR (qRT-PCR)

Primers for IL-6, IL-10, HMGB1, TLR4, and GFAP were designed using Primer3 software. The details of the primers are shown in Table 1. Primers and cDNA samples were diluted with nuclease-free water (NFW). Each primer was used along with the reference gene primer in a PCR reaction using Thunderbird™ Next SYBR® qPCR Mix (QPX-201, Toyobo) as described by the manufacturer and analyzed with Applied Biosystem® 7500 StepOnePlus™. The $2^{\Delta\Delta Ct}$ method was used to assess the expression level using β-actin (ACTB) as the reference gene.

Table 1. Primers Used in the PCR Reaction

Gene name	Genbank accession number		Primer sequence (5'-3')	Amplicon length (bp)
ß-actin	NM_001101.5	F	GCT GGA AGG TGG ACA GCG A	613
		R	GGC ATC GTG ATG GAC TCC G	
IL-6	NM_00600.5	F	CACTCACCTCTTCAGAACGAAT	107
		R	GCTGCTTTCACACATGTTACTC	
IL-10	NM_000572.3	F	GCTGGAGGACTTTAAGGGTTAC	106
		R	GATGTCTGGGTCTTGGTTCTC	
GFAP	NM_002055.5	F	GATCAACTCACCGCCAACA	107
		R	AGCCTCAGGTTGGTTTCATC	
HMGB1	NM_001313893.1	F	GGCCCGTTATGAAAGAGAAATG	119
		R	CAGAGCAGAAGAGGAAGAAGG	
TLR4	NM_003266.4	F	TTTCAGCTCTGCCTTCACTAC	107
		R	GACACCACAACAATCACCTTTC	

Detection of HMGB1, IL-6, IL-10, TLR4 and GFAP levels in serum samples by ELISA

The concentration of HMGB1, IL-6, IL-10, TLR4, and GFAP in the serum was measured using ELISA kits that are commercially available, as follows: HMGB1 (Cusabio, catalog number: CSB-E08223h), GFAP (Cusabio, catalog number: CSB-E08601h), TLR4 (Cusabio, catalog number: CSB-E12954h), IL-6 (Quantikine (R&D), catalog number: S6050), and IL-10 (Quantikine (R&D), catalog number: S1000B). The assays were carried out according to the manufacturer's instructions. The concentrations of cytokines were expressed as pg/ml.

Ethical Considerations

This study was approved by the Ethics Committee of the University of Indonesia and Cipto Mangunkusumo National Hospital (under permit number: KET-220/UN2.F1/ETIK/PPM.00.02/2021) and had informed consent from all patients.

Statistical Analysis

The statistical analyses were conducted using IBM SPSS statistics software for Windows, version 26.0 (SPSS Inc., USA). Categorical variables are analyzed with chi-square (or the alternatives: fisher's exact test, and Kolmogorov-Smirnov test for variables >2). Continuous data were validated for normality with Saphiro-Wilk test, presented as mean ± standard error of the mean (SEM), and were analyzed using unpaired t-tests or Mann-Whitney U tests for unpaired data and paired t-tests or Wilcoxon tests for paired data. The calculation of rank correlation coefficients according to Spearman. Values of $P<0.05$ were indicative of statistically significant differences.

Results

Characteristic of Subjects

Eighteen adult subjects with SE consisting of 12 DRV and 6 DRT were included in this study. The mean age of the subjects was 47.17±18.26. There were significant differences in age distribution between the DRV and DRT groups, with the DRT group having a younger age profile. Acute symptomatic etiology was present in most subjects. Overall, the majority of subjects were female and had intracranial etiology, with a seizure duration of under 30 minutes. The clinical characteristics of the subjects are shown in Table 2.

Table 2. Characteristics of Subjects

Characteristics of the subjects		DRV (N=12)	DRT (N=6)	P value
		Age (year), median (min-max)		
		56.5 (25–75)	22.5 (20–63)	0.013*
		PBMC count ($\times 10^6$), median (min-max)		
		8.53 (5.76–20.8)	7.59 (6.84–21.12)	0.851*
Gender N (%)	Male	2 (16.7)	0 (0)	0.529†
	Female	10 (83.3)	6 (100)	
History of epilepsy N (%)	Yes	2 (16.7)	2 (33.3)	0.569†
	No	10 (83.3)	4 (66.7)	
Etiology N (%)	Intracranial	8 (66.7)	2 (33.3)	0.766‡
	Metabolic	2 (16.7)	2 (33.3)	
	Mixed	2 (16.7)	2 (33.3)	
Seizure duration N (%)	<30 min	12 (100)	3 (50)	0.025†
	≥30 min	0 (0)	3 (50)	

DRV=Diazepam-responsive; DRT=Diazepam-resistant. *Mann-Whitney test; †Fisher's Exact test; ‡Kolmogorov-Smirnov test.

Concentration of Serum HMGB1, TLR4, IL-6, IL-10, and GFAP Protein Expression

A significant difference in serum HMGB1 and IL-6 concentration was found between the two groups (P=0.005 and P=0.013, respectively), whereas TLR4, IL-10, and GFAP and the ratio of IL-6 to IL-10 did not differ significantly between the two groups (Figure 1).

HMGB1 and Associated-genes Expression in PBMC in Response to LPS Stimulation

HMGB1 gene expression was found in all samples tested. There was a significant difference in HMGB1 mRNA expression between control and LPS samples in the DRV group (P=0.019), whereas it was not significantly increased in the DRT group (P=0.297) (Figure 2A). The highest expression, 40.162, was found in the DRV group stimulated with LPS. No difference was found between the LPS samples in both groups (P=0.51).

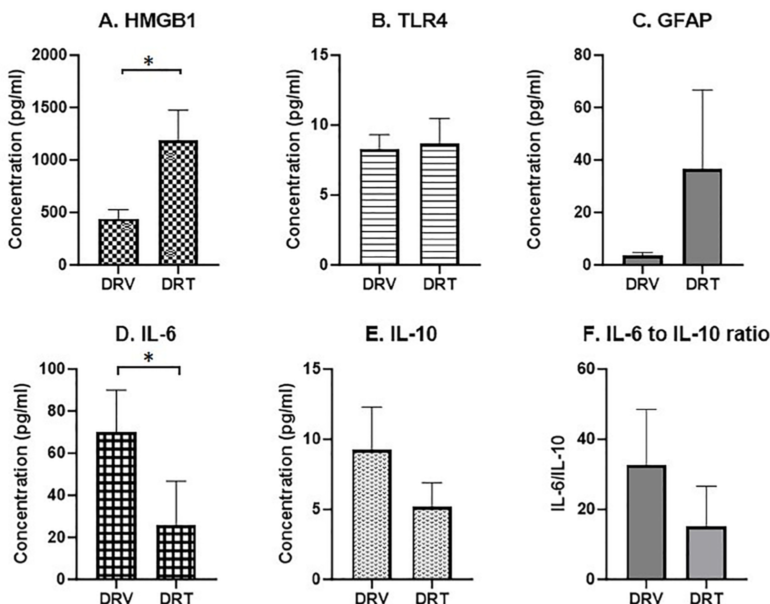
The mean expression of TLR4 did not significantly differ between control and LPS samples in both the DRV (P=0.844) and DRT groups (P=0.875) (Figure 2B). Additionally, there were no differences in TLR4 expression between LPS samples in both groups (P=0.7).

The expression of the IL-6 gene was low in the control-DRV group, and it was significantly different from the LPS-DRV group (P=0.001). However, there was no difference between the control and LPS samples in the DRT group (P=0.096), nor were there any differences in the LPS samples for either group (P=0.240) (Figure 2C).

There was no significant difference in the expression of the IL-10 gene in the control and LPS samples, either in the DRV (P=0.100) or in the DRT group (P=0.220). No difference was found in the LPS samples for either group (P=0.888) (Figure 2D). GFAP gene expression was found in only one subject (for each control and LPS-stimulated samples) in the DRV group, while none were found in the DRT group.

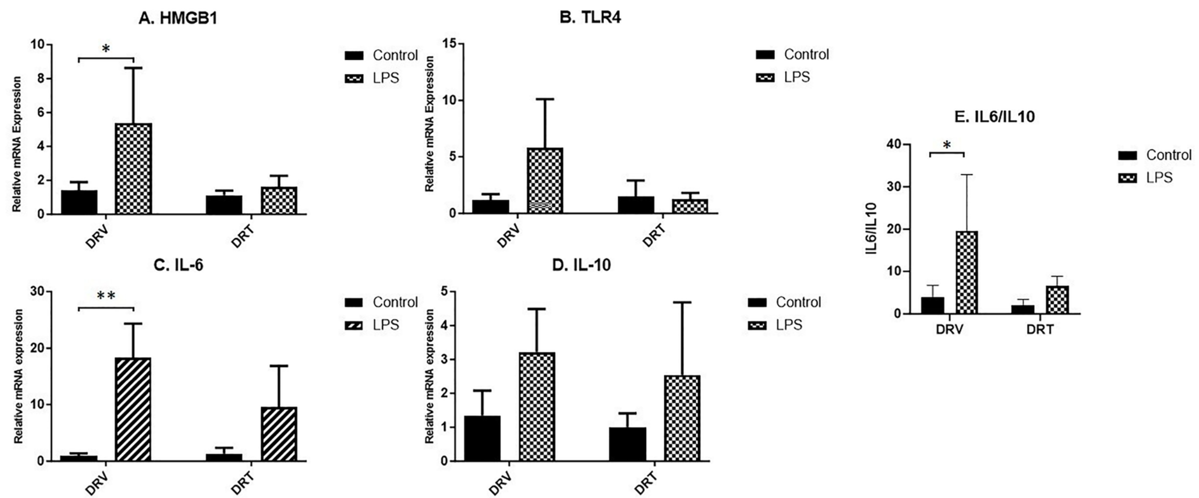
In the DRV group, the IL-6 to IL-10 ratio was 3.891±2.821 and 19.68±13.20 for the control and LPS, respectively (P=0.005), while in the DRT group the ratio was not different significantly between control and LPS samples (P=0.142). There was no discernible difference in the IL-6/IL-10 ratio between the LPS-stimulated samples of the DRV and DRT groups (P=0.981) (Figure 2E).

In terms of fold change, there was no difference in the fold change of any of the genes between the DRV and DRT groups (Figure 3).



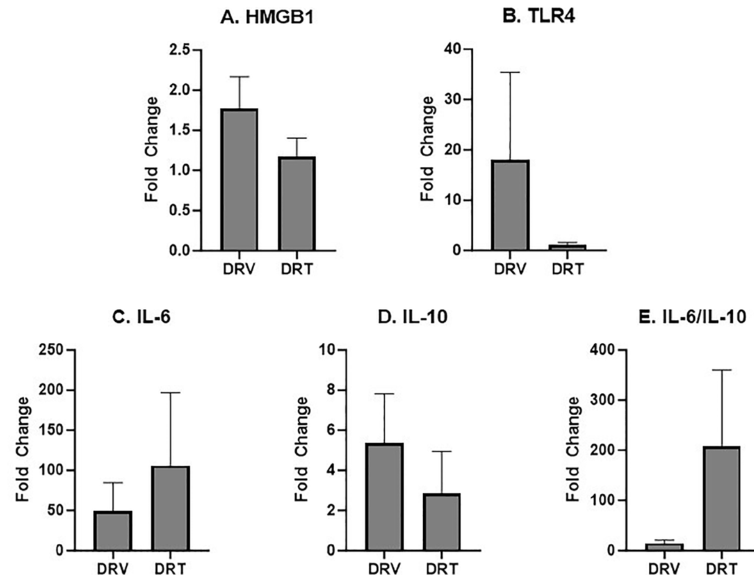
DRV=Diazepam-responsive; DRT=Diazepam-resistant; PBMC=Peripheral blood mononuclear cells; HMGB1=High-Mobility Group Box 1; TLR4=Toll-like receptor 4; GFAP=Glial fibrillary acidic protein; IL=Interleukin; Statistical analyses were performed using Mann-Whitney test. Data represent mean ± SEM. *P<0.05

Figure 1. Serum protein concentration of HMGB1, TLR4, IL-6, IL-10, GFAP and IL-6 to IL-10 Ratio (using ELISA method; DRV, N=18; DRT, N=6).



DRV=Diazepam-responsive; DRT= Diazepam-resistant; PBMC=Peripheral blood mononuclear cells; LPS= Lipopolysaccharide; HMGB1=High-Mobility Group Box 1; TLR4=Toll-like receptor 4; IL=Interleukin; Statistical analyses were performed using Wilcoxon test or paired t-test (paired data; compared control and LPS samples in each group) and Mann-Whitney test (unpaired data; compared LPS samples between DRV and DRT group). Data represent mean ± SEM. *P<0.05; **P<0.01

Figure 2. Gene expression of inflammatory markers from PBMC culture of SE patients. (using qPCR; LPS, N=18; Control, N=18, for each group).



DRV=Diazepam-responsive; DRT=Diazepam-resistant; PBMC=Peripheral blood mononuclear cells; LPS=Lipopolysaccharide; HMGB1=High-Mobility Group Box 1; TLR4=Toll-like receptor 4; IL=Interleukin. Statistical analyses were performed using Mann-Whitney test. Data represent mean ± SEM.

Figure 3. Fold Change between DRV and DRT groups. Fold change of LPS-stimulated and control samples, from PBMC culture of SE patients.

Correlation of Diazepam Response, Seizure Duration, Serum Protein Concentration and Fold Change of mRNA Expression

There were significant correlation between diazepam response with seizure duration (r_s=0.63; P=0.005), serum protein HMGB1 concentration (r_s=0.57; P=0.014) and IL-6 (r_s=–0.59; P=0.010); while seizure duration showed significant corellation with serum protein HMGB1 concentration (r_s=0.56; P=0.015) (Table 3). In addition, the serum IL-10 concentration was negatively correlated with the serum GFAP concentration (r_s=–0.51; P=0.031), and had moderate correlation with serum IL-6 concentration (r_s=0.42; P=0.077).

Table 3. Correlation of Diazepam Response, Seizure Duration, Fold Change of Mrna Expression, and Serum Protein Concetration

	Variables		DZP Response	Seizure Duration
Spearman's rho	DZP Response	r_s	1	0.632*
		p (2-tailed)	0	0.005
	Seizure Duration	r_s	0.632†	1
		p (2-tailed)	0.005	0
	FC HMGB1	r_s	-0.158	0.193
		p (2-tailed)	0.575	0.491
	FC TLR4	r_s	-0.087	0
		p (2-tailed)	0.825	1
	FC IL-6	r_s	0.267	0.171
		p (2-tailed)	0.377	0.577
	FC IL-10	r_s	-0.254	0
		p (2-tailed)	0.403	1
	Elisa HMGB1	r_s	0.568†	0.56†
		p (2-tailed)	0.014	0.015
	Elisa TLR4	r_s	0.091	-0.043
		p (2-tailed)	0.72	0.865
	Elisa IL6	r_s	-0.59†	-0.101
		p (2-tailed)	0.01	0.691
	Elisa IL-10	r_s	-0.091	-0.101
		p (2-tailed)	0.719	0.69
	Elisa GFAP	r_s	0.296	-0.072
		p (2-tailed)	0.233	0.776

DZP=Diazepam; FC=Fold Change of mRNA expression; *Correlation is significant at the 0.01 level (2-tailed); †Correlation is significant at the 0.05 level (2-tailed).

Discussion

Our study was done to investigate the correlation between diazepam response and several inflammatory biomarkers. To the best of our knowledge, this study represents the initial demonstration of the utility of mRNA and protein expression in HMGB1, TLR4, IL-6, IL-10, and GFAP from peripheral blood of adults SE patients in correlation with diazepam response. Our findings demonstrated that serum protein concentrations of HMGB1 were significantly higher in SE patients with diazepam resistance. Diazepam resistance in SE exhibits a robust correlation with seizure duration, as shown in the positive correlation in this study. As seizures become more prolonged, the exacerbation of inflammation occurs (46, 47), which can partially explain the significantly higher HMGB1 level in the DRT group. Neuroinflammation plays a significant role in various forms of epilepsy, including SE. According to reports, HMGB1 binds to TLR4 or RAGE, rapidly initiating pro-inflammatory signaling pathways, which disrupt the BBB and worsen seizure severity (7, 48). HMGB1 has the potential to intersect oxidative stress and inflammation as it enhances the production of reactive oxygen species (ROS), exacerbating the inflammatory process (1, 2).

Conversely, IL-6 was higher in responsive patients. This may be due to other factors such as the pro- and anti-inflammatory properties of IL-6 in certain condition such as in acute phase response (49), and other comorbidities including sepsis and hypoxia in DRV group, which may confound the results. IL-6 exhibits pro-inflammatory effects in acute inflammation whilst also displaying immunosuppressive and anti-inflammatory properties when expressed at lower concentrations.

Additionally, IL-6 may exhibit opposing functions in varying cell types. It is evident that IL-6 has a complex and multifaceted influence on inflammation and immune responses (49). IL-6 also helps regulate the production of anti-inflammatory protein such as IL-10 (49, 50), which explains the moderate correlation found between protein expression of IL-6 and IL-10, as well as the similar pattern of IL-6 and IL-10 protein expression, as well as the IL-6/IL-10 ratio that were found in this study.

There was no difference in serum TLR4 protein concentration between DRT and DRV groups in this study. The Heterogeneity of SE cases, and individual variation, in addition to timing of measurements, concurrent medications and comorbidities, as well as the presence of systemic inflammation, might affect the results, obscuring the association between sTLR4 and diazepam responsiveness (51–53).

We observed that the mRNA expression of HMGB1 in diazepam-responsive SE patients after LPS stimulation was about 3.5 times greater than in the control group. Conversely, there were subtle changes in the relative expression in diazepam-resistant subjects. Similar findings were noted with regards to IL-6 expression. These findings are consistent with the study results conducted by several researchers, which documented increased cytokine concentrations following LPS stimulation (54–56). LPS, the major component of Gram-negative bacterial walls can provoke acute inflammatory responses by stimulating the release of numerous inflammatory cytokines in various cell types, particularly monocytes/macrophages (57, 58). Resistant SE, which tended to be of longer duration than responsive SE, may have had more severe changes, including inflammation and BBB breakdown, as a result of the ongoing underlying pathology and co-morbidities in the patients (46). The mechanisms underlying diazepam-resistant SE are multifactorial and can involve changes in neuronal excitability, receptor function, and network properties (11, 46). These mechanisms along with the co-medication and other severe pathology in these conditions could potentially override the effects of LPS-inducing inflammation in the DRT group.

The IL-10 mRNA expression level increased after LPS stimulation in the DRV and DRT groups, but they did not differ significantly. This finding suggests that IL-10 expression may be a common response to this kind of stimulation (59, 60). The lack of significant difference between groups suggests that although both groups show this response, the level of IL-10 expression may not differentiate diazepam response or resistance in the context of SE.

There was no significant difference in TLR4 mRNA expression between control and LPS samples in either group, or between LPS samples in the DRV and DRT groups. This can be caused by the downregulation of TLR4. In response to prolonged inflammation, the body may downregulate the expression of TLR4 as a regulatory mechanism to avoid excessive immune activation and tissue damage. TLR4 is a key receptor involved in the recognition of LPS and chronic activation of TLR4 can lead to a state of immune tolerance or desensitisation, where cells become less responsive to the stimulus (e.g. LPS or bacterial products). This downregulation of TLR4 can result in lower mRNA levels (61, 62). Furthermore, TLR4 gene expression might be influenced by several things such as age, stimulus, environtment or patient's condition and cell part/type (63). The TLR4-dependent NF-–B signaling pathway is the primary pathway through which LPS induces an inflammatory response (44, 64). TLR4-independent host response to LPS has also been identified (65).

In this study, GFAP was only detected in one subject. GFAP is known to be expressed predominantly, but not exclusively, in astrocytes of the central nervous system (CNS), and to a lesser extent in other cells, including PBMCs (66).

Fold change indicates whether a gene is upregulated or downregulated between LPS-stimulated and control. In this study, except for TLR4 in the DRT group, there was an upregulated fold change for all other genes tested in both the DRV and DRT groups, but there was no significant difference between the groups. Some possible reasons for these findings were the heterogeneity within SE groups (6, 67), the small sample size in this

study, and the timing of measurements that might change overtime (68–71). Nevertheless, there were some trends in the results.

Serum IL-10 concentration was negatively correlated with the serum GFAP concentration. GFAP was exclusively expressed in astrocytes in the CNS white matter. Findings in the serum could potentially suggest BBB leakage in the neuroinflammation and gliosis that occur in the pathophysiology of SE (39). IL-10 is an anti-inflammatory cytokine that can modulate immune responses and reduce inflammation. Its role in epilepsy and SE is a subject of many ongoing researches, as the immune system and neuroinflammation play complex roles in these conditions (72). As the seizure activity is controlled, and the brain attempts to resolve the inflammatory response, the levels of IL-10 may increase as part of the body's anti-inflammatory response. This could potentially lead to a negative correlation between IL-10 and GFAP, as IL-10's anti-inflammatory effects might be associated with a reduction in astrocyte activation (GFAP expression) (66, 72, 73). On the contrary, serum IL-10 concentration had positive moderate correlation with the serum IL-6 concentration. IL-10 is a double-edged sword for the immune system: while it is a potent anti-inflammatory and immunosuppressive cytokine, it can also have immunostimulatory properties. To activate multiple signaling pathways that either inhibit or activate immune cells, it is crucial to consider the different sources of IL-10, the target cells on which it acts, as well as the timing and site of its secretion. Each of these features contributes to different functions (72).

In terms of clinical characteristics, the DRT group was found to have a younger age profile than the DRV groups. This may be attributed to several factors. Younger individuals may exhibit distinc underlying causes for SE (14, 74), or have different physiological responses to diazepam compared to older ones (75, 76). Additionally, age-related discrepancies may reveal diverse epilepsy types or other neurological ailments that are more prevalent in particular age categories (3, 5, 14). Further research is indispensable to investigate the particular mechanisms and factors that contribute to this observed age disparity in drug responsiveness among SE patients.

Limitation of Study

The small sample size is a constraint of this study. The limited resources and funding available at the time caused a small number of samples to be examined. To address this, further investigation is necessary with a bigger sample size in the future. Further research is required to study diazepam resistance in SE. This may include an investigation into GABA receptor and gene mutations, as well as other associated factors.

Conclusion

HMGB1 was highly expressed in the diazepam-resistant group and strongly correlated with response to diazepam treatment, and there was a significant increase in HMGB1 mRNA expression in response to LPS stimulation. These findings suggest that targeting HMGB1 may be a promising therapeutic strategy and that HMGB1 levels could serve as a valuable biomarker for predicting diazepam resistance in SE.

What Is Already Known on This Topic:

Time-dependent pharmacoresistance is still an issue in SE, and all the researchers are trying to look for alternative approaches to control the seizures. Recent studies have shown widespread brain inflammation in SE, suggesting that inflammation plays an essential role in the onset and development of SE. SE can cause damage to the BBB and activate various immune cells, releasing large amounts of pro-inflammatory mediators that can induce neuroinflammation through a variety of signaling pathways.

What This Study Adds:

This study provides new insights into the trends of various inflammatory biomarkers, particularly HMGB1, in relation to diazepam response in adult patients with status epilepticus, suggesting that it may be possible to regulate epileptic seizures by altering inflammatory signals in the brain.

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Author's Contributions: Acquisition, analysis, and interpretation of data: CNM, ML, FO, DAS, AB and HB; Drafting

the article: CNM and ML; Revising it critically for important intellectual content: CNM, ML, FO, DAS, AB, and HB; Approved final version of the manuscript: CNM and ML.

Conflict of Interest: The authors declare that they have no conflict of interest.

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