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Chitinase-3-like protein 1 may be a potential biomarker in patients with drug-resistant epilepsy

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ABSTRACT

The mechanisms of the pathogenesis of epilepsy remain unclear. Recent research shows that the inflammatory process occurring in the brain may be a common and critical mechanism of seizures. Chitinase-3-like protein 1 (CHI3L1 or YKL-40) is a newly discovered inflammatory factor. We aimed to evaluate the role of YKL-40 as a biomarker for epilepsy. 124 subjects were classified as control group (n = 23), new-diagnosis epilepsy group (NDE, n = 34), drug responsive epilepsy group (DPE, n = 37), and drug-resistant epilepsy group (DRE, n = 30) YKL-40 was measured by ELISA in serum and cerebrospinal fluid (CSF). The concentrations of serum and CSF YKL-40 and its diagnostic accuracy for epilepsy were analysed. Patients with DRE had higher concentrations of YKL-40 in serum and CSF, while patients with NDE and DPE had increased YKL-40 levels in CSF but not serum in comparison with control. Moreover, serum and CSF YKL-40 provide high diagnostic accuracy for DRE. YKL-40 may play a possible pathogenic role in epilepsy. YKL-40 may represent a potential biomarker of brain inflammation in patients with DRE.

1. Introduction

Epilepsy is known as a chronic brain disorder, which is responsible for the patients experiencing recurrent unprovoked seizures for a long time. This serious brain condition is one of the most common neurological disorders and is an important cause of disability and mortality in developing countries (Tian et al., 2016). However, the mechanisms involving in the pathogenesis of epilepsy are still not completely clear (Kwan et al., 2011). In recent years, the relations between epilepsy and immune system have attracted the attention of researchers (Granata et al., 2011). Studies have shown that inflammation plays an important role in generating and exacerbating epilepsy (Aronica et al., 2017; Gershen et al., 2015; Vezzani et al., 2011; Xu et al., 2013). Complement activation of the cerebral cortex or intracerebroventricular infusion of pro-inflammatory cytokines provoked and aggravated seizures in animal experiments (Dube et al., 2005; Xiong et al., 2003), while inducing systemic inflammation increased epilepsy susceptibility (Eun et al., 2015; Sayyah et al., 2003). In addition, active inflammation has been detected not only in prototypical inflammatory epilepsies, but also in drug-resistant epilepsy patients with various causes (Choi et al., 2009; Li et al., 2017; Wang et al., 2017). In clinical work, the diagnosis of epilepsy is mainly based on the patient's medical history and electroencephalogram (EEG) results, while there is a lack of objective biomarkers. Biomarkers reflecting the pathogenesis may be useful for studies of the underlying disease mechanisms contributing to epilepsy, to predict progression of disease and may serve as the surrogate markers in treatment trials.

Chitinase-3-like protein 1 (CHI3L1 or YKL-40), a member of the glycosyl hydrolase family 18, is mainly produced by macrophages, neutrophils, synovial and malignant cells. YKL-40 has been found to be increased in acute and chronic inflammatory diseases, which have a high remodeling of extracellular matrix, such as rheumatoid arthritis, asthma, inflammatory bowel disease, and systemic sclerosis (Johansen, 2006). Levels of cerebrospinal fluid (CSF) YKL-40 were increased in many central nervous system (CNS) diseases, such as amyotrophic lateral sclerosis, stroke, multiple sclerosis, and other neurological disorders (Hjalmarsson et al., 2014; Magdalinou et al., 2015; Martinez et al., 2015; Winer et al., 2013). In recent years, YKL-40 was considered a potential inflammation biomarker, but its value in epilepsy has not been described.

Abbreviations: AEDs, antiepileptic drugs; ANOVA, Analysis of covariance; AUC, area under the receiver operator characteristics curve; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy; EEG, electroencephalogram; ILAE, International League against Epilepsy; MRI, magnetic resonance imaging; ROC, Receiver operating curve; YKL-40, chitinase-3-like protein 1

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In this study, we investigated the potential clinical utility of YKL-40 as a biomarker for epilepsy. Because of the characteristics of epilepsy is a certain degree of inflammation, we aimed to verify whether serum and CSF YKL-40 concentrations are elevated in patients with epilepsy and whether serum and CSF YKL-40 provide high diagnostic accuracy for epilepsy.

2. Materials and methods

2.1. Study subjects

Participants were recruited from the Department of Neurology of the First Affiliated Hospital of Chongqing Medical University. We selected all subjects between 12 and 57 (inclusive) years of age who had completed lumbar puncture, venous blood extraction, detailed medical history, neurological and neuropsychological examinations, electroencephalogram (EEG), and cranial magnetic resonance imaging (MRI) or computed tomography (CT) scans. 124 subjects were classified as control group (n = 23), new-diagnosis epilepsy group (NDE, n = 34), drug responsive epilepsy group (DPE, n = 37), and drug-resistant epilepsy group (DRE, n = 30) according to the criteria proposed by the International League against Epilepsy (ILAE) in 2001 (Seino, 2006).

2.2. Classification criteria

The control group consisted of subjects with neurosis, which includes somatization and anxiety disorders, and had no any past history of seizures. Physical and laboratory examination, electrophysiologic study, brain MRI or CT were showed the normal results for all controls. NDE was defined as those who had at least one seizure attack before diagnosis and did not take any antiepileptic drugs (AEDs). In addition, DPE was defined as those who had been seizure-free for more than two years after taking one type of AEDs. We define DRE as those who had a seizure history of more than 3 years, and were on regular treatment with at least three types of common AEDs, but seizures have not been controlled. All the subjects were not found to have progressive lesions by MRI or CT in the CNS.

2.3. Ethics

The study was approved by the Research Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Informed written consent was obtained from all subjects. The research was performed in accordance with the Declaration of Helsinki of the World Medical Association.

2.4. Sample collection and storage

CSF and venous blood samples were obtained as previously described in detail (Wang et al., 2012). For each subject, 2 ml of CSF and 5 ml of veinous blood samples were collected. 2 ml of CSF samples were centrifuged at 2000 × *g* for 5 min at 4 °C and stored at −80 °C. 5 ml of venous blood samples which was used by a serum separator tube (SST) to collect, allow samples to clot for 2 h at room temperature or overnight at 4 °C before centrifugation for 5 min at t 3000 × *g*. Remove serum and aliquot and stored at −80 °C. Both CSF and blood samples were frozen until the samples were processed as one batch at the end of the study.

2.5. YKL-40 measurement

The concentrations of YKL-40 were measured by a commercial ELISA kit (Signalway Antibody, USA). Operation steps were performed according to the manufacturer's protocol. Dilution of the CSF and serum was 1:1 and 1:10, respectively. Then, 100 μL of CSF, serum samples were added to the 96-well microtiter plates. Before further washing, the plates were covered with an adhesive strip and incubated for 2 h at 37 °C. Then, 100 μL of the Biotin-antibody was added to each well and incubated for 1 h at 37 °C. After three washes, 100 μL of HRP-avidin was added to each well and incubated in the dark for 1 h at 37 °C. After the final four washes, 90 μL of substrate solution was added to each well and incubated for 30 min at 37 °C, protecting from light. To each well, 50 ml stop solution was added if color change does not appear uniform. Finally, the optical density of each well was determined at once. Optical densities were determined with a Multiskan Spectrum Microplate Spectraphotometer microplate reader set to 450 nm (Thermo Fisher Scientific, USA) as previous study (Ravizza et al., 2008). A standard curve was constructed by plotting absorbance values versus YKL-40 concentrations of calibrators.

2.6. Statistical analysis

Statistical analysis was performed by SPSS Statistics 20.0. Analysis of co-variance (ANCOVA) and chi-square analyses were used to test for significant differences between groups on demographics for continuous and categorical measurements respectively. Age and sex were the covariates for ANCOVA.

Spearman correlation was performed to test associations between CSF YKL-40 and serum- YKL-40.

Logistic regression models were used to test the diagnostic accuracies of YKL-40 at baseline against clinical diagnostic criteria for control versus NDE, control versus DPE, and control versus DRE, adjusted for age and gender. Overall diagnostic accuracy (area under the receiver operator characteristics curve, AUC) was also obtained from Receiver operating curve (ROC) analyses for YKL-40. Statistical significance was defined as $p < 0.05$.

Table 1
Demographics of subjects.

Characteristics	Control (n = 23)	NDE (n = 34)	DPE (n = 37)	DRE (n = 30)
Age (years)	31.3 (± 10.0)	28.0 (± 11.2)	26.9 (± 11.7)	28.3 (± 11.5)
Gender (F %)	12 (52.2%)	19 (55.9%)	18 (48.6%)	14 (46.7%)
Taking AEDs (n %)	0 (0%)[c,d]	0 (0%)[c,d]	37 (100%)[a,b]	30 (100%)[a,b]
Seizure types (n %)				
1.Secondarily generalized tonic-clonic seizure	0 (0%)[b,c,d]	11 (32.4%)[a]	11 (29.7%)[a]	8 (26.7%)[a]
2.Generalized tonic -clonic seizure	0 (0%)[b,c,d]	15 (44.1%)[a]	16 (43.2%)[a]	14 (46.7%)[a]
3.Complex partial seizure	0 (0%)[b,c,d]	6 (17.6%)[a]	7 (18.9%)[a]	6 (20.0%)[a]
4.Absence seizure	0 (0%)	2 (5.9%)	3 (8.1%)	2 (6.7%)

P values indicate the values assessed with contingency chi-square analyses of variance for each variable except age, where a analyses of variance was performed. Post hoc analysis provided significant differences between groups: [a]from Control; [b]from NDE; [c]from DPE; [d]from DRE. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy; AEDs, antiepileptic drugs.

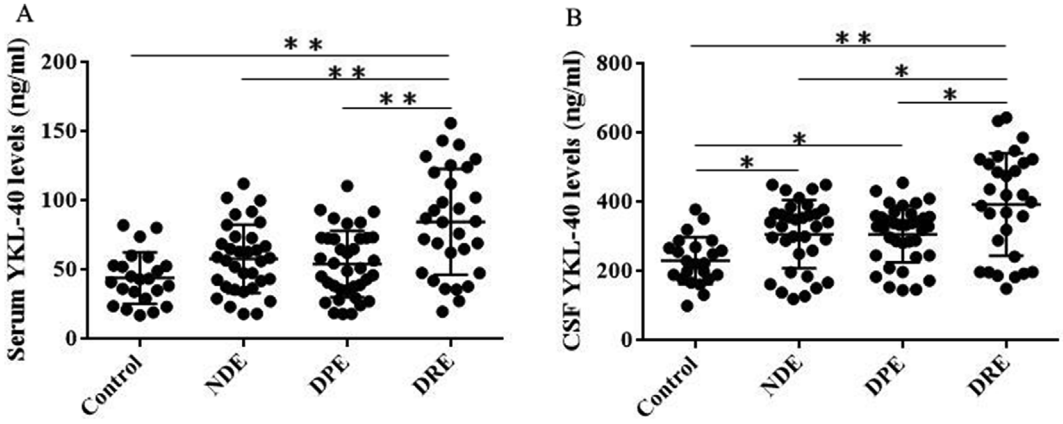

Fig. 1. Serum and CSF YKL-40 levels in different diagnostic groups. Serum YKL-40 levels (A) and CSF YKL-40 levels (B) in different diagnostic groups. Differences between groups were tested by ANOVA. *P < 0.01; **P < 0.001. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy; YKL-40, Chitinase-3-like protein 1.

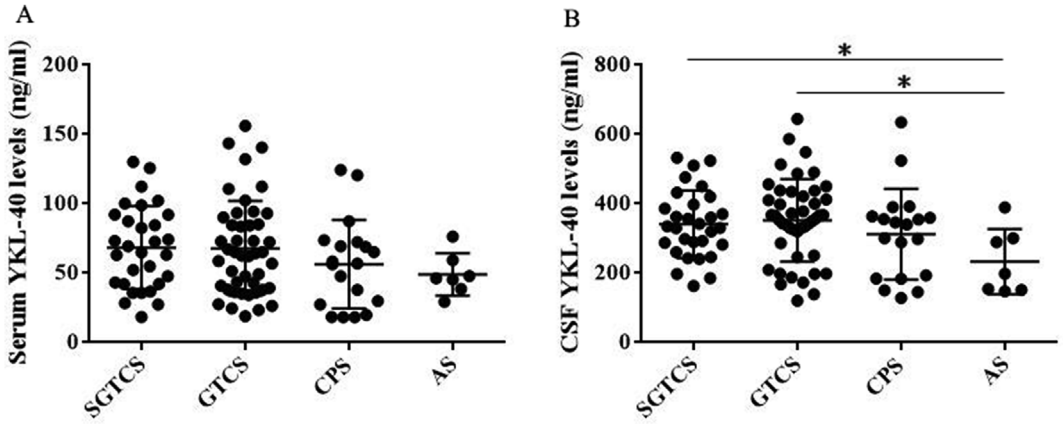

Fig. 2. Serum and CSF YKL-40 levels in different types of seizure. Serum YKL-40 levels (A) and CSF YKL-40 levels (B) in different types of seizure. Differences between groups were tested by ANOVA. *P < 0.05. Abbreviations: SGTCS, secondarily generalized tonic-clonic seizure; GTCS, generalized tonic -clonic seizure; CPS, complex partial seizure; AS, absence seizure; YKL-40, Chitinase-3-like protein 1.

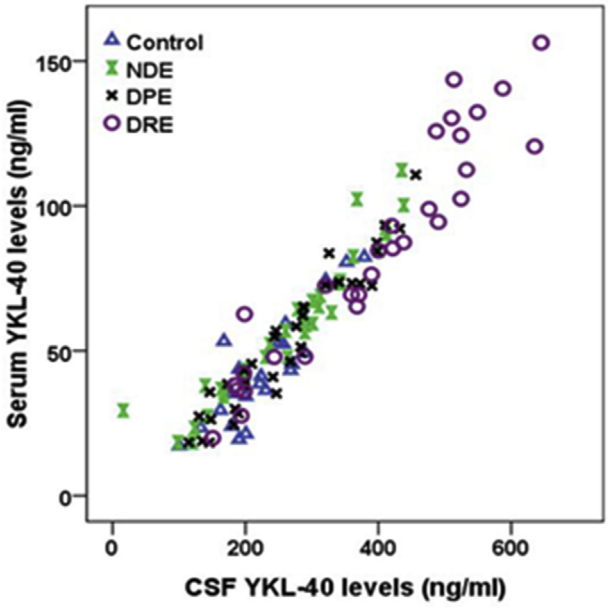

Fig. 3. Serum YKL-40 levels in relation to CSF YKL-40 levels. Correlations between Serum YKL-40 levels and CSF YKL-40 levels in different diagnostic groups. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy; YKL-40, Chitinase-3-like protein 1.

Table 2
Diagnostic accuracy of serum and CSF YKL-40.

Groups	Model	β (p)	AUC (95% CI)
Conrtol vs NDE	Serum YKL-40	**1.039 (0.012)**	0.663 (0.522–0.804)
	CSF YKL-40	1.006 (0.085)	0.610 (0.463–0.757)
Control vs DPE	Serum YKL-40	1.024 (0.078)	0.615 (0.471–0.758)
	CSF YKL-40	1.006 (0.106)	0.596 (0.452–0.739)
Control vs DRE	Serum YKL-40	**1.048 (0.001)**	0.813 (0.699–0.928)
	CSF YKL-40	**1.012 (0.001)**	0.804 (0.684–0.924)
NDE vs DPE	Serum YKL-40	0.992 (0.450)	0.457 (0.322–0.593)
	CSF YKL-40	1.000 (0.996)	0.491 (0.354–0.628)
NDE vs DRE	Serum YKL-40	**1.027 (0.004)**	0.714 (0.585–0.844)
	CSF YKL-40	**1.008 (0.001)**	0.751 (0.627–0.875)
DPE vs DRE	Serum YKL-40	**1.032 (0.001)**	0.738 (0.615–0.861)
	CSF YKL-40	**1.008 (< 0.001)**	0.754 (0.632–0.876)

Bold values indicate significant associations. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy; AUC, area under the receiver operator characteristics curve; YKL-40, Chitinase-3-like protein 1.

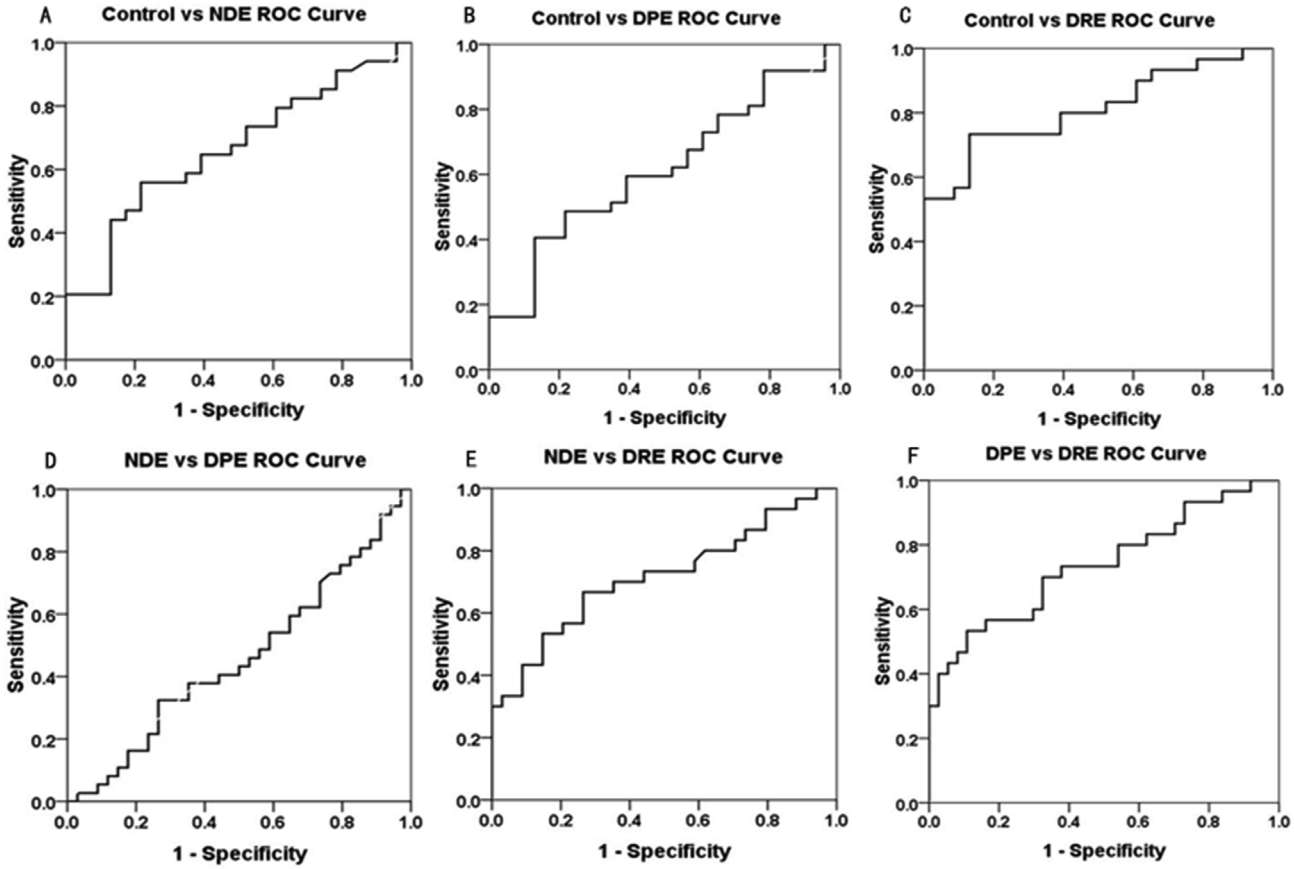

Fig. 4. Serum YKL-40 ROC analyses. ROC for the diagnostic utility of serum YKL-40 in differentiating NDE from control (A), DPE from control (B), DRE from control (C), DPE from NDE (D), DRE from NDE (E), DRE from DPE (F) by clinical diagnosis. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epilepsy.

3. Results

3.1. Baseline characteristics

The demographics of the study subjects are presented in Table 1. There were no differences in age and gender among the groups. None of the subjects had taken AEDs in the control and NDE groups, however, all participants had taken one or more kinds of AEDs in DPE and DRE groups. There were also no differences in seizure types between NDE, DPE, and DRE groups.

3.2. The levels of serum and CSF YKL-40 in every diagnostic group

Serum YKL-40 levels were significantly higher in patients with DRE ($p < 0.001$) as compared to control, NDE, and DPE. There were no differences between control, NDE, and DPE (Fig. 1A). Patients with DRE had increased CSF YKL-40 levels compared with control ($p < 0.001$), NDE ($P < 0.01$), and DPE ($P < 0.01$). Higher CSF YKL-40 levels were also found in both NDE ($P < 0.01$) and DPE ($P < 0.01$) compared with control. However, there were no differences between NDE and DPE (Fig. 1B). The intra-assay and inter-assay coefficients of variation were below 8% and 10%, respectively.

3.3. The levels of serum and CSF YKL-40 in different types of seizure

Serum YKL-40 levels were higher in patients with secondarily generalized tonic-clonic seizure (SGTCS) and generalized tonic-clonic seizure (GTCS) as compared to complex partial seizure (CPS) and absence seizure (AS). However, the difference between the groups was not statistically significant (Fig. 2A). Patients with SGTCS and GTCS had increased CSF YKL-40 levels compared with AS ($p < 0.05$ for both), but there were no differences between SGTCS, GTCS, and CPS (Fig. 2B).

3.4. Correlations between serum YKL-40 and CSF YKL-40

Serum YKL-40 was strongly correlated with CSF YKL-40 in control ($R = 0.794$, $P < 0.001$), NDE ($R = 0.974$, $P < 0.001$), DPE ($R = 0.960$, $P < 0.001$), and DRE subjects ($R = 0.955$, $P < 0.001$) (Fig. 3).

3.5. Diagnostic value of baseline serum and CSF YKL-40 for NDE, DPE, and DRE

Logistic regression models were used to test the accuracies for control versus NDE, control versus DPE, control versus DRE, NDE versus DPE, NDE versus DRE, and DPE versus DRE. The models were adjusted for age and gender. Serum YKL-40 was significant predictors of DRE ($\beta = 1.048$, $p = 0.001$ for control versus DRE; $\beta = 1.027$, $p = 0.004$ for NDE versus DRE; $\beta = 1.032$, $p = 0.001$ for DPE versus DRE) (Table 2 and Fig. 4C, E, and F). Serum YKL-40 had the higher accuracy for control versus DRE (AUC 0.813) (Table 2 and Fig. 4C). Serum YKL-40 was significant predictors of NDE ($\beta = 1.039$, $p = 0.012$) (Table 2 and Fig. 4A). However, Serum YKL-40 wasn't significant predictor for DPE (Table 2, Fig. 4B and D).

Similarly, CSF YKL-40 was significant predictors for DRE ($\beta = 1.012$, $p = 0.001$ for control versus DRE; $\beta = 1.008$, $p = 0.001$ for NDE versus DRE; $\beta = 1.008$, $p < 0.001$ for DPE versus DRE) (Table 2 and Fig. 5C, E, and F). CSF YKL-40 had the higher accuracy for control versus DRE (AUC 0.804) (Table 2 and Fig. 5C). However, CSF YKL-40 wasn't significant predictor for NDE and DPE (Table 2, Fig. 5A, B, and

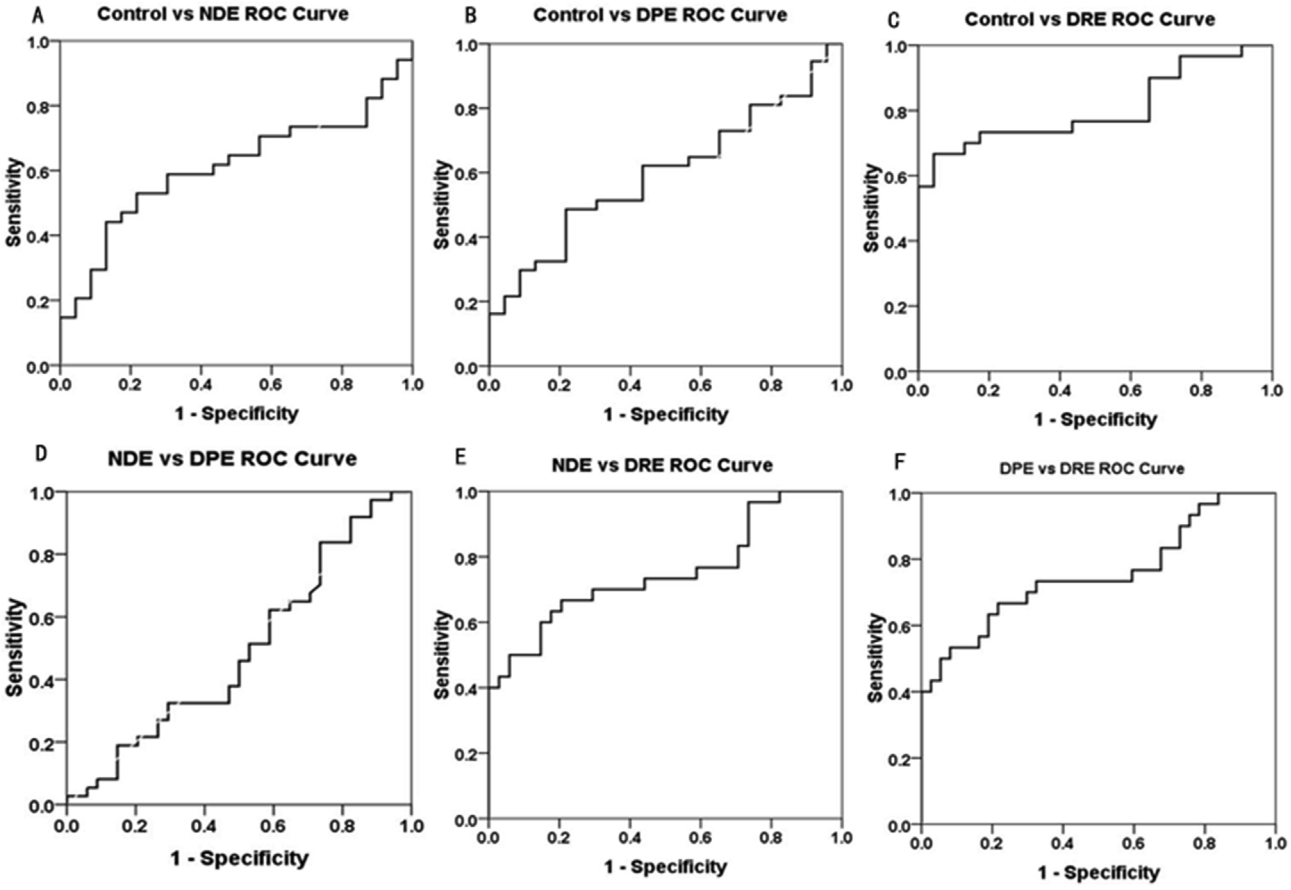

Fig. 5. CSF YKL-40 ROC analyses. ROC for the diagnostic utility of CSF YKL-40 in differentiating NDE from control (A), DPE from control (B), DRE from control (C), DPE from NDE (D), DRE from NDE (E), DRE from DPE (F) by clinical diagnosis. Abbreviations: NDE, new-diagnosis epilepsy; DPE, drug responsive epilepsy; DRE, drug-resistant epileps

D).

4. Discussion

In the present study, we found that patients with DRE had higher concentrations of YKL-40 in serum and CSF as compared with control, NDE, and DPE, while patients with NDE and DPE had increased YKL-40 levels in CSF but not serum in comparison with control. The serum YKL-40 levels correlated with the CSF YKL-40 levels. Moreover, serum and CSF YKL-40 was a significant predictor of DRE.

Inflammatory activity has been detected not only in prototypical inflammatory epilepsy such as limbic encephalitis and Rasmussen's encephalitis, but also in drug-resistant epilepsy patients with various causes (Aronica et al., 2005; Boer et al., 2006; Crespel et al., 2002; Maldonado et al., 2003; Ravizza et al., 2008; Vezzani and Granata, 2005). In temporal lobe epilepsy, it shows that nuclear factor-kappa B, proinflammatory cytokines, interleukin (IL) -1β and its signaling receptor IL-1R1 are highly expressed by neurons and glial cells (Crespel et al., 2002; Ravizza et al., 2008). Also, it demonstrates that increased cytokines levels such as IL-1β (Haspolat et al., 2002), IL-1-receptor antagonist (Peltola et al., 2000), and IL-6 (Peltola et al., 1998, 2000) have been detected in plasma and CSF in patients with recent seizures, but there was no evidence of infection. The physiological role of YKL-40 so far is not yet clear; however, it is presumed that it may be involved in inflammation in the process of tissue remodeling. Our study showed that the levels of CSF YKL-40 were significantly increased in NDE, DPE, and DRE groups, and the levels of serum YKL-40 were also significantly increased in DRE group. These results further indicate that the occurrence of epilepsy may be closely related to inflammation, and YKL-40 may play an important role in drug-resistant epilepsy.

In a study of Alzheimer's disease, it suggested that the levels of CSF and plasma YKL-40 were moderately correlated, and the levels of CSF YKL-40 were about 5 times higher than that of plasma YKL-40 (Craig-Schapiro et al., 2010). In the present study, it showed that CSF YKL-40 levels were significantly associated with serum YKL-40 levels. DRE group serum YKL-40 levels were significantly higher than the other groups. The levels of serum YKL-40 in NDE and DPE groups also increased compared with the control group, but there was no statistical difference. Similar coincident elevations of serum and CSF YKL-40 levels were reported in multiple sclerosis (Comabella et al., 2010) and aneurysmal subarachnoid hemorrhage (Kacira et al., 2008). Whether this increase in serum YKL-40 reflects passive or active output of central nervous system (CNS)-derived YKL-40 or coincident peripheral product that responds to systemic inflammatory signals is not clear (Craig-Schapiro et al., 2010). CSF YKL-40 levels were significantly higher than serum, suggesting that the relationship between epilepsy and inflammation may not depend on the periphery, but with excessive inflammation of the CNS. It has not yet been reported whether YKL-40 produced in the periphery can affect CSF levels. This issue is important in the future studies because peripheral inflammation is not uncommon in people most likely to screen for epilepsy.

Except for the levels of CSF YKL-40 in SGTCS and GTCS were significantly higher than those in AS, serum and CSF YKL-40 levels were not significantly different between the other groups, indicating that YKL-40 was not specific in different types of seizure.

We found that serum YKL-40 identified DRE in control versus DRE, NDE versus DRE, and DPE versus DRE after adjustment for age and gender, while serum YKL-40 had the higher accuracy for DRE in control

versus DRE. Interestingly, serum YKL-40 had the relatively high AUC for NDE in control versus NDE. Similarly, CSF YKL-40 provided diagnostic accuracy for DRE. Neither serum YKL-40 nor CSF YKL-40 could identify DPE. We speculate YKL-40 to be less accurate in DPE than in NDE and DPE because patients with DPE had no seizures for several years.

Finally, there are some limitations in the present study. First, the sample size of the study is too small to draw final conclusions. Second, the control group samples were from patients with neurosis, but not from healthy subjects. Therefore, further studies with a large samples and collecting healthy controls are needed to confirm these findings. Third, elevated YKL-40 levels may suggest that YKL-40 plays a potential role in the pathogenesis of seizures or that it is a compensatory survival response. However, it remains unknown whether interfering with the activity of YKL-40 in epilepsy represents a new possibility for reducing the loss of human neurons. In addition, it is also not clear whether increased YKL-40 levels would be detectable early in patients who will develop DRE later.

In conclusion, we suggest that serum and CSF YKL-40 may be a potential biomarker for predicting disease outcome in epilepsy. This marker may allow a better stratification of patients with epilepsy. However, further research is needed to gain a more complete understanding of the underlying mechanisms behind these findings.

Conflicts of interest

The authors declare that they have no competing interests.

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