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Expression of NLRP3 Inflammasome in Febrile Seizures and Clinical Significance

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KEYWORDS Children. Expression. Inflammasome. Protein. Seizure

ABSTRACT The aim of this study was to explore the expression of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome in children with febrile seizures (FS). Onset age at 1-3 years old, body temperature during attack ≥38.5°C, complex FS, family history of seizures, duration of attack >15 min, electroencephalographic abnormality, serum NLRP3 level >1.75 ng/L and interleukin-1β (IL-1β) level >48.01 pg/mL were risk factors for recurrence after first attack (P<0.05). The area under the receiver operating characteristic curve, sensitivity and specificity of the prediction model in forest plot were 0.821 (95% confidence interval: 0.771-0.878), 0.922 and 0.695, respectively, suggesting high accuracy. The standard curve fitted well with the prediction curve, indicating high concordance between predicted recurrence and actual condition. Serum NLRP3 level >1.75 ng/L is a risk factor for recurrence after first attack in children, and NLRP3 level is accurate for predicting recurrence.

INTRODUCTION

Febrile seizure (FS) (Hashimoto et al. 2021), which is a common epileptic syndrome in neurology, frequently occurs in children aged between 6 months and 3 years old (Hautala et al. 2021). This disease severely affects the intelligence and cognitive development of children, but it can be relieved beyond 6 years old generally because of the brain development (Sheppard et al. 2021). Normally, FS is induced by upper respiratory tract infection or other infectious diseases (Sawires et al. 2022), and children have rapidly raised body temperature and seizure signs at the same time in the initial stage. Currently, the specific pathogenic factors for pediatric FS remain elusive, and it is rather difficult to prevent and to treat this disease timely and effectively due to complicated influencing factors. It has previously been reported that the expression levels of various inflammatory indices, such as interleukin-1β (IL-1β), procalcitonin and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome, were elevated in patients with seizures or epilepsy (Talebian et al. 2020).

NLRP3 inflammasome is a multiprotein complex in the cytoplasm and an important component of the innate immune system (Li et al. 2020). It mainly comprises NOD-like receptors, apoptosis-associated speck-like protein containing a CARD and phosphorylated caspase-1 precursors (Liu et al. 2020; Zhao et al. 2021). Since it is capable of dephosphorylating or activating phosphorylated caspase-1 to promote the maturation of IL-1β, NLRP3 inflammasome is the key to IL-1β maturation (Mishra et al. 2019). Moreover, as a vital constituent of innate immunity, NLRP3 inflammasome can be activated by various pathogens or danger signals (Ratajczak et al. 2020). It plays pivotal roles in immune responses and occurrence of many diseases, including familial periodic autoinflammatory response, type 2 diabetes mellitus, Alzheimer's disease, atherosclerosis and epilepsy (Fusco et al. 2020). Therefore, NLRP3 inflammasome may work as a new target for the treatment of inflammatory diseases.

Objectives

In this study, 268 FS children were selected to explore the expression of NLRP3 inflammasome and the clinical significance, aiming to provide novel insights into the clinical prevention and treatment of this disease.

MATERIAL AND METHODS

General Data

The clinical data of 268 FS children admitted to and treated in our hospital from January 2015

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to February 2018 were retrospectively analyzed. There were 152 boys and 116 girls aged between 3 months-6 years old, (3.7±1.6) years on average. There were 96 children with recurrence after first attack, and 172 children did not have recurrence, with a recurrence probability of 35.82 percent. Epilepsy ultimately occurred in 9 children (9.38%). This study was reviewed and approved the ethics committee of our hospital, and the guardians of the children were informed of the study and signed the informed consent.

The inclusion criteria involved: (1) children meeting the diagnostic criteria of pediatric FS (Tavasoli et al. 2014), (2) those with complete clinical data, (3) those who had the first attack at the age of 3 months-6 years old, and (4) those suffering from seizures during fever. The following exclusion criteria were used: (1) children with intracranial infection or other metabolic or organic diseases that may lead to seizures, (2) those with central nervous system infection accompanied by FS, or (3) those complicated with genetic diseases, neurocutaneous syndrome, birth defect or inborn error of metabolism.

Collection of General Data

The general information and data of the children were collected, including onset age, gender, body temperature during attack, type of attack, with or without family history of epilepsy, with or without family history of seizures, mode of attack, duration of attack, presence or absence of electroencephalographic abnormality, primary infectious disease, presence or absence of recurrence after first attack, recurrence frequency, with or without final progression into epilepsy, and levels of serum NLRP3 and IL-1β.

Methods

Fasting venous blood (3 mL) was collected from each child in the early morning and centrifuged to obtain the serum which was stored at -20°C for detection. The levels of NLRP3 inflammasome and NLRP3 in the serum were measured through double-antibody sandwich enzyme-linked immunosorbent assay according to the kit's instructions (TPI, USA).

Follow-up

The children were followed up by outpatient examination or telephone interview after first attack every 6 months, and the follow-up was terminated after 3 years or final progression into epilepsy. The content of follow-up involved the presence or absence of seizures (with time of attack recorded), body temperature during attack, frequency of attack, mode of attack, duration of attack, concomitant condition, presence or absence of electroencephalographic abnormality, and with or without progression into epilepsy.

Statistical Analysis

SPSS 23.0 software (IBM Inc., USA) was employed for statistical analysis. The normally distributed measurement data were expressed as mean ± standard deviation ($\overline{x} \pm s$) and compared between groups using the independent-samples *t*-test. The measurement data not in line with normal distribution were represented as $M\ (P_{25}, P_{75})$, and the Mann-Whitney U-test was used for intergroup comparison. The count data were expressed as frequency (%), and the χ^2 test was conducted for intergroup comparison. The risk factors for recurrence after first attack were analyzed using a multivariate logistic regression model. Forest plot was drawn by Empower Stats and "R" software package. The Bootstrap method was utilized for internal validation of the model calibration, and the discrimination of model was assessed by plotting receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) of NLRP3 level for predicting recurrence was calculated. The effects of NLRP3 level on recurrence were assessed by Kaplan-Meier survival curves. $P<0.05$ suggested that a difference was statistically significant.

RESULTS

Clinical Characteristics of FS Children

There were more children (67.91%) with FS at 1-3 years old. The boy/girl ratio was 1.3:1. The proportions of children with body temperature during attack >38°C (74.63%), simple FS (64.93%), no family history of epilepsy (88.43%), no family history of seizures (79.10%) and generalized sei-

zures (64.55%) were higher. Most children had a duration of attack <15 min (79.85%), normal electroencephalogram (78.36%) and upper respiratory tract infection as the primary infectious disease (76.49%). Additionally, the children with recurrence after first attack accounted for 35.82 percent of the total cases (Table 1).

Table 1: Clinical characteristics of FS children

Item	*n (268)*	*Constituent ratio (%)*
Onset Age [n (%)]		
3 months-1 year old	56	20.90
1-3 years old	182	67.91
3-6 years old	30	11.19
Gender [n (%)]		
Boy	152	56.72
Girl	116	43.28
Body Temperature During Attack [n (%)]		
≤38.5°C	68	25.37
>38.5°C	200	74.63
Type of Attack [n (%)]		
Simple	174	64.93
Complex	94	35.07
Family History of Epilepsy [n (%)]		
Yes	31	11.57
No	237	88.43
Family History of Seizures [n (%)]		
Yes	56	20.90
No	212	79.10
Mode of Attack [n (%)]		
Generalized	173	64.55
Focal/asymmetric	95	35.45
Duration of Attack [n (%)]		
<15 min	214	79.85
≥15 min	54	20.15
Electroencephalogram [n (%)]		
Normal	210	78.36
Abnormal	58	21.64
Primary Infectious Disease [n (%)]		
Upper respiratory tract infection	205	76.49
Pneumonia	6	2.24
Viral diarrhea	22	8.21
Bronchitis	30	11.19
Herpangina	5	1.87
Recurrence After First Attack [n (%)]		
Yes	96	35.82
No	172	64.18

a: χ^2 value, rest: *t* value

Influencing Factors for Recurrence after First Attack

Among the 96 children with recurrence, 48 (50.00%), 33 (34.38%) and 15 (15.63%) suffered from once, twice and 3 times of recurrence, respectively. Moreover, 9 (9.38%) children ultimately had epilepsy. Recurrence was correlated with onset age at 1-3 years old, body temperature during attack ≤38.5°C, complex FS, family history of seizures, duration of attack >15 min, electroencephalographic abnormality, and serum NLRP3 and IL-1β levels. Additionally, there were significant differences from those of the non-recurrence group (P<0.05) (Table 2).

Results of Multivariate Logistic Regression Analysis

Multivariate logistic regression analysis was conducted with recurrence after first attack as the dependent variable, and onset age, body temperature during attack, type of attack, family history of seizures, duration of attack, electroencephalogram, NLRP3 and IL-1β as independent variables. The results showed that onset age at 1-3 years old, body temperature during attack ≤38.5°C, complex FS, family history of seizures, duration of attack >15 min, electroencephalographic abnormality, serum NLRP3 level >1.75 ng/L and IL-1β level >48.01 pg/mL were risk factors for recurrence after first attack (P<0.05) (Table 3 and Fig. 1).

Model Evaluation Results

The prediction model was evaluated by plotting ROC curves. AUC was 0.821 (95% CI: 0.771-0.878), the sensitivity was 0.922, and the specificity was 0.695 (Fig. 2). According to internal data validation results, the standard curve fitted well with the prediction curve, suggesting high concordance between the predicted recurrence after first attack and the actual condition (Fig. 3).

Predictive Values of NLRP3 and IL-1β for Recurrence after First Attack

The NLRP3 and IL-1β levels rose remarkably in the recurrence group compared with those of the non-recurrence group. The AUC, optimal cut-off value, sensitivity and specificity of NLRP3 level for predicting recurrence after first attack were 0.810 (95% CI: 0.781-0.839, P<0.001), 1.75 ng/L, 94.01 percent and 66.42 percent, respectively. In terms of IL-1β level for predicting recurrence after first attack, AUC was 0.792 (95% CI: 0.776-0.808, P<0.001), the optimal cut-off value was 48.01 pg/L, the sensitivity was 88.05 percent, and the specificity was 64.03 percent. These results sug-

Table 2: Influencing factors for recurrence after first attack

Item	*Recurrence group (n=96)*	*Non-recurrence group (n=172)*	*Recurrence probability (%)*	*Statistical value*	*P*
Onset Age [n (%)]				9.567	0.008
3 months-1 year old	15 (15.63)	41 (23.84)	26.79	/	/
1-3 years old	76 (79.17)	106 (61.63)	41.76	/	/
3-6 years old	5 (5.21)	25 (14.53)	16.67	/	/
Gender [n (%)]				1.308	0.253
Boy	50 (52.08)	102 (59.30)	32.89	/	/
Girl	46 (47.92)	70 (40.70)	39.66	/	/
Body Temperature During Attack [n (%)]				9.707	0.002
≤38.5°C	35 (36.46)	33 (19.19)	51.47	/	/
>38.5°C	61 (63.54)	139 (80.81)	30.50	/	/
Type of Attack [n (%)]				4.944	0.026
Simple	54 (56.25)	120 (69.77)	31.03	/	/
Complex	42 (43.75)	52 (30.23)	44.68	/	/
Family History of Epilepsy [n (%)]				3.803	0.051
Yes	16 (16.67)	15 (8.72)	51.61	/	/
No	80 (83.33)	157 (91.28)	33.76	/	/
Family History of Seizures [n (%)]				16.443	0.000
Yes	33 (34.38)	23 (13.37)	58.93	/	/
No	63 (65.63)	149 (86.63)	29.72	/	/
Mode of Attack [n (%)]				2.528	0.112
Generalized	56 (58.33)	117 (68.02)	32.37	/	/
Focal/asymmetric	40 (41.67)	55 (31.98)	42.11	/	/
Duration of Attack [n (%)]				9.407	0.002
≤15 min	67 (69.79)	147 (85.47)	31.31	/	/
>15 min	29 (30.21)	25 (14.53)	53.70	/	/
Electroencephalogram [n (%)]				8.143	0.004
Normal	66 (68.75)	144 (83.72)	31.43	/	/
Abnormal	30 (31.25)	28 (16.28)	51.72	/	/
Primary Infectious Disease [n (%)]				1.240	0.744
Upper respiratory tract infection	77 (80.21)	128 (74.42)	37.56	/	/
Pneumonia	2 (2.08)	4 (2.33)	33.33	/	/
Viral diarrhea	7 (7.29)	15 (8.72)	31.82	/	/
Bronchitis	9 (9.38)	21 (12.21)	30.00	/	/
Herpangina	1 (1.04)	4 (2.33)	20.00	/	/
NLRP3 (ng/L)	2.41 ±0.53	1.10 ±0.29	/	26.170[a]	0.000
IL-1β (pg/mL)	60.69±10.48	35.34 ±6.56	/	24.329[a]	0.000

a: *t* value, rest: χ^2 value

Table 3: Results of multivariate logistic regression analysis on influencing factors for recurrence after first attack

Variable	*β*	*SE*	*Wald* χ^2	*OR (95% CI)*	*P*
Onset age at 1-3 years old	0.955	0.313	6.102	1.635 (1.107,2.281)	0.014
Body temperature during attack ≤38.5°C	0.819	0.293	5.590	2.121 (1.256,3.103)	0.005
Complex FS	1.145	0.306	7.484	1.428 (1.027,1.947)	0.032
With family history of seizures	1.196	0.300	7.973	2.110 (1.375,2.963)	0.001
Duration of attack >15 min	1.300	0.317	8.202	2.018 (1.207,2.946)	0.005
Electroencephalographic abnormality	1.026	0.292	7.024	1.882 (1.132,2.750)	0.009
NLRP3 level >1.75 ng/L	0.518	0.202	5.129	2.751 (1.533,4.087)	<0.001
IL-1β level >48.01 pg/mL	0.518	0.202	5.129	2.393 (1.532,3.372)	<0.001

CI: Confidence interval; OR: odds ratio; SE: standard error

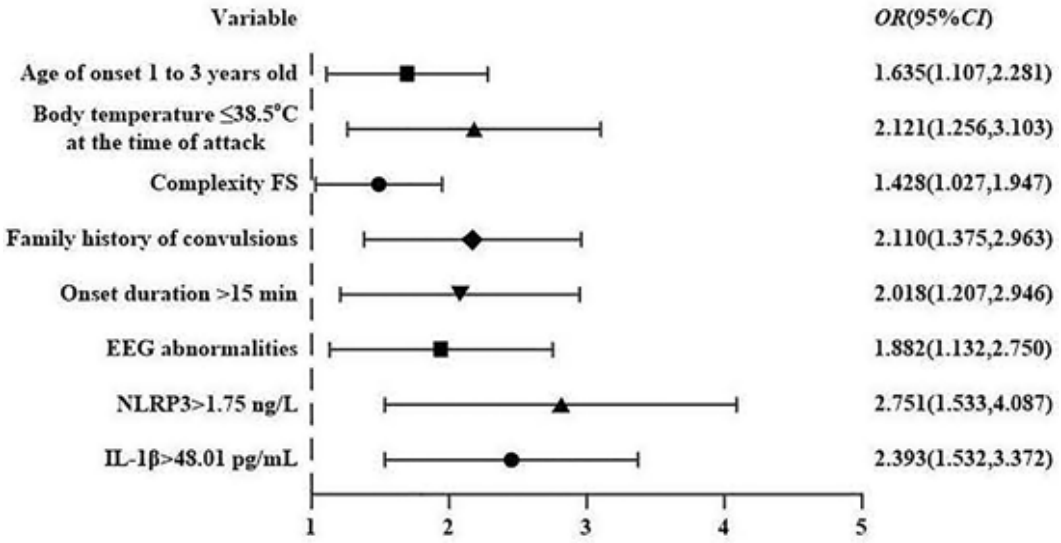

Fig. 1. Forest plot of risk factors for recurrence after first attack
Multivariate logistic regression analysis was conducted with recurrence after first attack as the dependent variable, and onset age, body temperature during attack, type of attack, family history of seizures, duration of attack, electroencephalogram, NLRP3 and IL-1β as independent variables. CI: Confidence interval; EEG: electroencephalogram; FS: febrile seizures; IL-1β: interleukin-1β; NLRP3: nucleotide-binding oligomerization domain-like receptor protein 3; OR: odds ratio

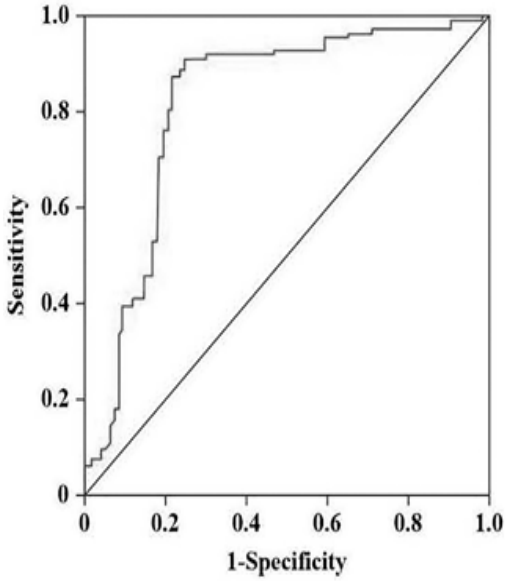

Fig. 2. ROC curve of prediction model
The prediction model was evaluated by plotting ROC curves. ROC: receiver operating characteristic

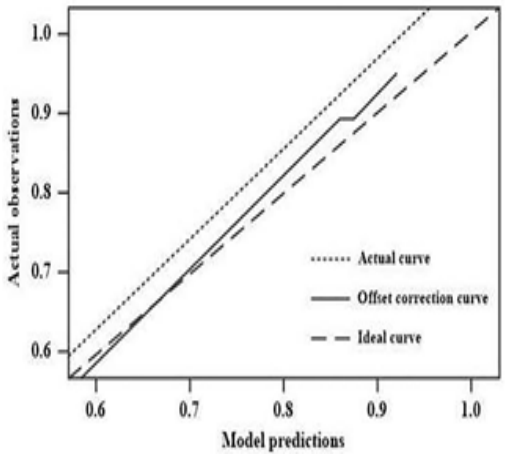

Fig. 3. Calibration chart of internal validation of model
The Bootstrap method was utilized for internal validation of the model calibration

gested that NLRP3 was superior to IL-1β in diagnostic efficiency (Fig. 4).

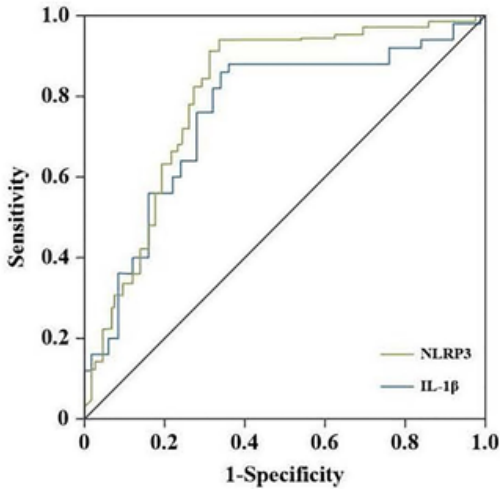

Fig. 4. ROC curve of NLRP3 level for predicting recurrence after first attack in children
The area under the ROC curve of NLRP3 level for predicting recurrence was calculated. IL-1β: Interleukin-1β; NLRP3: nucleotide-binding oligomerization domain-like receptor protein 3; ROC: receiver operating characteristic

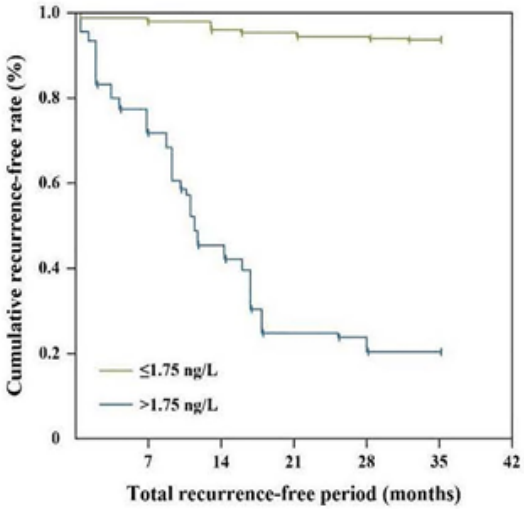

Fig. 5. Survival curves of children
The effects of NLRP3 level on recurrence were assessed by Kaplan-Meier survival curves. NLRP3: Nucleotide-binding oligomerization domain-like receptor protein 3

Analysis Results of Survival Curves

The children with NLRP3 level >1.75 ng/L had a recurrence probability of 79.63 percent (86/108), and those with NLRP3 level ≤1.75 ng/L had a recurrence probability of 6.25 percent (10/160), showing a significant difference ($P<0.05$) (Fig. 5).

DISCUSSION

Pediatric FS is mainly manifested as sudden violent spasm and convulsion of systemic or local muscle groups, accompanied by short-term mental confusion, which has a good prognosis in most children but may threaten patients' life in severe cases (Akbayram et al. 2012; Menze et al. 2021). The specific pathogenesis of FS remains largely unknown, so finding the risk factors as well as targeted preventive and therapeutic methods is crucial for reducing the probabilities of FS attack and recurrence (Yang et al. 2021).

The roles of immune inflammasome-mediated inflammatory responses in the onset and progression of various diseases have attracted widespread attention (Zhang et al. 2020). In particular, NLRP3 inflammasome is most widely studied and regarded as a potential therapeutic target (Wang et al. 2019). However, the expression of NLRP3 inflammasome in FS cases or the clinical significance has never been reported hitherto. In this study, therefore, the clinical data of 268 FS children were retrospectively analyzed. Of all the children, 96 suffered from recurrence after first attack, but 172 did not. Recurrence had correlations with onset age, body temperature during attack, type of attack, with or without family history of seizures, duration of attack, presence or absence of electroencephalographic abnormality, and levels of serum NLRP3 and IL-1β. Besides, onset age at 1-3 years old, body temperature during attack ≤38.5°C, complex FS, family history of seizures, duration of attack >15 min, electroencephalographic abnormality, serum NLRP3 level >1.75 ng/L and IL-1β level >48.01 pg/mL were risk factors for recurrence after first attack.

For children aged 1-3 years old, the brain is developing at a rapid but unstable rate. The neuronal structure is simple, but the functional differentiation, dendrites, axons and myelin sheaths are underdeveloped (Bartkowska et al. 2022). In addition, the chemical composition and enzymat-

ic activity in the tissues of these children are different from those in mature brain tissues, and the threshold of seizures is low, so they are vulnerable to FS (Juul et al. 2023). The children aged below 1 year old have highly immature brain development, with dominant inhibitory activities, and those with an age above 3 years old have gradually well-developed brain. As a result, FS rarely occurs in the two age groups. Besides, the patients with a lower body temperature during the initial episode of FS are more susceptible to recurrence (Kubota et al. 2021). In addition, complex FS has a higher probability of progression into secondary epilepsy than simple FS (Smith et al. 2019). Moreover, FS is an autosomal dominant inherited disease accompanied by incomplete penetrance or polygenic inheritance, with a tendency to familial inheritance (Holm et al. 2012; Çomak et al. 2015). Furthermore, electroencephalogram is a crucial auxiliary examination method for FS diagnosis, and abnormal results indicate certain injury of brain function. The combined action of seizures and fever can influence the brain function of children, and a longer time results in severer brain injury (Gunawardena et al. 2022). Hence, FS cases are prone to recurrence. Lastly, the high levels of serum NLRP3 and IL-1β indicate the occurrence of inflammatory responses (Cai et al. 2021).

The results of ROC curve analysis exhibited that the prediction model in forest plot had high accuracy. The internal data validation results of the model showed that the predicted recurrence was consistent with the actual condition. Furthermore, the AUC, optimal cut-off value, sensitivity and specificity of NLRP3 level for predicting recurrence were 0.810, 1.75 ng/L, 94.01 percent and 66.42 percent, respectively. In terms of IL-1β level for predicting recurrence after first attack, the AUC (0.792), sensitivity and specificity were lower than those of NLRP3 level. Hence, NLRP3 level has higher diagnostic efficiency than that of IL-1β level. Furthermore, the recurrence probability was 79.63 percent in children with NLRP3>1.75 ng/L and 6.25 percent in those with NLRP≤31.75 ng/L, with a significant difference.

CONCLUSION

In conclusion, serum NLRP3 level >1.75 ng/L is a risk factor for recurrence after first attack in children, and NLRP3 level is accurate for predicting recurrence. Children with NLRP3 level >1.75 ng/L have a recurrence probability of 79.63 percent, and those with NLRP3 level ≤1.75 ng/L have a recurrence probability of 6.25 percent.

RECOMMENDATIONS

NLRP3 inflammasome is associated with FS recurrence after first attack in children, so it can be employed as a novel target for FS prevention and treatment.

ABBREVIATIONS

AUC: Area under the ROC curve; CI: confidence interval; FS: febrile seizures; IL-1β: interleukin-1β; NLRP3: nucleotide-binding oligomerization domain-like receptor protein 3; ROC: receiver operating characteristic.

REFERENCES

Akbayram S, Cemek M, Büyükben A et al. 2012. Major and minor bio-element status in children with febrile seizure. *Bratisl Lek Listy,* 113: 421-423.

Bartkowska K, Tepper B, Turlejski K et al. 2022. Postnatal and adult neurogenesis in mammals, including marsupials. *Cells*, 11: 2735.

Cai J, Guan H, Jiao X et al. 2021. NLRP3 inflammasome mediated pyroptosis is involved in cadmium exposure-induced neuroinflammation through the IL-1β/IkB-α-NF-κB-NLRP3 feedback loop in swine. *Toxicology*, 453: 152720.

Çomak E, Tüfekçi Ö, Kiliçbay F et al. 2015. Febrile seizures in children with familial Mediterranean fever: Coincidence or association? *Eur J Paediatr Neurol,* 19: 572-576.

Fusco R, Siracusa R, Genovese T et al. 2020. Focus on the role of NLRP3 inflammasome in diseases. *Int J Mol Sci*, 21: 4223.

Gunawardena S, Chikkannaiah M, Stolfi A et al. 2022. Utility of electroencephalogram in the pediatric emergency department. *Am J Emerg Med*, 54: 26-29.

Hashimoto R, Suto M, Tsuji M et al. 2021. Use of antipyretics for preventing febrile seizure recurrence in children: A systematic review and meta-analysis. *Eur J Pediatr,* 180: 987-997.

Hautala M, Arvila J, Pokka T et al. 2021. Respiratory viruses and febrile response in children with febrile seizures: A cohort study and embedded case-control study. *Seizure*, 84: 69-77.

Holm IA, Poduri A, Crandall L et al. 2012. Inheritance of febrile seizures in sudden unexplained death in toddlers. *Pediatr Neurol*, 46: 235-239.

Juul HM, Brooks-Kayal AR, Talos DM 2023. Why do febrile seizures involve specifically the developing

brain? In: TZ Baram, S Shinnar, CE Stafstrom (Eds.): *Febrile Seizures*. London, United Kingdom: Academic Press, pp. 155-178.

Kubota J, Higurashi N, Hirano D et al. 2021. Body temperature predicts recurrent febrile seizures in the same febrile illness. *Brain Dev*, 43: 768-774.

Li Z, Guo J, Bi L 2020. Role of the NLRP3 inflammasome in autoimmune diseases. *Biomed Pharmacother*, 130: 110542.

Liu Z, Xian H, Ye X et al. 2020. Increased levels of NLRP3 in children with febrile seizures. *Brain Dev*, 42: 336-341.

Menze ET, Ezzat H, Shawky S et al. 2021. Simvastatin mitigates depressive-like behavior in ovariectomized rats: Possible role of NLRP3 inflammasome and estrogen receptors' modulation. *Int Immunopharmacol*, 95: 107582.

Mishra N, Schwerdtner L, Sams K et al. 2019. Cutting edge: protein arginine deiminase 2 and 4 regulate NLRP3 inflammasome–dependent IL-1β maturation and ASC speck formation in macrophages. *J Immunol*, 203: 795-800.

Ratajczak MZ, Bujko K, Cymer M et al. 2020. The Nlrp3 inflammasome as a "rising star" in studies of normal and malignant hematopoiesis. *Leukemia*, 34: 1512-1523.

Sawires R, Buttery J, Fahey M 2022. A review of febrile seizures: Recent advances in understanding of febrile seizure pathophysiology and commonly implicated viral triggers. *Front Pediatr*, 9: 801321.

Sheppard E, Lalancette E, Thébault Dagher F et al. 2021. Cognitive and behavioural development in children presenting with complex febrile seizures: At onset and school age. *Epilep Disord*, 23: 325-336.

Smith DK, Sadler KP, Benedum M 2019. Febrile seizures: Risks, evaluation, and prognosis. *Am Fam Physician*, 99: 445-450.

Talebian A, Hassani F, Nikoueinejad H et al. 2020. Investigating the relationship between serum levels of Interleukin-22 and Interleukin-1 Beta with febrile seizure. *Iran J Allergy Asthma Immunol*, 19: 409-415.

Tavasoli A, Afsharkhas L, Edraki A 2014. Frequency of meningitis in children presenting with febrile seizures at Ali-Asghar Children's Hospital. *Iran J Child Neurol*, 8: 51-56.

Wang B, Cao X, Lin J et al. 2019. Up-regulation of microRNA-223 inhibits brain injury and hippocampal neuron apoptosis of rats after febrile seizure through the NLRP3-Caspase-1 signaling pathway. *Biomed Pharmacother*, 114: 108683.

Yang JH, Villegas R, Khanna S et al. 2021. The utility of infectious and neurodiagnostic testing in children with complex febrile seizures requiring mechanical ventilation. *J Child Neurol*, 36: 735-742.

Zhang Y, Zhao Y, Zhang J et al. 2020. Mechanisms of NLRP3 inflammasome activation: its role in the treatment of Alzheimer's disease. *Neurochem Res*, 45: 2560-2572.

Zhao P, Cao L, Wang X et al. 2021. Extracellular vesicles secreted by Giardia duodenalis regulate host cell innate immunity via TLR2 and NLRP3 inflammasome signaling pathways. *PLoS Negl Trop Dis*, 15: e0009304.

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