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Original Article

Serum level of IL-1β and IL-18 in patients with traumatic brain injury and their effects on cognitive impairment

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Abstract: Objective: The purpose of this study was to evaluate the changes of cognitive function for patients undergoing traumatic brain injury (TBI) and its association with interleukin (IL)-1β and IL-18 concentration within 6 months post-injury. Methods: 102 patients with TBI were recruited in test group, while 56 persons undergoing orthopedic operation and 50 healthy volunteers were divided into control group. Cognitive function and IL-1β and IL-18 concentration in control group was assessed at the beginning of the study (T0). For the test group, the endpoint indexes were evaluated at 7 days (T0), 3 months (T1) and 6 months (T2) post-injury. We compared the changes of cognitive function and its association with interleukin concentration with time going in test group. Results: Patients in test group exhibited poor cognitive status and increased IL-1β and IL-18 level (18.39 ± 4.21, 99.73 ± 13.49 pg/mL, respectively), compared with those in the control group (7.62 ± 1.81, 54.78 ± 6.26 pg/mL, respectively). The cognitive impairment occurrence and interleukin level were significantly associated with injury intensity ($P<0.05$ for all). Moreover, cognitive function of patients with TBI was improved with time going. And the improvement was significantly associated with down-regulated IL-1β and IL-18 level ($P<0.05$ for all). Conclusion: cognitive impairment occurrence is significantly correlated with GCS score and IL-1β and IL-18 level. Additionally, with time going cognitive function is improved and interleukin may be involved in.

Keywords: Traumatic brain injury, cognitive impairment, IL-1β, IL-18

Introduction

Traumatic brain injury (TBI) is a public health and socio-economic problem in the world, which is the major cause of death and lifelong disability, especially among the young [1]. What's worse, with the widely used of motor vehicles, the occurrence rate of TBI is increasing, particularly in the developing countries [2]. Survivors of TBI may frequently undergo low quality of life, due to the cognitive, physiological and psychiatric impairment, as well as the huge economic burden caused by TBI [3]. The damage followed by TBI includes the primary injury and secondary injury. The primary injury results from the direct mechanical force. It causes compression and shearing of neural, glial, and vascular cells [4, 5]. The secondary injury usually lasts for months' post-injury, including the brain cell apoptosis, tissues damage and atrophy [6].

Cognitive impairment is the one of the common sequelae of TBI, which can affect attention, memory, executive functions, language functions and information-processing speed [7]. A lot of studies had investigated the mechanism of cognitive impairment caused by TBI and growing evidence had proved that post-injury neuroinflammation may contribute to the long term sequelae following TBI [8, 9]. It was reported that increased expression level of interleukin such as interleukin (IL)-1β, IL-6, IL-18, might be correlated with cerebral damage [10, 11]. In the study of Mina et al., IL-1β was proved to be correlated with cognitive impairment caused by sepsis via animal experiments [12]. A study carried out by Salani et al. demonstrated that IL-18

Table 1. Demographic characteristics of the study groups

Characteristics	Test group (n=102)	Control group (n=106)	*P*
Age (year)			0.525
≥50	69	76	
<50	33	30	
Gender			0.434
Male	66	63	
Female	36	43	
Duration of education (year)			0.349
≥12	68	77	
<12	34	29	
Handedness			0.756
Right	82	87	
Left	20	19	
GCS			
13-15	35	-	
9-12	39	-	
<9	28/	-	
MMSE T0			0.000
≥24	55	14	
<24	47	92	
IL-1β T0 (pg/mL)	18.39 ± 4.21	7.62 ± 1.81	0.000
IL-18 T0 (pg/mL)	99.73 ± 13.49	54.78 ± 6.26	0.000

Note: -: no available data. T0: for test group T0 time meant 7 days after TBI, in control group T0 represented the beginning of the study.

Table 2. MMSE values varying over time

MMSE score	Time		
	7 days after TBI	3 months after TBI	6 months after TBI
≥24	47	62	77
<24	55	40	25
P value	-	0.035*	0.000*

Note: -: no available data. *: indicated significant differences compared with 7 days after TBI.

expressed abnormally in pre-clinical state of mild cognitive impairment and Alzheimer's disease, which confirmed that IL-18 may be involved in the early phase of Alzheimer's pathophysiology [11]. However, the long term changes of IL-1β and IL-18 concentration in patients undergoing TBI and their association with cognitive impairment had been rarely reported in the previous studies.

In the present study, we aimed to evaluate the cognitive changes of patients undergoing TBI in 6 months. In addition, we assessed the serum concentration of IL-1β and IL-18 in patients with TBI and analyzed their correlation with cognitive impairment occurrence. This study may be useful for treatment of cognitive impairment following TBI.

Materials and methods

Patients

The study was carried out from March 2010 to 2014 May in Tianjin Medical University General Hospital. The patients enrolled in this study were accorded with the following inclusion criterion: (1) The patients were pathologically diagnosed with TBI. (2) Their age rang was 18-75 years. (3) The patients collected in this study could understand and complete the cognitive function test. (4) The eligible participators had not taken any antipsychotic drugs or other drugs which could influence the central nervous system within 4 weeks. (5) The persons in this study had not been diagnosed with cerebral, mental disease or other serious body disease. (6) The eligible volunteers who were with substance abuse such as alcohol, narcotics or other drugs affecting the central nervous system function would not been collected in this study.

102 patients who were accorded with the inclusion criterion were collected in the present study, as test group. While 56 persons who had underwent orthopedic operation and 50 healthy volunteers were recruited in control group. The consent forms were obtained from all the participators before the study. This study was approved by the Ethics Committee of Tianjin Medical University General Hospital.

Cognitive function assessment

Mini-mental state examination (MMSE) was applied for evaluating cognitive functions of the subjects [13]. The test was used to assess the global cognitive function of the study objects, including orientation to place and time, the short-term memory, episodic long-term memory, subtraction, the ability to construct a sentence and oral language. The total score was 30 and patients with the score less than 24 were considered as cognitive impairment [14].

Table 3. Association between GCS score and MMSE at 7 days after TBI

GCS score	Case (n)	MMSE score		*P*
		≥24	<24	
13-15	35	21	14	0.000
9-12	40	24	16	
<9	28	3	25	

The cognitive function of volunteers in control group was assessed at the beginning of the study (T0), while patients in test group were measured at 7 days (T0), 3 months (T1) and 6 months (T2) after surgery.

Collection of serum specimens

5 mL blood specimens were collected from all the subjects after an overnight fast. Serum specimens were obtained after being centrifuged the collected blood specimens with 2 h from the collection. The obtained serum specimens were stored at -80°C until used.

The serum specimens of control group were collected at the time of the study beginning (T0). In test group, serum specimens were collected separately at T0, T1 and T2.

IL-1β and IL-18 detection

Enzyme-linked immunosorbent assay (EILISA) kit was used for detection the concentration of IL-1β and IL-18 in collected serum specimens according to the manufacturing instructions. ELISA assay was applied for all the collected serum specimens.

Statistic analysis

In the present study, the continuous variables were presented as average ± SD. Student t test was applied for continuous variables analysis and discontinuous variable analysis was performed with chi-square assay. All the statistic analysis was performed in SPSS 18.0 software. $P<0.05$ was considered statistically significant.

Results

Demographic characteristics of the included patients

102 patients including 66 male and 36 female diagnosed with TBI were assigned into test group, while the control group included 63 male and 43 female volunteers. The demographic characteristics such as age, gender, education and handedness were similar between the study groups ($P>0.05$ for all) (**Table 1**).

Cognitive function assessment at T0 and its changing over time

We detected the cognitive function of all the persons in the control group at the beginning of the study. Cognitive function of patients in TBI group were firstly assessed at 7 days followed TBI. The results indicated that the morbidity of cognitive impairment was higher in TBI group than that in the control group ($P<0.001$, **Table 1**).

The cognitive function of patients in TBI group was evaluated at 3 months and 6 months followed injury. Results indicated that with the time developing, the rate of cognitive impairment was obviously decreased, compared with the 7 days after injury ($P<0.05$ for all) (**Table 2**).

Morbidity of cognitive impairment in TBI group according to injury intensity

According to Glasgow Coma Scale score (GCS), patients in TBI group were divided into mild TBI group (GCS score 13-15), moderate TBI group (9-12) and severe TBI group (<9). Analysis indicated that MMSE score was significantly different in the three groups (**Table 3**). The occurrence rate of cognitive impairment was higher for patients with severe TBI than those with mild and moderate TBI.

Serum IL-1β and IL-18 levels and their changes with time

The serum concentration of IL-1β and IL-18 was detected for test group and control group at T0. The results indicated that serum levels of IL-1β and IL-18 were up-regulated in TBI group, compared with control group ($P<0.001$ for all, **Figure 1**).

We detected the interleukin levels of patients with TBI at T1 and T2. The results demonstrated that IL-1β and IL-18 expression levels were significantly down-regulated with the time developing ($P<0.05$ for all, **Figure 2**).

Association between interleukin levels and GVS score

We evaluated the relationship between injury severity and IL-1β and IL-18 levels. The results shown in **Table 4** suggested that levels of IL-1β

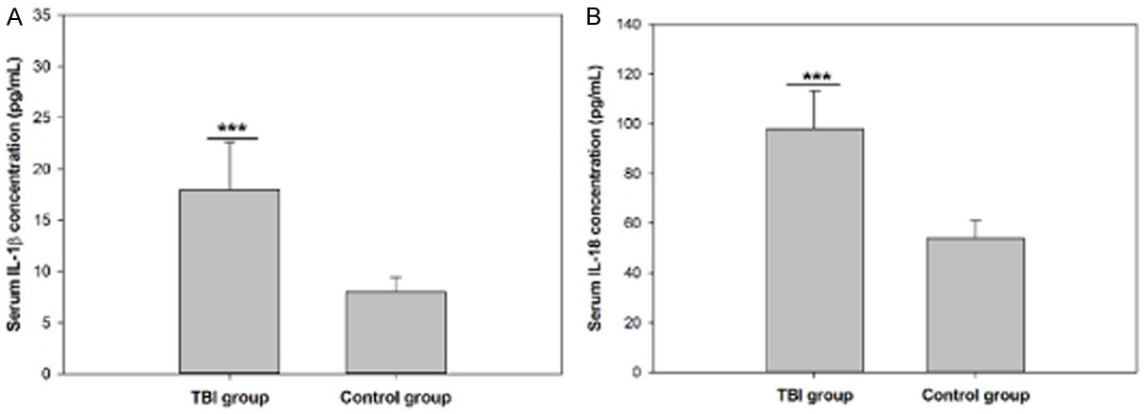

Figure 1. Serum IL-1β and IL-18 concentration in TBI group and control group at T0. A: IL-1β expression level in TBI group and control group. B: Serum concentration of IL-18 in study groups. T0: for test group T0 time meant 7 days after TBI, in control group T0 represented the beginning of the study. Serum IL-1β and IL-18 levels of test group were obviously increased, compared with control group. *: indicated a significant difference, compared with T0, $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

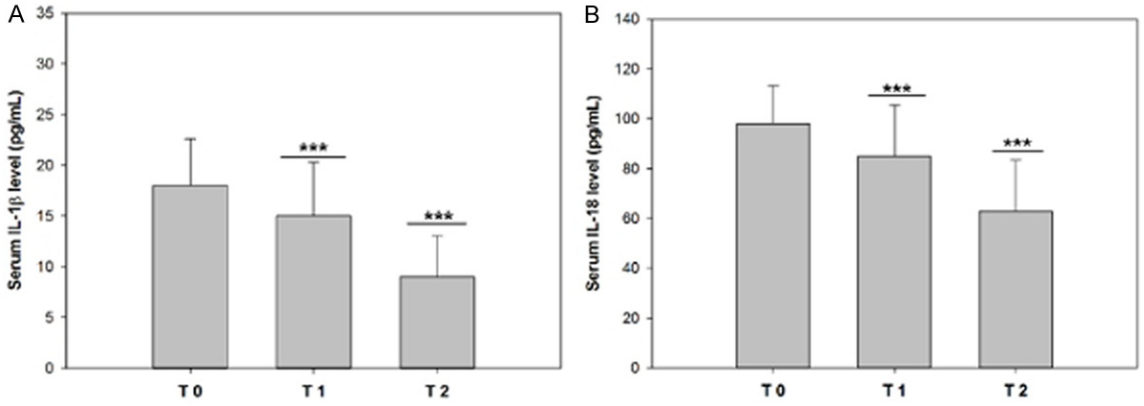

Figure 2. Changes of serum IL-1β and IL-18 concentration in TBI group over time. A: Changes of IL-1β concentration in patients with TBI. B: Changes of IL-18 with time in TBI group. With time going, IL-1β and IL-18 concentration was significantly down-regulated in TBI group. T0: 7 days post-injury, T1: 3 months after injury, T2: 6 months post-injury. *: indicated significant differences, compared with T0, $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

Table 4. Relationship between serum IL-1β and IL-18 concentration and GCS score

GCS score	IL-1β (pg/mL)	IL-18 (pg/mL)
13-15	14.46 ± 1.34	89.11 ± 8.27
9-12	17.82 ± 1.37	97.41 ± 8.84
<9	24.11 ± 2.63	116.21 ± 6.96
P value	0.000	0.000

and IL-18 were significantly correlated with the degree of trauma ($P<0.05$ for all).

Relationship between IL-1β and IL-18 levels and MMSE score

The association between IL-1β and IL-18 levels and MMSE score was assessed in this study via student t test. Analysis indicated that patients with cognitive impairment in TBI group had higher levels of IL-1β and IL-18 than those with MMSE score more than 24 (**Table 5**).

In addition, we analyzed the relationship between interleukin expression level and cognitive impairment at different times. The results were accorded with the results obtained at T0 (**Table 5**), which indicated that with the decreased of IL-1β and IL-18 expression level, cognitive function of patients undergoing TBI was significantly improved.

Discussion

Cognitive impairment after TBI was a troublesome problem for public health and various fac-

Table 5. Association between cognitive impairment and interleukin and its changing over time

Time	MMSE score	Case (n)	Interleukin	
			IL-1β (pg/mL)	IL-18 (pg/mL)
T0	≥24	47	16.64 ± 3.10	96.21 ± 10.78
	<24	55	19.89 ± 4.47	102.73 ± 14.88
	P	-	0.000	0.014
T1	≥24	62	15.19 ± 3.08	78.42 ± 24.16
	<24	40	18.00 ± 4.68	88.35 ± 20.28
	P	-	0.000	0.034
T2	≥24	77	9.62 ± 2.80	64.52 ± 15.64
	<24	25	11.32 ± 3.36	74.16 ± 17.63
	P	-	0.014	0.011

Note: T0: 7days after TBI. T1: 3 months after TBI. T2: 6 months after TBI. -: no available data.

tors were reported to be associated with neurodegeneration. Han et al. had reported that using propofol at a recommended or higher dose for anesthesia may lead to the cognitive defects [15]. Cytokineses were proposed as the leading cause of cognitive impairment and various members were proved to contribute to cognitive dysfunction, such as IL-6, IL-8, IL-18, tumor necrosis factor (TNF)-α, IL-1β and so on [3, 16-18]. However, a meta-analysis constructed by Saleem et al. indicated that there were no significant differences in inflammatory factors between patients with mild cognitive impairment and healthy control [19]. The purpose of this study was to evaluate cognitive functions of patients with TBI and the relationship between IL-1β and IL-18 concentration and cognitive impairment occurrence.

In the present study, we proved that patients undergoing TBI had a higher morbidity of cognitive impairment than the healthy volunteers. The results indicated that TBI can significantly influence cognitive function. Moreover, cognitive function was significantly associated with injury intensity. In addition, we found that with time going, the occurrence rate of cognitive impairment was decreased. This funding was accorded with the study of Skandsen et al. [20]. They had reported that half of patients with moderate TBI and even one third of those with severe TBI had a normal cognitive assessment 3 months post-injury. However, the mechanism for cognitive improvement post-injury was needed further investigation.

The effects of IL-1β and IL-18 on cognitive function had been reported in the previous studies. Fredrik et al. via an animal experiment proved that anti-IL-1β treatment could improve cerebral edema, tissues loss and late cognitive function following TBI, which might be a treatment option for TBI in the further [3]. A study carried out by Gorska-Ciebiada et al. had indicated that up-regulated IL-1β in elderly diabetic patients was significantly associated with high occurrence rate of cognitive impairment than those with normal level [21]. The effect of IL-18 had also been proved in the previous studies. It was reported that IL-18 was expressed highly in patients with Alzheimer's disease, compared with healthy controls [22].

In the present study, we proved that compared with healthy volunteers, serum concentration of IL-1β and IL-18 were obviously increased in patients undergoing TBI. The increased levels were significantly correlated with GCS score. Moreover, with the time developing, cognitive function was improved and serum levels of IL-1β and IL-18 were down-regulated. Statistical analysis indicated that cognitive impairment occurrence rate was significantly associated with IL-1β and IL-18 serum concentration. The cognitive function could recovery after injury and various factors may contribute to the improvement. Mondello et al. had indicated that serum level of MAP-2 was significantly increased at 6 months after TBI, which was correlated with improved outcomes in survivors of severe TBI [23]. Cytochrome c oxidase (COX) was proved to play an important role in cognitive improvement induced by exercise following TBI [24]. A study constructed by Briones et al. indicated that pro-inflammatory cytokines IL-1β, TNF-α and enhanced levels of the anti-inflammatory cytokine IL-10 after mild TBI were associated with improvement in cognitive function [25]. This conclusion was accorded with our founding. However, inflammatory response was a complex process and various factors may influence the cytokines expression levels, such as age, GOS score, gender and history of drugs. Further study should consider more correlated factors when analyzing effect of IL-1β and IL-18 on cognitive improvement.

In conclusion, patients undergoing TBI are easily to process cognitive impairment, compared with healthy controls. Moreover, cognitive impairment morbidity is significantly correlated with injury intensity and IL-1β and IL-18 concentration. In addition, cognitive function may be

improved within 6 months post-injury and IL-1β and IL-18 may be involved in. This study may be useful for therapy of cognitive impairment following TBI.

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Disclosure of conflict of interest

None.

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