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Longitudinal Change of Serum Inter-α-Trypsin Inhibitor Heavy Chain H4 and its Relation with Inflammation, Disease Recurrence, and Mortality in Acute Ischemic Stroke Patients

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Inter-α-trypsin inhibitor heavy chain H4 (ITIH4) modulates atherosclerosis, lipid, and inflammation, which is involved in the development of acute ischemic stroke. Hence, this study aimed to investigate the longitudinal change and prognostic role of ITIH4 in acute ischemic stroke. In 267 patients with acute ischemic stroke, serum ITIH4 after admission (baseline), the 1st day after admission (D1), D3, D7, and D30, and inflammatory cytokines at baseline were detected by enzyme-linked immunosorbent assay (ELISA). Additionally, serum ITIH4 of 30 controls after enrollment was detected by ELISA. ITIH4 was reduced in acute ischemic stroke patients than controls [median (interquartile range, IQR): 131.0 (95.5-194.3) vs. 418.6 (241.5-506.8) ng/mL] ($P < 0.001$). Among acute ischemic stroke patients, ITIH4 was negatively associated with tumor necrosis factor-alpha ($r = -0.211$, $P = 0.001$), interleukin (IL)-1β ($r = -0.164$, $P = 0.007$), IL-6 ($r = -0.121$, $P = 0.049$), and IL-17A ($r = -0.188$, $P = 0.002$). ITIH4 presented a decreased trend from admission to D3, then increased from D3 to D30 ($P < 0.001$). The 1-year, 2-year, and 3-year cumulative recurrence rate was 7.5%, 18.0%, and 19.1%, respectively; meanwhile, 1-year, 2-year, and 3-year cumulative death rate was 2.2%, 7.1%, and 7.1%, accordingly. The further analysis presented that ITIH4 at baseline ($P = 0.002$), D1 ($P = 0.049$), D3 ($P = 0.003$), D7 ($P < 0.001$), and D30 ($P < 0.001$) was decreased in recurrent patients than non-recurrent patients; besides, ITIH4 at D3 ($P = 0.017$), D7 ($P = 0.004$), and D30 ($P = 0.002$), but not at baseline ($P = 0.151$) or D1 ($P = 0.013$), was decreased in deaths than survivors. Serum ITIH4 declines at first and then elevates with time, and its reduction is correlated with higher inflammation, increased risk of recurrence and mortality in acute ischemic stroke patients.

Keywords: acute ischemic stroke; inflammation; inter-α-trypsin inhibitor heavy chain H4; recurrence; survival
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Introduction

Acute ischemic stroke (AIS) is a common cerebral vascular disease that induces a huge social burden worldwide (Hurford et al. 2020). In China, the prevalence and mortality of AIS is approximately 2 million and 1.1 million per year, respectively (Wu et al. 2019). AIS not only causes both permanent cognitive and functional impairment, but is also viewed as one of the key reasons for deaths globally (Virani et al. 2020; Hasan et al. 2021). Over the decades, even though plenty of intensive treatment strategies are applied in AIS patients (including mechanical thrombectomy, intravenous thrombolysis, revascularization therapy, etc.), a proportion of patients still face high rates of recurrence and mortality (Herpich and Rincon 2020; Li et al. 2021; Petty et al. 2021). Considering that there still exist intractable challenges in the management of AIS, it is imperative to explore potential biomarkers to reflect disease risk and prognosis of AIS.

Inter-α-trypsin inhibitor heavy chain H4 (ITIH4), a

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member of the serine protease inhibitor family, is a liver-originated plasma glycoprotein (Zhuo and Kimata 2008; Ma et al. 2021). It has been reported that ITIH4 modulates atherosclerosis and lipids, which are involved in the development of AIS (Malaud et al. 2012; Hao et al. 2022; Hsu et al. 2022). Importantly, ITIH4 has the capability of regulating inflammation, and the latter is detrimental to the severity and functional outcomes of AIS (Shi et al. 2019; Ma et al. 2021; Huang 2023). For instance, ITIH4 is able to regulate inflammation through modulating Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway in *Escherichia coli* infected mice model (Ma et al. 2021). In the clinical field, one study has reported that ITIH4 is linked to decreased infarct volume; meanwhile, ITIH4 is declined in 72 h after admission and then elevated with the improvement of AIS, while the sample size of the study is limited (only 5 patients) (Nayak et al. 2012).

Thus, the present study aimed to explore the longitudinal change of ITIH4 and its linkage with pro-inflammatory cytokines, recurrence, and survival in 267 AIS patients.

Methods

Participants

Between February 2018 and September 2020, a total of 267 first episode AIS patients were consecutively enrolled in this study. The enrollment criteria contained: (a) diagnosis of AIS via Guidelines of American Stroke Association (Jauch et al. 2013); (b) over 18 years old; (c) with no intracranial hemorrhage; (d) volunteered to provide serum samples and comply with the study assessment. Patients who were complicated with cancers or hematologic malignant diseases were excluded. Additionally, a total of 30 subjects with a high risk of stroke were also enrolled as controls. The age and sex of controls were matched to them of AIS patients. The inclusion criteria were: (a) had high risk of stroke (had at least 2 stroke risk factors, which included smoke, hypertension, hyperlipidemia, diabetes mellitus, chronic kidney disease, cardiovascular disease, shortage of exercise, etc.); (b) elder than 18 years old; (c) had no history of stoke; (d) willing to participate in the study. The present study was approved by the Ethics Committee of Lishui Municipal Central Hospital with approval number of 201706. Each participant signed the informed consent.

Data collection and sample processing

After enrollment, demographics were obtained from all participants, and disease characteristics were collected from AIS patients. Serum samples of AIS patients were gained at admission, the 1st day after admission (D1), the 3rd day after admission (D3), the 7th day after admission (D7), and the 30th day after admission (D30); meanwhile, serum samples of controls were collected after inclusion. Following that, the levels of ITIH4 and inflammatory cytokines were examined by enzyme-linked immunosorbent assay (ELISA). The Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China) was commissioned to produce ELISA kits and complete the corresponding assays. The kits used in the study were as follow: Human ITIH4 ELISA Kit (No. Cat. ml037314), Human TNF-α ELISA Kit (No. Cat. ml061140), Human IL-1β ELISA Kit (No. Cat. ml058059), Human IL-6 ELISA Kit (No. Cat. ml058097), and Human IL-17A ELISA Kit (No. Cat. ml058052).

Assessment

AIS patients received standardized follow-up until March 2022. The median follow-up was 19.0 months, with a range of 4.0-38.0 months. Based on the follow-up, the disease status (recurrence or death) of AIS patients was recorded. The cumulative recurrence rate and cumulative death rate were calculated. Patients who did not experience recurrence or death at analysis were censored at their last date of disease assessment.

Statistics

SPSS (version 22.0, IBM Corp., Armonk, NY, USA) was applied for analysis, and GraphPad Prism (version 7.01, GraphPad Software Inc., San Diego, CA, USA) was applied for graphing. Normal distributed continuous variable was presented as mean value ± standard deviation (SD). Skewed distributed continuous variable was presented as median (interquartile range). Categorized variable was presented as count (percentage). Comparisons of data from participants were determined by Wilcoxon rank sum test, Chi-square test, or Student's t-test. Associations of variables were evaluated by Spearman's rank correlation test. The change in ITIH4 over time was assessed by Friedman's test. Multivariate Cox's proportional hazards regression analysis was performed for predictive factor screening. $P < 0.05$ was considered significant.

Results

Clinical characteristics of controls and AIS patients

The study flow was illustrated in Fig. 1. The mean age of 30 controls and 267 AIS patients were 65.4 ± 8.7 and 65.1 ± 9.1 years, accordingly. There were 6 (20.0%) females and 24 (80.0%) males among the controls; meanwhile, AIS patients included 77 (28.8%) females and 190 (71.2%) males. From the comparison analyses, no difference in demographics (including age, sex, history of smoking, etc.) was found between controls and AIS patients (all $P > 0.05$). Regarding disease characteristics among AIS patients, the mean National Institute Health of Stroke Scale score was 9.4 ± 5.3; besides, the median (interquartile range; IQR) value of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-17A were 70.5 (58.7-93.7), 2.6 (1.5-3.8), 37.3 (27.6-55.5), and 65.4 (54.0-78.5) pg/mL, respectively (Table 1).

Comparison of ITIH4 between controls and AIS patients

In order to compare the difference in ITIH4 between

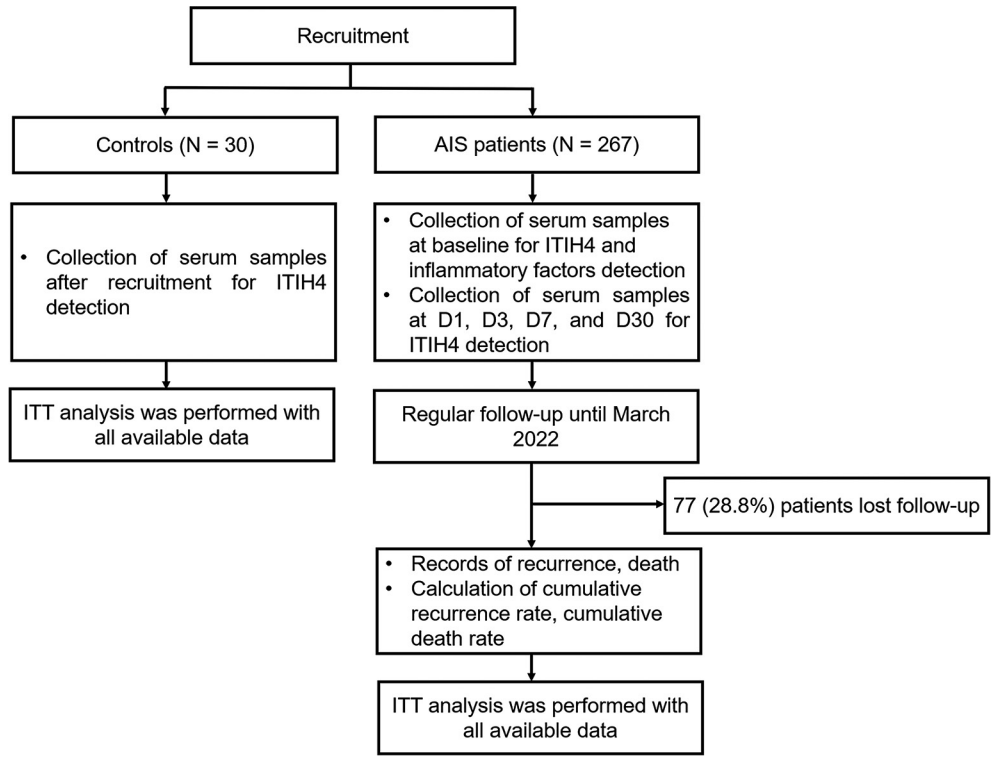


Fig. 1. Study flow chart.
AIS, acute ischemic stroke; ITIH4, inter-*α*-trypsin inhibitor heavy chain H4; D1, the 1st day after admission; D3, the 3rd day after admission; D7, the 7th day after admission; D30, the 30th day after admission; ITT analysis, intention-to-treat analysis.

Table 1. Clinical characteristics.

Items	Controls (N = 30)	AIS patients (N = 267)	*P* value
Age (years), mean ± SD	65.4 ± 8.7	65.1 ± 9.1	0.869
Sex, n (%)			0.306
Female	6 (20.0)	77 (28.8)	
Male	24 (80.0)	190 (71.2)	
BMI (kg/m^2), mean ± SD	24.1 ± 2.6	24.4 ± 2.5	0.448
History of smoke, n (%)	14 (46.7)	142 (53.2)	0.498
History of hypertension, n (%)	21 (70.0)	217 (81.3)	0.142
History of hyperlipidemia, n (%)	12 (40.0)	133 (49.8)	0.308
History of diabetes mellitus, n (%)	6 (20.0)	66 (24.7)	0.567
History of chronic kidney disease, n (%)	3 (10.0)	52 (19.5)	0.205
History of cardiovascular disease, No. (%)	6 (20.0)	84 (31.5)	0.195
Time since symptom to admission (hours), median (IQR)	-	4.0 (3.0-6.0)	-
Treatment, No. (%)			-
Thrombolysis	-	217 (81.3)	
Mechanical embolectomy	-	50 (18.7)	
NIHSS score, mean ± SD	-	9.4 ± 5.3	-
TNF-α (pg/mL), median (IQR)	-	70.5 (58.7-93.7)	-
IL-1β (pg/mL), median (IQR)	-	2.6 (1.5-3.8)	-
IL-6 (pg/mL), median (IQR)	-	37.3 (27.6-55.5)	-
IL-17A (pg/mL), median (IQR)	-	65.4 (54.0-78.5)	-

AIS, acute ischemic stroke; SD, standard deviation; BMI, body mass index; IQR, interquartile range; NIHSS, National Institute Health of Stroke Scale; TNF-*α*, tumor necrosis factor alpha; IL-1*β*, interleukin-1*β*; IL-6, interleukin-6; IL-17A, interleukin 17A.

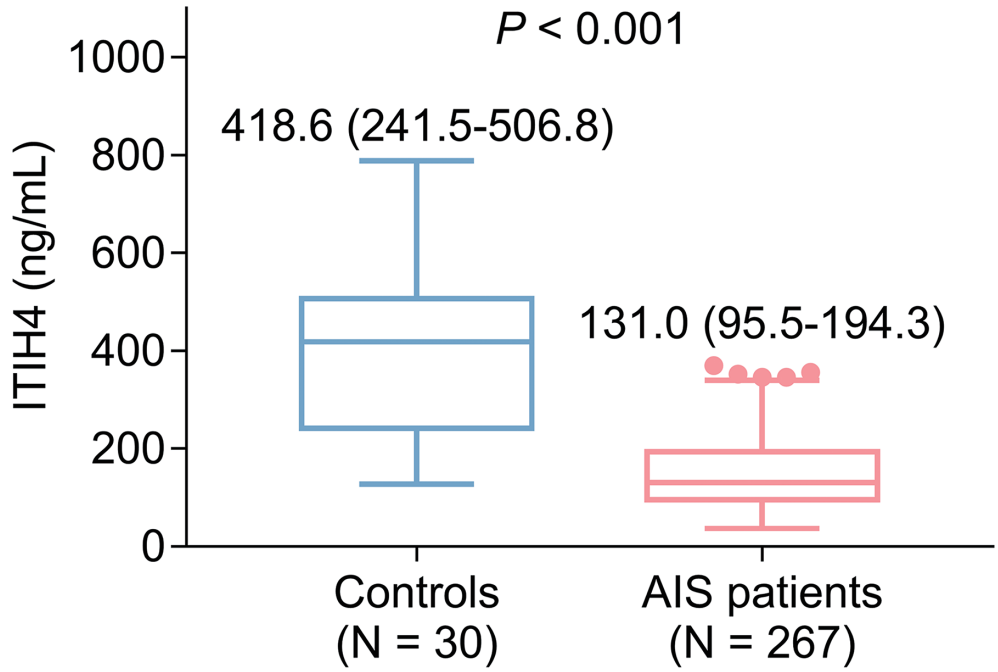


Fig. 2. ITIH4 was decreased in acute ischemic stroke (AIS) patients than in controls. Data are shown as median (IQR).

controls and AIS patients, serum samples were collected among controls after enrollment and AIS patients at baseline, which illustrated that a reduction of ITIH4 was found in AIS patients than in controls [median (IQR): 131.0 (95.5-194.3) vs. 418.6 (241.5-506.8) ng/mL] ($P < 0.001$) (Fig. 2).

Correlation between ITIH4 and inflammation in AIS patients

To investigate the relation between inflammation and ITIH4 in AIS patients, inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-17A) from serum samples of AIS patients at admission were collected, which presented that ITIH4 was negatively related to TNF-α ($r = -0.211$, $P = 0.001$) (Fig. 3A), IL-1β ($r = -0.164$, $P = 0.007$) (Fig. 3B), IL-6 ($r = -0.121$, $P = 0.049$) (Fig. 3C), and IL-17A ($r = -0.188$, $P = 0.002$) (Fig. 3D).

Longitudinal change of ITIH4 in AIS patients

ITIH4 presented a decreasing trend from admission to D3, then turned into an increasing trend from D3 to D30 among AIS patients ($P < 0.001$). Specifically, the median (IQR) levels of ITIH4 at admission, D1, D3, D7, and D30 were 131.0 (95.5-194.3) ng/mL, 107.0 (74.7-155.1) ng/mL, 105.5 (68.6-151) ng/mL, 149.5 (87.0-212.3) ng/mL, and 184.5 (123.7-257.4) ng/mL, respectively (Fig. 4).

Correlation of ITIH4 with recurrence and death in AIS patients

The 1-year, 2-year, and 3-year cumulative recurrence rates were 7.5%, 18.0%, and 19.1%, respectively; meanwhile, 1-year, 2-year, and 3-year cumulative death rates were 2.2%, 7.1%, and 7.1%, accordingly (Table 2). Then, further analysis presented that ITIH4 at baseline ($P = 0.002$), D1 ($P = 0.049$), D3 ($P = 0.003$), D7 ($P < 0.001$), and D30 ($P < 0.001$) were decreased in recurrent patients compared to non-recurrent patients (Fig. 5A); besides, ITIH4 at D3 ($P = 0.017$), D7 ($P = 0.004$), and D30 ($P = 0.002$), but not at baseline ($P = 0.151$) or D1 ($P = 0.113$), was decreased in deaths compared to survivors (Fig. 5B). Multivariate Cox's proportional hazards regression analysis presented that ITIH4 at D30 independently predicted higher recurrence-free survival [$P < 0.001$, hazard ratio (HR) = 0.992] and prolonged overall survival ($P = 0.006$, HR = 0.991) among AIS patients (Supplementary Table S1).

Discussion

So far, even though AIS diagnosis (such as magnetic resonance imaging and computed tomography) has made great progress, the exploration of potential biomarkers to reflect the disease risk of AIS is still imperative (Ren et al. 2022; Yang et al. 2022). Previous studies have reported that ITIH4 is dysregulated in cerebral-cardiovascular diseases. For instance, ITIH4 is declined in patients with coronary heart disease (Xu et al. 2013); moreover, it also has been reported that ITIH4 is elevated in atherosclerotic plaque samples (Malaud et al. 2012). In addition, research has presented that ITIH4 protein is reduced in AIS patients compared to healthy populations (Kashyap et al. 2009). In the present study, ITIH4 was decreased in AIS patients than in controls, which was in line with the previous study (Kashyap et al. 2009). The potential explanations might be that: (1) ITIH4 might play a protective role in the central nervous system by suppressing the extent of cerebral infarction and neuron death (Kashyap et al. 2009; Nayak et al.

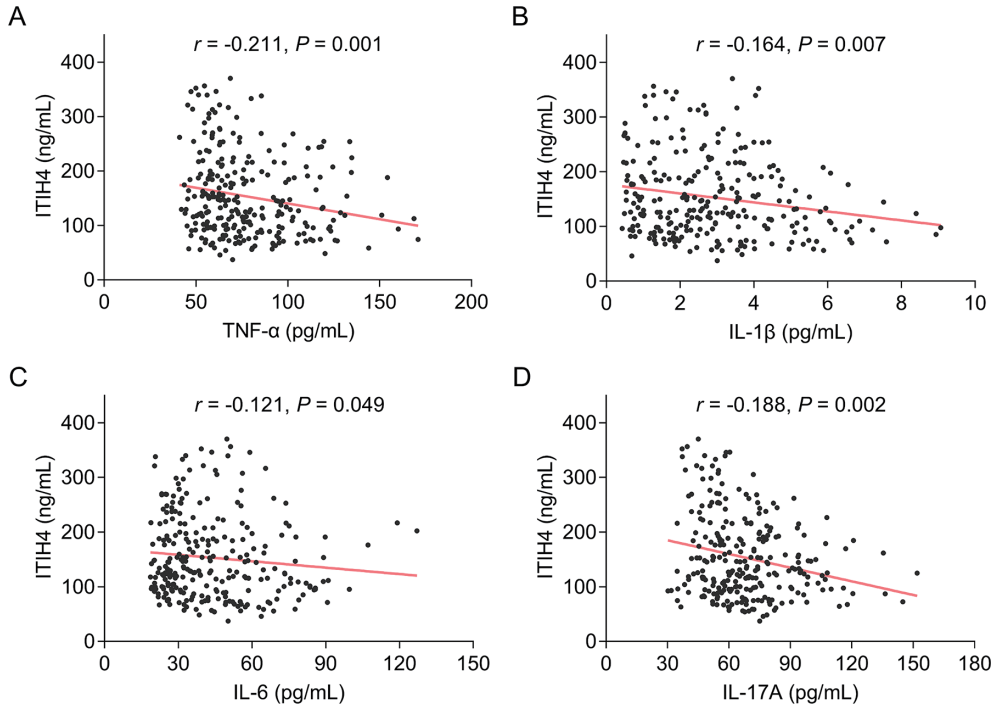
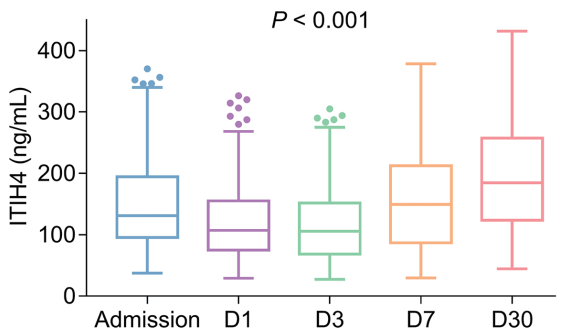


Fig. 3. ITIH4 was negatively linked with pro-inflammatory cytokines. Correlation of ITIH4 with TNF-α (A), IL-1β (B), IL-6 (C), and IL-17A (D).



ITIH4 (ng/mL)	Median (IQR)
Admission	131.0 (95.5-194.3)
D1	107.0 (74.7-155.1)
D3	105.5 (68.6-151.5)
D7	149.5 (87.0-212.3)
D30	184.5 (123.7-257.4)

Fig. 4. ITIH4 was initially decreased from admission to day 3 (D3), and then increased from D3 to D30 among acute ischemic stroke (AIS) patients.

Table 2. Cumulative recurrence rate and cumulative death rate of acute ischemic stroke (AIS) patients.

Items	Cumulative recurrence rate	Cumulative death rate
1-year	7.5%	2.2%
2-year	18.0%	7.1%
3-year	19.1%	7.1%

2012; McCullough et al. 2021); thus, declined ITIH4 implied that the central nervous system got damaged, leading to the pathogenesis of AIS; (2) ITIH4 could inhibit the occurrence of atherosclerosis and consequently accelerate the pathogenesis of AIS (Malaud et al. 2012); thereby, reduced ITIH4 might be related to a higher risk of atherosclerosis, which resulted in a higher possibility of AIS (Riggs et al. 2022). Thus, declined ITIH4 was correlated with a higher risk of AIS.

Accumulating studies have illustrated that inflammation is involved in the progression of AIS (Kashyap et al. 2009; Nayak et al. 2012; Hinterdobler et al. 2021; Libby 2021; Huang 2023). Thus, it is crucial to identify potential biomarkers to reflect inflammation and then promote the management of AIS. Inspired by previous studies, we investigated the relation of ITIH4 with inflammation in AIS, which revealed that ITIH4 was negatively related to TNF-α, IL-1β, IL-6, and IL-17A among AIS patients. The potential reason might be that the light chain of ITIH4 could inhibit inflammation through several signalings, such

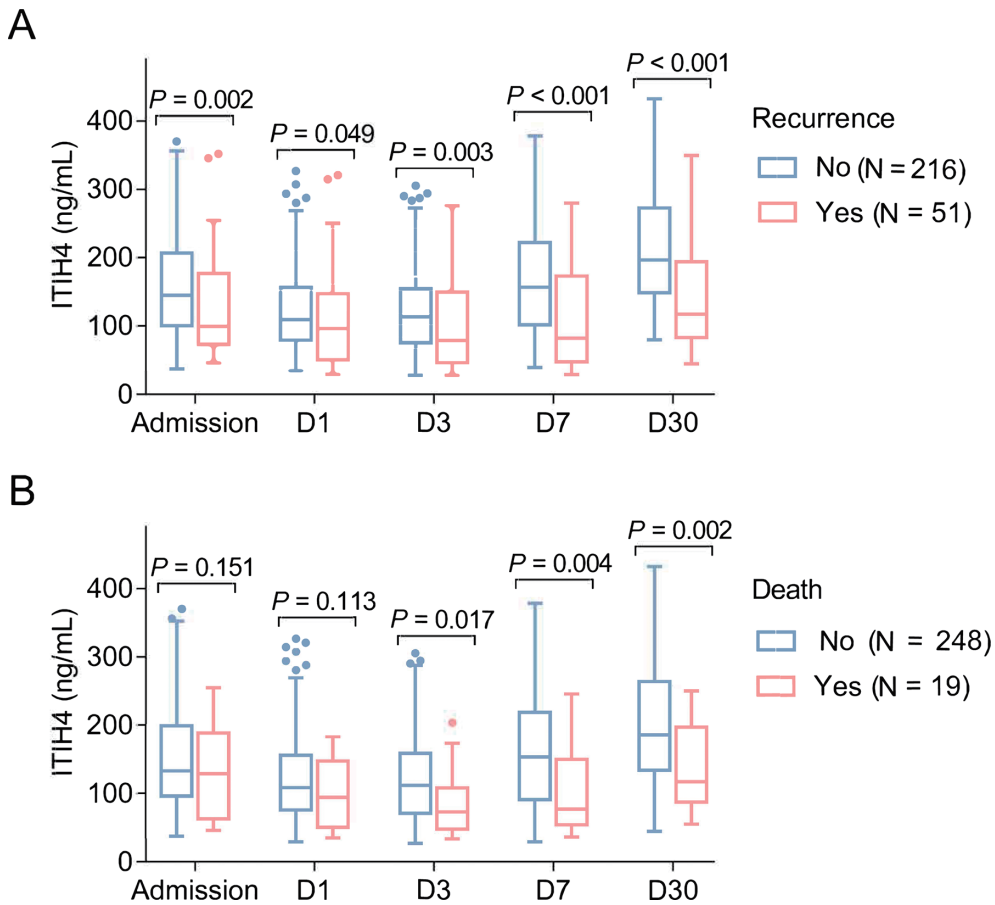


Fig. 5. ITIH4 was declined in acute ischemic stroke (AIS) patients with recurrence and death. Comparison of ITIH4 from baseline to day 30 (D30) between recurrent patients and non-recurrent patients (A), and between in deaths and survivors (B).

as JAK-STAT signaling and the nuclear factor kappa-B signaling (Stober et al. 2019; Ma et al. 2021). Thereby, a negative association was discovered between ITIH4 and inflammatory cytokines in AIS patients.

Apart from that, we also discovered that ITIH4 was decreased at first and then increased with time among AIS patients, which might be induced by the elevated neuroinflammation after the onset of the disease and then amelioration after treatment (Kashyap et al. 2009); meanwhile, ITIH4 could reflect the level of inflammation among AIS patients (above-mentioned); thus, ITIH4 was reduced and then increased with time in AIS. In addition, we also explored the linkage between ITIH4 and prognosis in AIS patients, which illustrated that ITIH4 was declined in recurrent patients and deaths. The potential explanation might be that ITIH4 could play a protective role in the central neuvous system and an anti-inflammatory role in AIS; thereby, reduced ITIH4 implied central nervous system damage and severe inflammation (Shi et al. 2019); thus, AIS patients with decreased ITIH4 might have more possibility of recurrence and death. Meanwhile, we also explored the prognostic value of ITIH4 at each time point for AIS patients, which showed that ITIH4 at D30 independently predicted higher survival, indicating ITIH4 at D30 possessed a relatively strong prognostic value among these patients.

Nevertheless, there existed several limitations in the present studies: (1) the role of ITIH4 in the regulatory mechanism of AIS could be investigated, which could contribute to the development of ITIH4-based treatment; (2) the correlation of ITIH4 with adhesion molecules in AIS patients could be further explored; (3) the follow-up period could be prolonged in the further study to evaluate the long-term prognostic value of ITIH4 in AIS patients.

In conclusion, ITIH4 is decreased at first and then increased with time, and its reduction is related to higher inflammation, elevated recurrence and increased mortality in AIS patients, which indicates that its vertical monitoring may help to promote the management of these patients.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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