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ORIGINAL ARTICLE

The Effect and Clinical Implications of IL-1β on the Development of Aneurysmal Subarachnoid Hemorrhage

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SUMMARY

Background: **This study aimed to investigate the effect and clinical implications of IL-1β on the development of aneurysmal subarachnoid hemorrhage.**
Methods: **This retrospective study included a total of 80 participants, and these participants were divided into the following two groups: control group (healthy participants) and experimental group (aneurysmal subarachnoid hemorrhage patients). Then, all of the participants received digital subtraction angiography or computed tomography angiography. Participants' general data were collected and analyzed. IL-1β expression in blood samples were determined by ELISA, and then IL-1β protein were determined by western blotting.**
Results: **A total of 80 participants was included in this study, and the participants` general data, including gender, age, and previous medical history, showed no significant differences between the experimental group and control group. The IL-1β value in the experimental group was significantly increased, and the difference was statistically significant ($p < 0.05$).**
Conclusions: **Upregulated IL-1β can promote the development of aneurysmal subarachnoid hemorrhage, indicating that IL-1β is a key factor in evaluating the prognosis of patients with aneurysmal subarachnoid hemorrhage.**
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KEYWORDS

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INTRODUCTION

Aneurysmal subarachnoid hemorrhage (ASH), which is a cerebrovascular disease that seriously endangers human health, accounts for about 85% of all spontaneous subarachnoid hemorrhages. Epidemiological study results [1,2] suggest that ASH has an incidence of 10.5/100,000 and a cumulative mortality as high as 24.6% after ASH [3,4] and that ASH easily leads to related secondary complications, such as cell death in the brain, brain edema, microvascular dysfunction, and declined cerebral blood flow [5]. Although some therapeutic methods, such as microsurgical clipping or endovascular coiling treatment, are utilized to treat patients with ASH and these treatment methods for ASH have a protective effect in preventing the development of ASH,

the delayed cerebral ischemia and cerebral vasospasm after ASH seriously threaten the life of patients with ASH and ASH also easily causes permanent disability and neurological deficits in patients with ASH [6]. In our study, we highlighted that the therapeutic outcomes of current treatments for aneurysmal subarachnoid hemorrhage (ASH) often fall short of the expectations and needs of patients and their families. Specifically, these requirements include the need for more effective treatments that enhance recovery, minimize long-term complications, and improve overall quality of life [7]. Therefore, it is urgent to conduct related studies to find promising treatment methods for ASH, but the detailed pathogenesis of ASH is still elusive, so we need to carry out association studies to clarify the mechanisms of ASH.

Currently, common therapeutic regimens for ASH include microsurgical clipping and endovascular coiling treatment [8]. With therapeutic approaches increasing and improving, the therapeutic effect in ASH has been improved and these therapeutic approaches have prolonged patients` life [9,10]. However, due to serious secondary complications, the mortality of ASH is still high. It is imperative to prolong patient's life and improve patient prognosis, but related studies on how to prolong patient's life are still limited. Currently, only Bakker [11] reported that genetic risk score was used to assess the prognosis of ASH. Interestingly, some studies have reported that a good prognostic method for ASH would be a promising method to prolong patient's life. In addition, diagnostic biomarkers for ASH are associated with the patient's life and prognosis, and interleukin-1β (IL-1β) is more often used to evaluate the prognosis of ASH. To better treat the patients with ASH, diagnostic biomarkers for ASH are vital, as they play an important role in decreasing mortality of ASH.

Interleukin-1β (IL-1β), which is the first member of the IL-1 family to be detected, can control the cell biological function through the receptor IL-1R [12,13]. In recent years, we have made a lot of progress in understanding the mechanism of IL-1β in numerous human diseases and its function in ASH [14]. More and more studies have pointed out that IL-1β is associated with cell growth, cell differentiation, cell division, proliferation, migration, and invasion by controlling the receptor IL-1R [15,16]. Therefore, IL-1β plays an important role in the occurrence and development of various tumors. The results by Devlin [17] suggest that the main pro-inflammatory cytokine, IL-1β, in patients with ASH is clearly increased compared to healthy participants, and these study results showed that IL-1β is a potential biomarker, which can be used to diagnose ASH. In addition, a trial by Xu [18] also pointed out that downregulation of IL-1β can be considered a potential candidate when relieving neuroinflammatory responses triggered by SAH. We can see from previous studies that IL-1β is a potential diagnostic biomarker which is closely related to ASH development process, and this study initially provided some evidence of IL-1β as a biomarker for assessing the prognosis of ASH.

Previous studies have suggested that IL-1β expression level has been associated with ASH development, and downregulation of IL-1β level can inhibit the development of ASH by regulating cell biological processes, but these results still lack sufficient evidence. In addition, the effect and clinical implications of IL-1β on the development of aneurysmal subarachnoid hemorrhage is also elusive. Therefore, this study aimed to investigate the effect and clinical implications of IL-1β on the development of ASH, so that we can improve the quality of life in patients with ASH and prolong patients' life.

MATERIALS AND METHODS

Study design and participants

We conducted a retrospective observational pilot trial, which enrolled a total of 40 patients with ASH from our hospitals from June 2021 to May 2023, and all these patients were considered as experimental group (n = 40). A check-up population from the same time period was considered as control group (n = 40). The participants in the experimental group and the control group received digital subtraction angiography or computed tomography angiography. The hospital´s Ethics Committee has approved all protocols used in this study, and written informed consent forms were obtained from all patients with ASH.

Inclusion criteria for all patients with ASH were as follows: 1) head CT in patient indicates subarachnoid hemorrhage; 2) the results by digital subtraction angiography or computed tomography angiography confirmed the presence of the aneurysm; 3) patient received craniotomy clipping or interventional surgery; 4) complete routine blood test data upon admission were recorded; 5) no history of surgical treatment within 30 days before diagnosis of ASH; and 6) the patient or patients' guardians have signed the written informed consent.

Exclusion criteria for all patients with ASH were as follows: 1) the progression of the ASH is alarming, and the patient even died in the emergency room or during hospitalization; 2) serious complications occur during the treatment process; 3) patient requests to withdraw from the study; and 4) patient has not signed the written informed consent.

Inclusion criteria for control group were as follows: all participants in control group received computed tomography angiography or digital subtraction angiography and all participants in the control group have signed the written informed consent.

Exclusion criteria for control group were as follows: participant did not receive computed tomography angiography or digital subtraction angiography detection and participant has not signed the written informed consent.

Collection of clinical material from patients

After patients of the experimental group were admitted to the hospital, age, gender, aneurysm size, clinical classification, previous medical history, and the severity of brain edema were collected and analyzed. Additionally, the demographic data of the control group, such as age, gender, and previous medical history, were collected and analyzed. All participants of the control group and experimental group received computed tomography angiography or digital subtraction angiography detection, and blood samples from the two groups were collected simultaneously. Following the collection of the blood samples, they were preserved for subsequent experimental analyses, and the angiography is presented in Figure 1.

The detection of blood samples by ELISA method

To measure the IL-1β levels in the blood samples from experimental group and control group, the enzyme linked immunosorbent assay method was used. The blood samples were grinded and lysed using cell lysate, and then the samples were centrifuged at 4℃, 10,000 rpm for 10 minutes, and after that an appropriate amount of supernatant was collected for protein quantification detection. The ELISA kit (MAB2741, R & D Systems, Co.) was used to detect IL-1β levels in blood samples from experimental group and control group, according to the protocol provided by manufacturer.

Western Blotting

The BALF samples were lysed using 150 - 250 μL/20 mg RIPA buffer containing 1% phosphotransferase inhibitor and 1% proteinase inhibitor, and then the samples were centrifuged at 12,000 g at 4℃ for 15 minutes, and total protein was collected and stored at -80℃. Proteins were quantified by using BCA Protein assay kit. Then 180 μL BCA working solution was added to each well. The purification of proteins was performed by using gel electrophoresis to transfer the protein into polyvinylidene difluoride (PVDF) membrane. The PVDF membrane was then incubated overnight with the primary antibody at 4℃. Next, the PVDF membrane was incubated for two hours with secondary antibodies (1:1,000) at room temperature, followed by washing, three times/5 minutes each. Finally, the blots were observed in the imaging system by using enhanced chemiluminescence.

Statistical analysis

SPSS 20.0 statistical software was used for statistical analysis. The descriptive data were presented by n (%), and measurement data are expressed by mean ± standard deviation (x ± s). Chi-squared test was performed for evaluation of descriptive data and *t*-test was used to compare the difference of measurements between the two groups. $p < 0.05$ shows that the difference is significant.

RESULTS

Participants` general data

Participants` clinical medical data of the experimental group and healthy participants (control group) are presented in Table 1. The gender, age, aneurysm size, clinical classification, previous medical history, and the severity of brain edema are recorded. The outcomes suggest that participants` general data, including gender, age, and previous medical history, show no significant difference between the experimental group and control group.

Comparison of IL-1β level by ELISA

The IL-1β level between the experimental group and control group were calculated by using the ELISA test method. Figure 2 shows that the IL-1β value in the control group was 15.1 ± 5.3 ng/mL and the IL-1β value in the experimental group was 29.6 ± 5.8 ng/ml. Compared to the IL-1β value in the control group, the IL-1β value in the experimental group was significantly increased, and the difference was statistically significant ($p < 0.05$). These findings suggest that the IL-1β value is associated with the development process of ASH.

Comparison of IL-1β protein level by western blotting

IL-1β protein levels were determined by western blotting and are presented in Figure 3. Those outcomes suggest that the experimental group showed high expression of IL-1β protein, and the control group showed low expression of IL-1β protein. Compared to IL-1β protein level in the control group, the IL-1β protein level in the experimental group were clearly increased. These outcomes suggest that upregulated IL-1β protein could be associated with the development of ASH.

DISCUSSION

Patients with ASH are at high risk of dying. To improve the quality of life in patients with ASH and to prolong the patients' lives, we conducted this study to record the participants' general data and IL-1β protein levels in the blood samples of the two groups. Our results show that the preoperative general data of the patients, including gender, age, and previous medical history, between experimental group and control group show no significant difference. Aneurysm size in experimental group was 6.23 ± 3.21 mm, and the numbers of ASH patients with Hunt-Hess I, Hunt-Hess II, Hunt-Hess III, and Hunt-Hess IV in the experimental group were 7 (17.5%), 14 (35%), 11 (27.5%), and 8 (20%), respectively. The numbers of ASH patients with mild brain edema, moderate brain edema, and severe brain edema were 8 (20%), 23 (57.5%), and 9 (22.5%), respectively. Compared to IL-1β protein levels in blood samples of the control group, IL-1β protein levels of the experimental group were significantly increased, and IL-1β protein

Table 1. Demographic and participants general data.

Variable	Experimental group	Control group	p-value
Gender, male (%)	18 (45%)	15 (37.5%)	0.496
Age (year)	56.6 ± 10.8	57.2 ± 11.3	0.572
Aneurysm size (mm)	6.23 ± 3.21	-	-
Clinical classification			
Hunt-Hess I	7 (17.5%)	-	
Hunt-Hess II	14 (35%)	-	
Hunt-Hess III	11 (27.5%)	-	
Hunt-Hess IV	8 (20%)	-	
Previous medical history			
Hypertension	17 (42.5%)	10 (25%)	0.098
Diabetes	4 (10%)	3 (7.5%)	0.692
Smoking history	5 (12.5%)	4 (10%)	0.723
Severity of brain edema			
Mild	8 (20%)	-	
Moderate	23 (57.5%)	-	
Severe	9 (22.5%)	-	

A chi-squared test was performed for the evaluation of the descriptive data, and measurement data was compared using the *t*-test; compared to measurement data in the control group, $p < 0.05$.

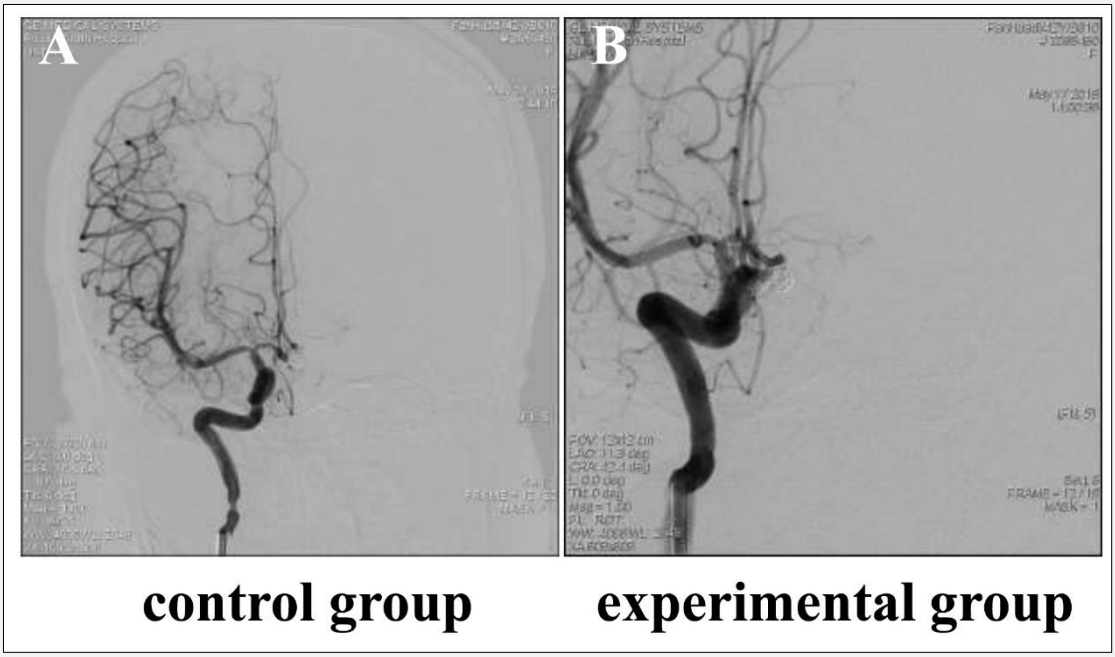

Figure 1. Representational pictures of the control group and experimental group.

A shows angiography of healthy participants; B shows angiography of aneurysmal subarachnoid hemorrhage patients.

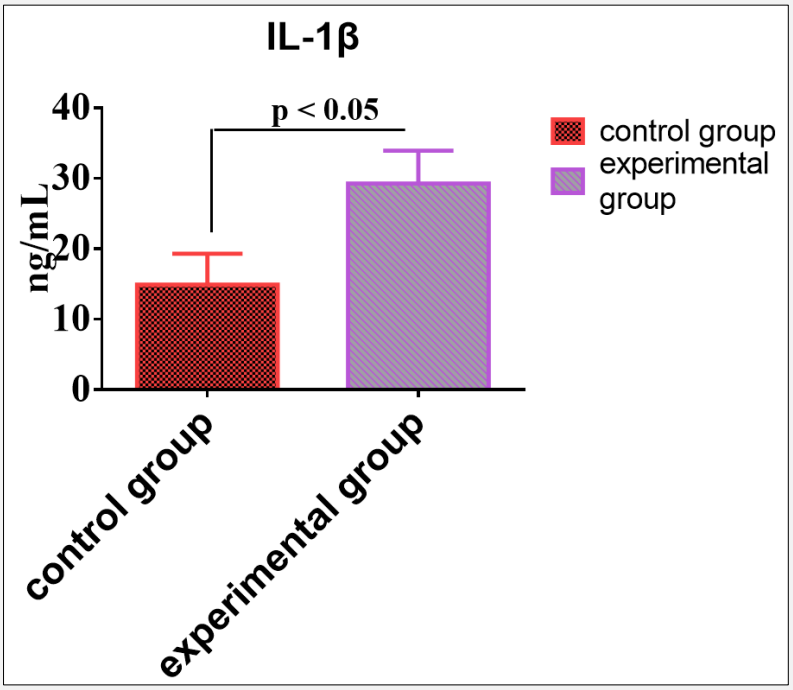

Figure 2. The levels of IL-1β.

The IL-1β levels in blood samples from the control group and experimental group were determined by ELISA and the results are presented in Figure 2. The value is presented as means ± standard deviation, and measurement data was compared by using the *t*-test. * p < 0.05 versus control group shows that the difference is statistically significant.

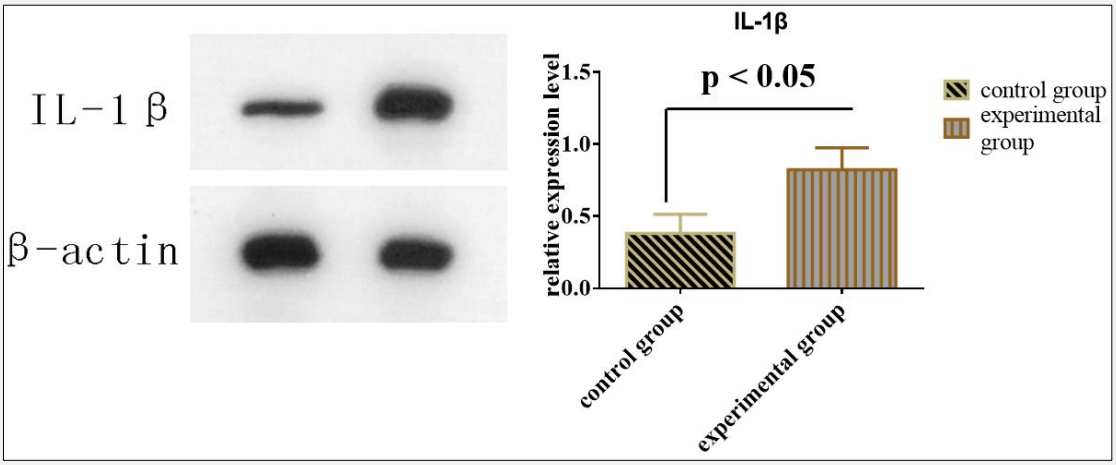

Figure 3. The relative levels of IL-1β protein.

The IL-1β protein levels in the blood samples from the control group and experimental group were determined by western blotting and the results are presented in Figure 3. The value is presented as means ± standard deviation, and measurement data was compared by using the *t*-test. * p < 0.05 versus control group shows that the difference is statistically significant.

levels between the two groups were significantly statistically different. These outcomes suggest that IL-1β protein level may be associated with the development of ASH by controlling the cell physiological functions.

Aneurysm size, as a key factor of aneurysm rupture risk, has been widely used to evaluate the effect of microsurgical clipping and endovascular coiling treatment in patients with ASH [19]. Roethlisberger [20] reported that aneurysm size is an independent factor that can be used to evaluate the neurological deficit in ASH patients and that aneurysm size in the ASH patients was 5 to 25 mm. Aneurysm size in our study was 6.23 ± 3.21 mm, and these results are similar to the results described in previous studies [20,21]. Some trials [22,23] have reported that the percentage of ASH patients with Hunt-Hess are similar with those reported in our study.

Interleukin-1β (IL-1β), as a proinflammatory factor, plays an important role in patients with ASH by controlling cell biological processes, but studies relating to IL-1β in aneurysmal subarachnoid hemorrhage remain limited. An *in vivo* and *in vitro* trial by Xu [18] pointed out that upregulation of IL-1β expression level is a potential contributor, which was expressed in an ASH rat model, and that downregulation of IL-1β can attenuate ASH development, and the results in this study show that IL-1β is a biomarker which can be used to relieve neuroinflammatory responses triggered by SAH. In addition, some trials [17,24,25] reported that main proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, are significantly elevated in patients with alcoholic steatohepatitis (ASH), and these proinflammatory cytokines are promising targets for treating ASH patients [26]. However, the clinical information described in these studies remain limited and block the application of these proinflammatory cytokines in ASH patients. In our study, we aimed to clarify the role of IL-1β in aneurysmal subarachnoid hemorrhage (ASH) by referencing a retrospective clinical study by Luo [27]. This study found that IL-1β levels are significantly higher in ASH patients compared to those with a good prognosis. Based on these findings, we suggest that IL-1β could be considered a valuable diagnostic biomarker for ASH [28]. In addition, Tian [29] investigated the mechanism of ASH and pointed out that hemoglobin and heme increased neuronal apoptosis to promote ASH development by aggravating the destruction of the blood-brain barrier and vasogenic and cytotoxic brain edema, and we presumed that IL-1β was implicated in ASH by increasing the neuronal apoptosis. The relationship between IL-1β expression and aneurysmal subarachnoid hemorrhage was investigated in our study; the results in the present trial suggest that overexpression of IL-1β can promote the development of aneurysmal subarachnoid hemorrhage. Our findings have been reported in related fields as well, and our study provides a probable method for diagnosing early aneurysmal subarachnoid hemorrhage.

CONCLUSION

We can conclude that upregulated IL-1β expression can promote the development of aneurysmal subarachnoid hemorrhage.

Ethical Approval and Consent to Participate:
The research protocol was reviewed and approved by the Ethics Committee and Institutional Review Board of the Rui Jin Hospital North, Shanghai Jiao Tong University School of Medicine.

Declarations of Interest:
The authors declare that they have no competing interests.

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