Am J Transl Res 2023;15(6):4033-4044
www.ajtr.org /ISSN:1943-8141/AJTR0148906

Original Article

Mitochondrial Omi/HtrA2 signaling pathway is involved in neuronal apoptosis in patients with cerebral hemorrhage

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Received January 3, 2023; Accepted May 18, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: Objective: This study aimed to analyze the role of mitochondrial Omi/HtrA2 signaling pathway in neuronal apoptosis in patients with cerebral hemorrhage (CH). Methods: In this retrospective analysis, the clinical data of 60 patients with CH who received craniotomy or minimally invasive intracranial hematoma (MIIH) were included in the case group, which was sub-divided into a craniotomy group (n=22) and a minimally invasive group (n=38) depending on the type of surgery. The brain tissue specimens of the above patients were retained in the surgical specimen repository of Yuhuan Second People's Hospital. Another 15 normal brain tissue samples retained in the surgical specimen repository were included in the normal group. The expression levels of Omi/HtrA2, X-linked inhibitor of apoptosis protein (XIAP), poly-adenosine diphosphate-ribose polymerase (PARP), pro-caspase 3, and pro-caspase 9 were determined using Western blotting. Results: The case group exhibited a higher proportion of neuronal apoptosis, higher expression levels of Omi/HtrA2, PARP, and pro-caspase 3 and 9, higher activities of caspase 3 and caspase 9 ($P < 0.05$), and lower XIAP expression ($P < 0.05$) in brain tissue than the normal group. The proportion of neuronal cell apoptosis in brain tissues was positively correlated with the expression of Omi/HtrA2, PARP, and pro-caspase 3 and pro-caspase 9 ($r > 0$, $P < 0.05$), and the activity of caspase 3 and caspase 9 was negatively correlated with XIAP expression ($r < 0$, $P < 0.05$). Compared with the craniotomy group, the minimally invasive group demonstrated higher efficacy and hematoma removal rate, shorter hematoma removal time, hematoma drainage time, operation time, and hospital stay, less intraoperative bleeding, and lower postoperative complication rates ($P < 0.05$). The minimally invasive group showed higher expression level of serum XIAP and lower levels of serum caspase 3 and caspase 9 than the craniotomy group ($P < 0.05$). Conclusions: Mitochondrial Omi/HtrA2 signaling pathway may be involved in neuronal apoptosis. MIIH has the advantages of high efficacy, high hematoma clearance rate, and few complications for the treatment of CH.

Keywords: Cerebral hemorrhage, Omi/HtrA2 signaling pathway, neuronal cell apoptosis, minimally invasive intracranial hematoma

Introduction

Cerebral hemorrhage (CH) refers to non-traumatic intracerebral hemorrhage, characterized by blood vessel rupture caused by hypertension combined with arteriolosclerosis [1, 2]. CH is characterized by subtle symptoms, sudden onset, and rapid progression, and the patients may present with elevated blood pressure, vomiting, and headache. If not treated in time, the occupying effect of the CH can lead to midline displacement and brain hernia, which may result in acute death [3]. Currently, surgical treatment is commonly used in CH treatment. Craniotomy can effectively improve intracranial ischemia and reduce the occupying effect of hematoma. Minimally invasive intracranial hematoma (MIIH) is a novel surgical method used to treat CH [4]. It is characterized by simplicity, short operation time, minimal trauma, and safety. MIIH can effectively restore normal cerebral blood supply and reduce the degree of neurological damage [5].

Apoptotic mechanism of CH may be one of the main pathways of neuronal cell death [6, 7].

Omi/HtrA2 is an oligomeric serine protease produced by mitochondria, which plays a key role in the process of apoptosis in perfusion and cardiac ischemia/reperfusion injury [8]. Under conditions of stimulated stress, mitochondrial release of Omi/HtrA2 into the cytoplasm occurs, and it can mediate apoptosis through both caspase-dependent and non-caspase-dependent pathways. X-linked inhibitor of apoptosis protein (XIAP) is an endogenous inhibitor of caspases. Omi/HtrA2 can degrade XIAP to increase the activity of caspase protein and promote cell apoptosis [9]. However, whether mitochondrial Omi/HtrA2 signaling pathway is involved in neuronal apoptosis after CH remains unclear. Therefore, this study aimed to analyze the correlation between mitochondrial Omi/HtrA2 signaling pathway and neuronal apoptosis, comprehensively explore the role and mechanism of the Omi/HtrA2 signaling pathway in mediating CH, and assess the efficacy of MIIH.

Materials and methods

Data collection

The clinical data of 60 patients with CH underwent craniotomy or MIIH in Yuhuan Second People's Hospital from January 2019 to February 2020 were retrospectively analyzed, and the brain tissue specimens of the patients were collected from the surgical specimen repository of our hospital and set as the case group. Another 15 normal brain tissue samples from the surgical specimen repository were set as the normal group.

Case inclusion criteria: patients who met the criteria for the diagnosis and treatment of hypertensive CH based on the *Chinese Guidelines for the Diagnosis and Treatment of Cerebral Hemorrhage (2019)* [10], and the diagnosis was then confirmed by cranial CT; patients with hematoma volume of 25-60 mL, course of disease < 24 h, and clear consciousness upon admission; patients who received craniotomy or MIIH; patients or who voluntarily provided informed consent to the entire surgical procedure and the retention of brain tissue after surgery.

Exclusion criteria: patients with coagulation disorders; patients with acute and chronic infections; patients with dysfunction of vital organs, such as the heart, liver, or kidneys; patients with CH resulting from brain trauma or a tumor; patients with connective tissue disease; patients with subarachnoid hemorrhage; patients with contraindications to surgery; patients who had undergone surgical treatment within one month prior to enrollment; patients with immune system or hematological disorders; patients with vascular malformations.

The included 60 subjects of the case group consisted of 42 males and 18 females aged 49-78 years, with a mean age of 62.09±4.24 years. Among them, 22 patients who underwent craniotomy hematoma removal were set as a craniotomy group, and 38 patients who underwent MIIH removal were set as a minimally invasive group. Additionally, 15 normal brain tissue samples in the normal group composed of 11 from males and 4 from females. They were aged 47-75 years, with the mean age of 61.15±4.87 years. In this retrospective study, medical ethical issues and informed consent of patients were taken into consideration when selecting brain tissue specimens. The study was conducted in accordance with the requirements of the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Yuhuan Second People's Hospital.

Surgical methods

(i) In this craniotomy group, a dehydrating agent was applied, and an 8 × 10 cm horseshoe-shaped incision was made at the temporal bone. The hematoma was removed through the superior temporal gyrus approach. Hemostasis was performed by electrocoagulation or compression with a gelatin sponge, followed by cranial closure and sutures. (ii) In the minimally invasive group, the head of patient was shaved to expose the skin, and the puncture point was determined under CT. The needle length was determined as the distance from the puncture point to the target point, which was at the center of the hematoma. The disposable intracranial hematoma puncture needle (YL-1) was positioned at the designated drill site and inserted into the dura mater until it reached the edge of the hematoma. One aspiration was performed intervals of 0.5 cm as the needle was advanced until reaching the center of the hematoma. The first removal volume was 30%-60%, followed by the injection of a mixture of b.i.d. 20,000 U of urokinase + 4 mL of 0.9% sodium chloride into the hematoma cavity.

Tissues processing and detection

After crushing the brain tissue, the cells were filtered with nylon mesh and centrifuged to remove the supernatant. Then, the cells were fixed with 700 ml/L of ethanol, and the apoptosis of neuronal cells was measured by flow cytometry (MoFloAstrios EQ, Beckman Coulter). Afterwards, 10,000 cells were acquired using Cell Quest software for the identification of apoptotic cell counts. Mod Fit software was used to analyze the cell cycle and fit apoptotic peaks and map the DNA distribution. The apoptotic peaks were the G1 subpeaks before the G1 phase, and the results of neuronal cell apoptosis were expressed as apoptotic percentages.

Western blot was utilized to detect the protein expression of mitochondrial Omi/HtrA2 signaling pathway. The brain tissue homogenate was prepared, and the total protein was extracted. Bicinchoninic acid protein quantitative kit (Thermo Scientific; Lot No. 23256) was used to determine protein concentration. After electrophoresis utilizing BG-caTANK electrophoresis instrument (Beijing Baygene Biotech Company Limited), 5 μg of protein was transferred to a PVDF membrane and sealed with TBST buffer containing 5% skim milk at room temperature for 1 h. The corresponding monoclonal antibodies were added and incubated at 4°C overnight, and the membrane was washed with TBST 3 times. Horseradish peroxidase labeled IgG was added and incubated in a shaker at room temperature for 1 h, followed by the membrane being washed 3 times with TBST. The membrane was exposed to chemiluminescent reagent for 5 min. After scanning the results, the grayscale values of the strips were analyzed by Quantity One software. With β-actin being the internal control, the relative grayscale values were calculated, that is, the protein expression of Omi/HtrA2, XIAP, poly-adenosine diphosphate-ribose polymerase (PARP), pro-caspase 3, and pro-caspase 9.

The protein activity of caspase 3 and caspase 9 (Lot No. T9281 and T9275, Livemore, USA) in brain tissue was determined spectrofluorometrically using specific fluorogenic substrates. Brain tissue homogenates (100 μL) were combined with caspase-3 substrate AC-DEVD-AMC or caspase-9 substrate AC-LEHD-AFC (10 μL) in HEPES buffer to make a final volume of 1 mL. The mixture was incubated at 37°C for 1 h. The fluorescence intensity was measured by fluorescence spectrophotometer at an excitation wavelength of 400 nm and a release wavelength of 505 nm. The fluorescence intensity was calculated using the fluorescence intensity without brain tissue as the reference value. The results were expressed by the relative fluorescence intensity.

Outcome measures

(1) Therapeutic efficacy and hematoma clearance rate: The efficacy criteria were formulated according to the *Chinese Cerebral Hemorrhage Diagnosis and Treatment Guidelines (2019)*. The absence of post-operative clinical symptoms and a hematoma clearance rate of > 90% were considered as excellent response. Significant improvements of post-operative symptoms and a hematoma clearance rate of > 70% were defined as good response. Non-improved or even deteriorative post-operative symptoms and a hematoma clearance of < 70% were considered as poor response. Total response rate = (excellent + good) response/total number of cases. Hematoma clearance rate = the difference in hematoma volume before and after surgery/preoperative hematoma volume × 100%.

(2) Surgery-related indicators: The duration of hematoma removal, time of hematoma drainage, duration of surgery, length of hospital stay, and intraoperative blood loss were recorded in patients with CH.

(3) Indicators of nerve injury and oxidative stress: 3 mL of fasting venous blood was collected before and 7 days after the operation. The levels of neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF), S100B protein, glutathione peroxidase (GSH-Px), malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OhdG), cortisol (Cor), XIAP, Caspase-9, and Caspase-3 were determined using enzyme-linked immunosorbent assay kits (Beijing North Institute of Biotechnology Co., Ltd.). The level of neuron-specific enolase (NSE) was determined using NSE kit (Shanghai Xinfan Biological Technology Co., Ltd.).

(4) Prognosis: Scandinavian Stroke Scale (SSS) and Barthel Scale/Index (BI) were used to assess the neurological function and activity of daily living in patients before and 6 months after the operation. The SSS consists of 9 items, including facial expression, language,

Table 1. Comparison of baseline data between the craniotomy and minimally invasive groups

Baseline data	Craniotomy group (n=22)	Minimally invasive group (n=38)	t/χ^2	*P*
Sex (M/F)	15/7	27/11	0.055	0.815
Age (years)	62.54±4.37	61.09±5.11	1.115	0.270
Duration of illness (h)	5.57±1.25	6.01±1.16	1.376	0.174
Hematoma volume (mL)	37.84±3.49	38.16±4.02	0.311	0.757
History of hypertension	19	34	0.0031	0.956
Site of hemorrhage				
Basal nucleus	13	20	0.235	0.628
thalamus	3	6		
cerebellum	0	1		
lobes	6	11		

Table 2. Comparison of efficacy and hematoma clearance rate between the craniotomy and minimally invasive groups

Group	Cases	Excellent	Good	Poor	Response rate	Hematoma clearance rate
Craniotomy group	22	7 (31.82)	8 (36.36)	7 (31.82)	15 (68.18)	17 (77.27)
Minimally invasive group	38	16 (42.11)	19 (50.00)	3 (7.89)	35 (92.11)	37 (97.37)
χ^2	-	-	-	-	4.183	4.219
P	-	-	-	-	0.042	0.040

level of consciousness, limb paralysis, eye movement, orientation, etc., with a total score of 46 points. Low scores indicate poor neurological function. The BI scale consists of 10 items, including bathing, eating, toileting, dressing, etc., with a total score of 100 points. High scores indicate better performance in activity of daily living. The Montreal Cognitive Assessment Scale (MoCA-B) [11] was used to assess the cognitive function of patients, in the aspects of delayed memory, attention, abstraction, naming, orientation, language fluency, visual space, and executive ability, with a total score of 30 points, and a score of < 26 indicates cognitive dysfunction.

(5) Complications: Postoperative complications including lung infection, gastrointestinal bleeding, hyperthermia, and renal insufficiency were recorded.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) 23.0 was used for data analysis. Measurement data were described by mean ± standard deviation (SD) and the between-group comparisons were performed by independent sample t tests and intragroup comparisons by paired t tests. Counting data described by percentages (%) were compared by χ^2 test. Pearson's correlation analysis was used to analyze the correlation between two factors. $P < 0.05$ indicated a statistically significant difference.

Results

Comparison of baseline data between the craniotomy and minimally invasive groups

There were no significant differences in sex, age, course of disease, history of hypertension, hematoma volume, or bleeding site between the craniotomy and minimally invasive groups ($P > 0.05$) (**Table 1**).

Comparison of efficacy and hematoma clearance rate between the craniotomy and minimally invasive groups

The minimally invasive group exhibited higher efficacy and hematoma clearance rate than the craniotomy group ($P < 0.05$). It is indicated that MIIH was more effective in the treatment of CH (**Table 2**).

Comparison of surgery-related indices between the craniotomy and minimally invasive groups

The minimally invasive group showed shorter hematoma removal time, hematoma drainage

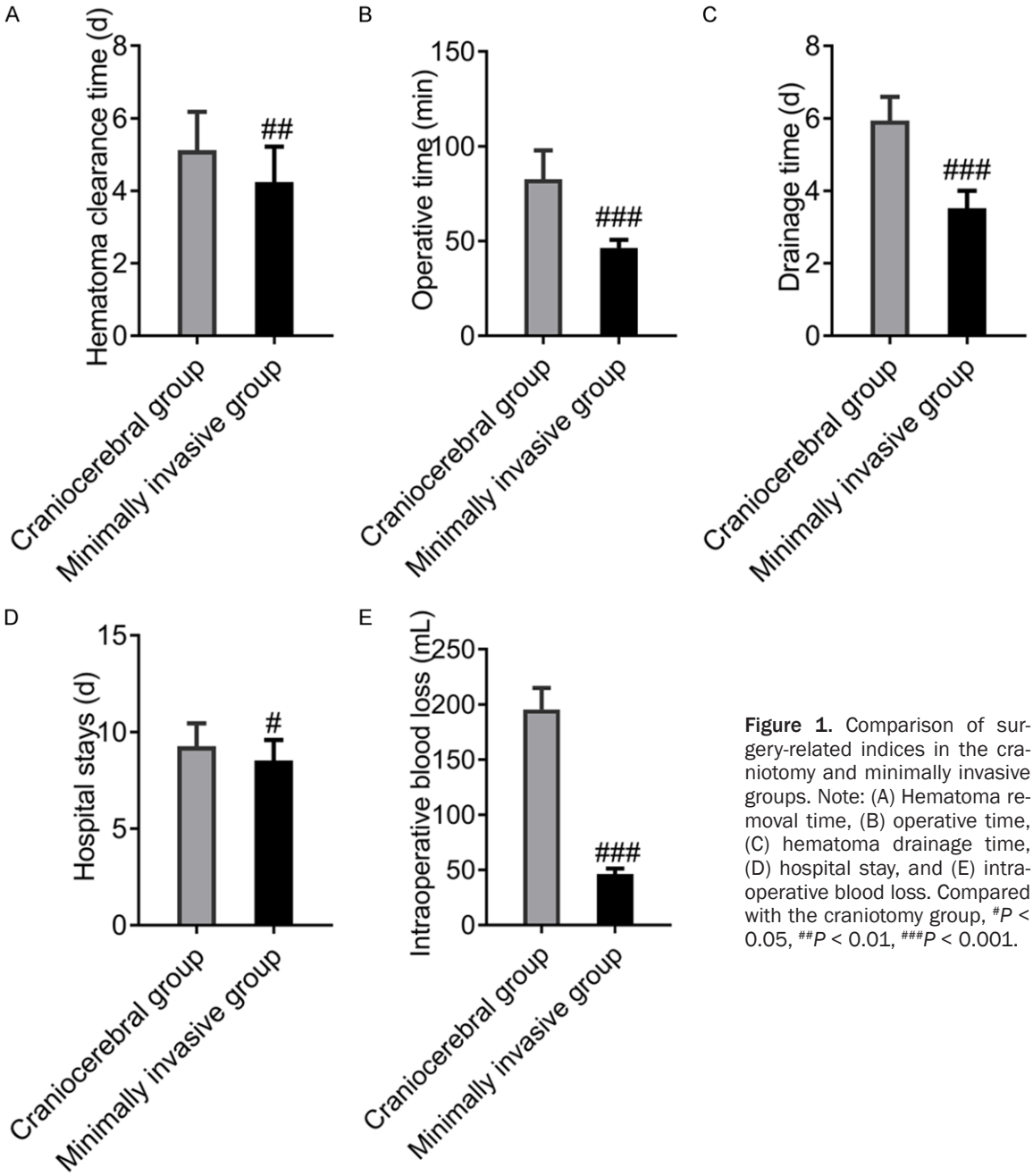

Figure 1. Comparison of surgery-related indices in the craniotomy and minimally invasive groups. Note: (A) Hematoma removal time, (B) operative time, (C) hematoma drainage time, (D) hospital stay, and (E) intraoperative blood loss. Compared with the craniotomy group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

time, operative time, length of hospital stay, and less intraoperative blood loss than the craniotomy group ($P < 0.05$). This suggests advantages of MIIH in operative time, bleeding loss, and treatment time (**Figure 1**).

Comparison of neurological injury indicators between the craniotomy and minimally invasive groups

Serum BDNF levels were higher and NPY, NSE, and S100B levels were lower in the minimally invasive group than those in the craniotomy group ($P < 0.05$). This indicates that MIIH could improve the neurological injury indicators and protect nervous system in patients with CH (**Figure 2**).

Comparison of oxidative stress between the craniotomy and minimally invasive groups

Serum GSH-Px levels were higher and MDA, 8-OhdG, and Cor levels were lower in the minimally invasive group than those in the cranioto-

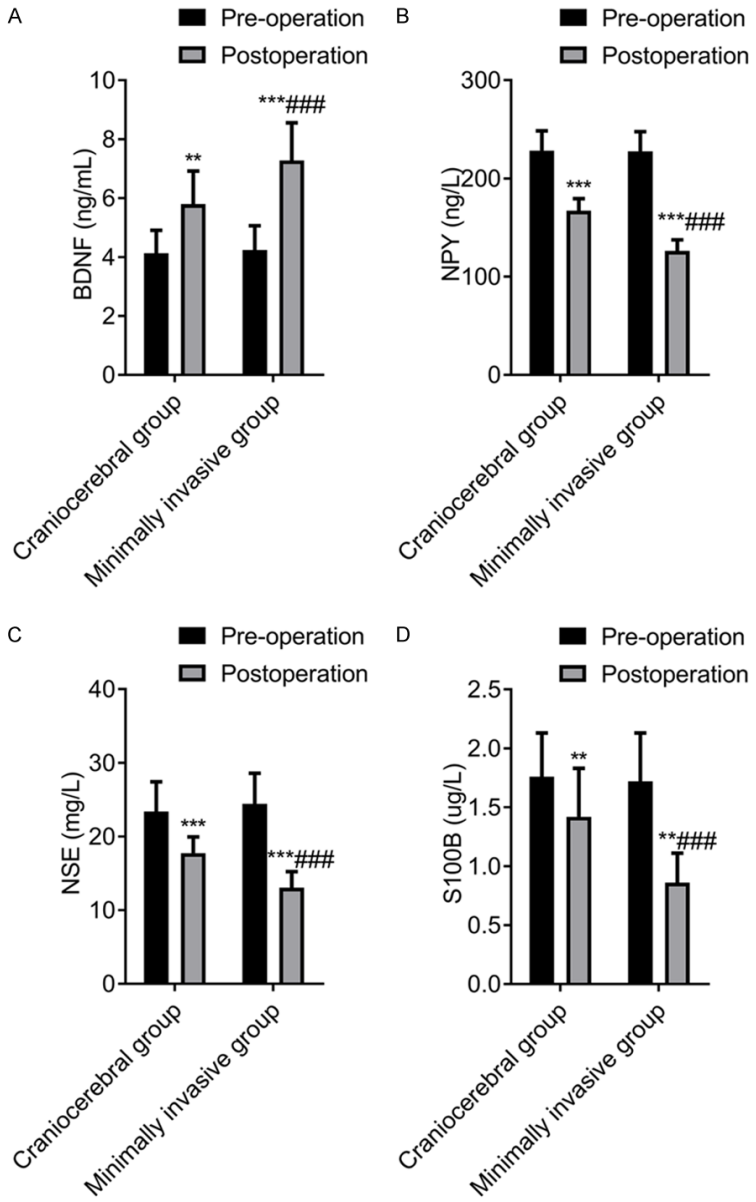

Figure 2. Comparison of nerve injury indices between the craniotomy and minimally invasive groups. Notes: (A) Brain-derived neurotrophic factor (BDNF); (B) neuropeptide Y (NPY); (C) neuron-specific enolase (NSE); and (D) S100B. Compared with the craniotomy group, $^{\#\#\#}P < 0.001$; compared with the group before surgery, $^{**}P < 0.01$, $^{***}P < 0.001$.

my group ($P < 0.05$).This indicates that MIIH could reduce oxidative stress in patients with CH (**Figure 3**).

Comparison of serum apoptosis-related molecules between the craniotomy and minimally invasive groups

There were no significant differences in the expression levels of caspase-3, caspase-9 and XIAP between the minimally invasive group and craniotomy group before surgery ($P > 0.05$). After surgery, the expression level of serum XIAP was higher, whereas serum caspase 3 and caspase 9 levels were lower in the minimally invasive group than those in the craniotomy group ($P < 0.05$). This indicated that MIIH could significantly reduce serum apoptosis-related molecules in patients with CH (**Figure 4**).

Comparison of SSS, BI, and MoCA-B scores between the craniotomy and minimally invasive groups

Postoperative SSS and BI scores in the minimally invasive group were higher than those in the craniotomy group ($P < 0.05$). Postoperative MoCA-B scores were not significantly different between the two groups ($P > 0.05$). This indicated that MIIH could improve neurological impairment and performance in activities of daily living in CH patients without affecting their cognitive functions (**Figure 5**).

Comparison of complications between the craniotomy and minimally invasive groups

The total incidence of postoperative complications in the minimally invasive group (5.26%) was lower than that in the craniotomy group (27.27%) ($P < 0.05$), indicating the safety of MIIH (**Table 3**).

Comparison of brain tissue indicators between the case and normal groups

The case group exhibited a higher proportion of neuronal apoptosis, higher expression of Omi/HtrA2, PARP, and pro-caspase 3 and pro-caspase 9, higher activities of caspase 3 and cas-

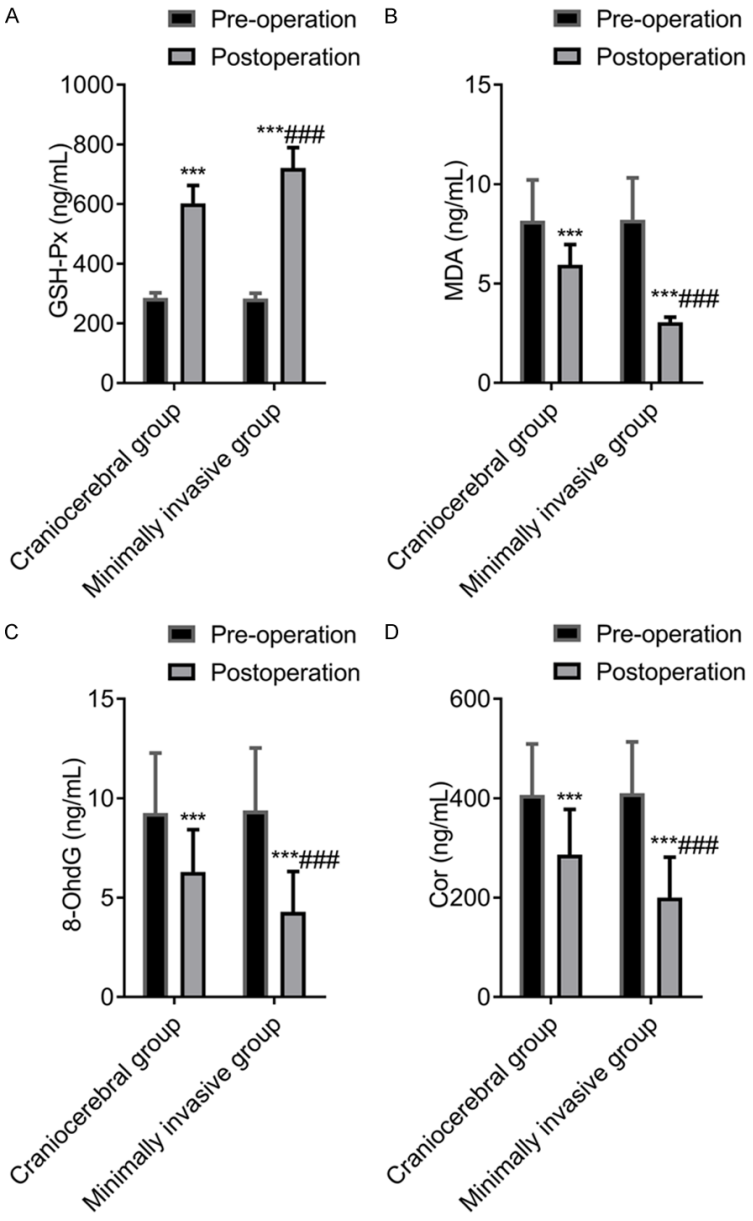

Figure 3. Comparison of oxidative stress between the craniotomy and minimally invasive groups. Notes: (A) Glutathione peroxidase (GSH-Px); (B) malondialdehyde (MDA); (C) 8-hydroxydeoxyguanosine (8-OhdG); and (D) cortisol (Cor). Compared with the craniotomy group, $^{###}P < 0.001$; compared with the group before surgery, $^{***}P < 0.001$.

pase 9 ($P < 0.05$), and lower level of XIAP ($P < 0.05$) in brain tissue than the normal group (**Figure 6**).

Correlation between the proportion of neuronal apoptosis in brain tissue and each indicator

Pearson's correlation analysis showed that the proportion of neuronal apoptosis in brain tissue was positively correlated with the expression of Omi/HtrA2, PARP, and pro-caspase 3 and pro-caspase 9 as well as the activity of caspase 3 and caspase 9 ($r > 0$, $P < 0.05$). However, it was negatively correlated with the XIAP expression ($r < 0$, $P < 0.05$). It is indicated that the proportion of neuronal apoptosis in brain tissue was closely related to each indicator (**Table 4**).

Discussion

Secondary injury is the main cause of brain tissue damage after CH [12, 13]. Mitochondrial apoptosis is a highly conserved process regulated by apoptotic genes. Apoptotic signals from hypoxia, ischemia, trauma, or inflammatory mediators can affect mitochondria, change mitochondrial membrane permeability, release mitochondria-associated substances into the intracellular compartment, and mediate mitochondrial and cellular apoptosis [14-16]. In this study, the increased expression of proteins such as pro-caspase 3 and pro-caspase 9 could accelerate the process of neuronal apoptosis [17]. Su et al. [18] found that the expression levels of caspase 3, p-p38, and p-ERK were higher in a rat cerebral ischemia-reperfusion group than those in a sham-operated group, which was evidenced by pathological damage and neurological deficits, indicating that pro-caspase 3 and pro-caspase 9 can reflect the apoptosis of nerve cells. Qureshi et al. [19] reported that 10 of the 12 patients with spontaneous CH showed signs of apoptosis, and apoptotic cells accounted for more than half of the total number of cells in 5 of the patients. The proportions of apoptotic and necrotic cells were similar in the other 5 specimens, indicating that apoptosis was a form of cell death associated with CH around

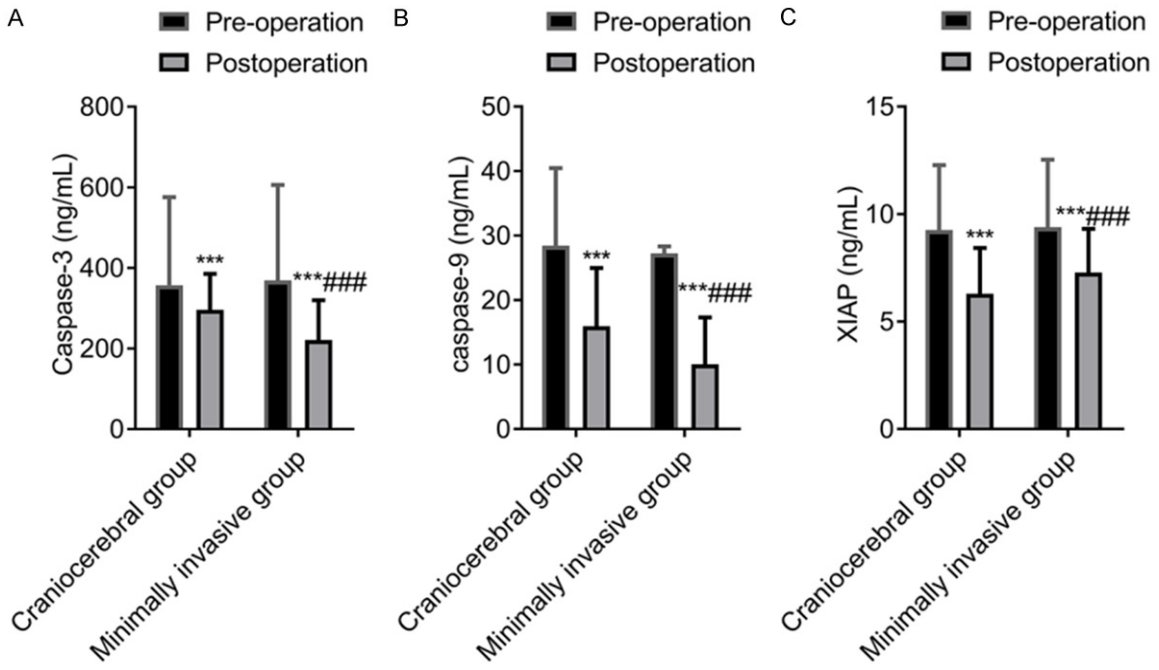

Figure 4. Comparison of serum apoptosis-related molecules between the craniotomy and minimally invasive groups. Notes: (A) Caspase 3; (B) caspase 9; and (C) X-linked inhibitor of apoptosis protein (XIAP). Compared with the craniotomy group, $^{###}P < 0.001$; compared with the group before surgery, $^{***}P < 0.001$.

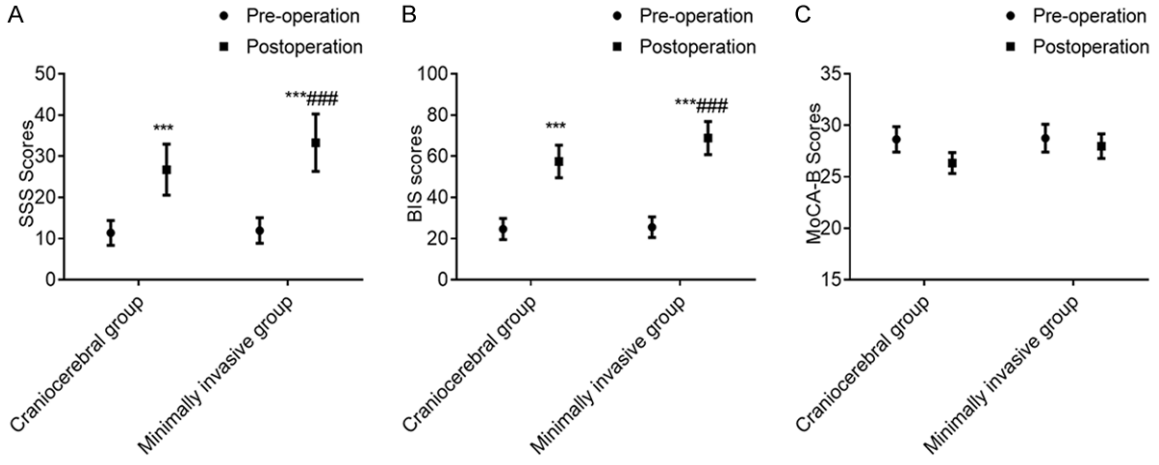

Figure 5. Comparison of SSS and BI scores in craniotomy and minimally invasive groups. Note: (A) Scandinavian Stroke Scale (SSS); (B) Barthel Scale/Index (BI); and (C) Montreal Cognitive Assessment Scale (MoCA-B). Compared with the craniotomy group, $^{###}P < 0.001$; compared with the group before surgery, $^{***}P < 0.001$.

Table 3. Comparison of complications between the craniotomy and minimally invasive groups (%)

Group	Cases	Lung infection	Bleeding from the digestive tract	Fever	Renal insufficiency	Incidence rate
Craniotomy group	22	2 (9.09)	1 (4.55)	2 (9.09)	1 (4.55)	6 (27.27)
Minimally invasive group	38	1 (2.63)	0	1 (2.63)	0	2 (5.26)
χ^2	-	-	-	-	-	4.092
P	-	-	-	-	-	0.043

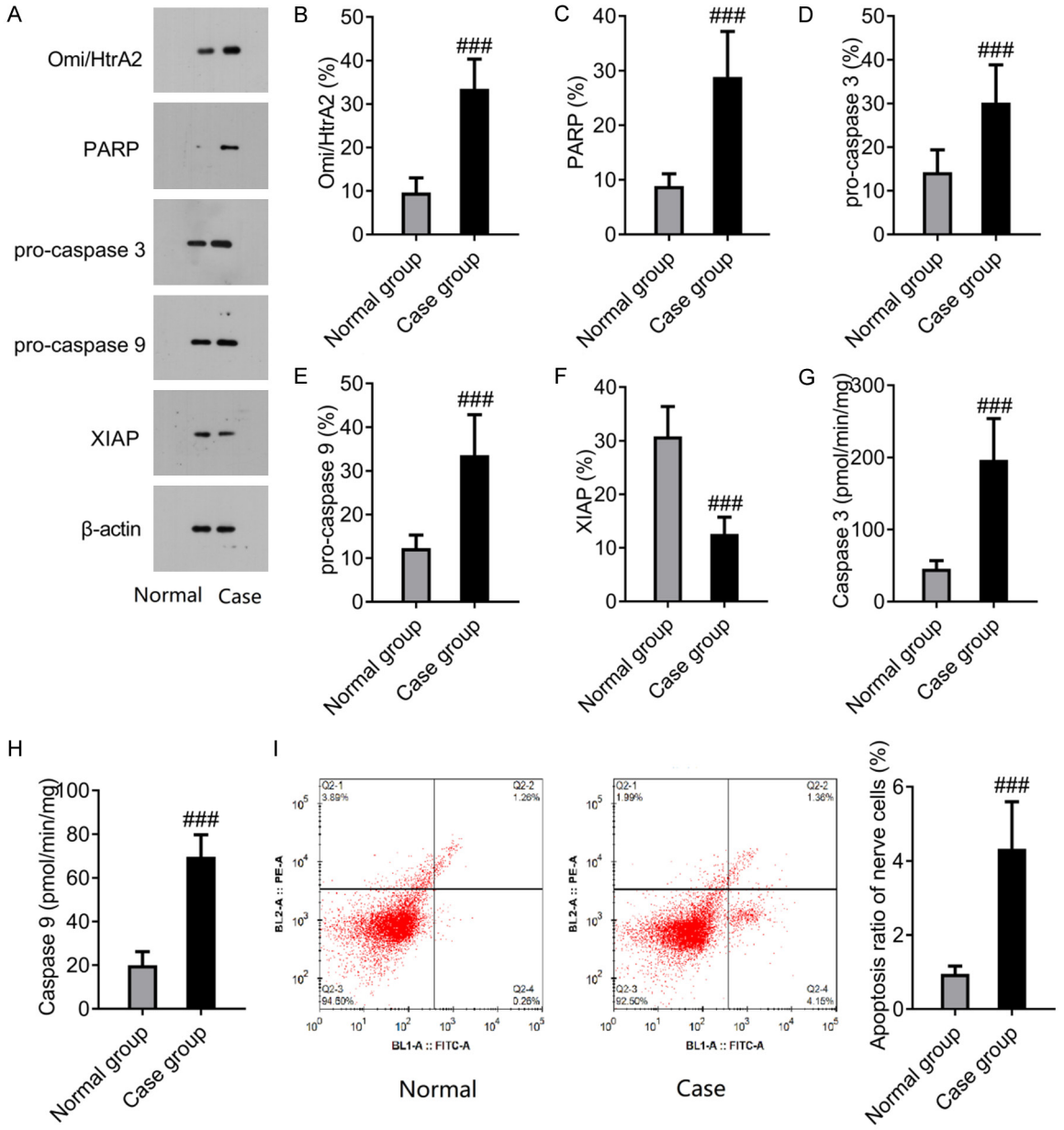

Figure 6. Comparison of brain tissue parameters in the normal and case groups. Note: (A) Western blotting images of Omi/HtrA2, poly-adenosine diphosphate-ribose polymerase (PARP), pro-caspase 3, pro-caspase 9, and X-linked inhibitor of apoptosis protein (XIAP); (B) Omi/HtrA2; (C) PARP; (D) pro-caspase 3; (E) pro-caspase 9; (F) XIAP; (G) caspase 3; (H) caspase 9; and (I) apoptosis rate. Compared with the normal group, $^{###}P < 0.001$.

hematoma. In this study, the proportion of apoptosis, Omi/HtrA2, PARP, and pro-caspase 3 and pro-caspase 9 expression levels, and caspase 3 and caspase 9 activities were higher and XIAP expression was lower in the case group, indicating that the apoptotic mechanism played a crucial role in neural cell injury after CH.

This study further analyzed the correlation between mitochondrial Omi/HtrA2 signaling pathway and neuronal apoptosis. We found that the proportion of neuronal apoptosis in brain tissue was positively correlated with Omi/HtrA2, PARP, pro-caspase 3 and pro-caspase 9 expression levels as well as the activities of caspase 3 and caspase 9, and was negatively correlated with XIAP expression, indicating that neuronal apoptosis in brain hemorrhages is closely related to the expression level and protein activity of Omi/HtrA2. Omi/HtrA2 is a pro-apoptotic protein mainly distributed in mito-

Table 4. Correlation between the proportion of neuronal apoptosis in the brain tissue and each indicator

Indicator	Coefficient	
	r	*P*
Omi/HtrA2	6.742	0.000
PARP	5.683	0.000
Pro-caspase 3	4.264	0.004
Pro-caspase 9	4.597	0.001
Caspase 3	7.251	0.000
Caspase 9	6.923	0.000
XIAP	-5.987	0.000

Note: PARP, poly-adenosine diphosphate-ribose polymerase; XIAP, X-linked inhibitor of apoptosis protein.

chondria, which mediates apoptosis through caspase-dependent and non-caspase dependent pathways. (1) Caspase-dependent pathway: XIAP is located at the Xq25 chromosome and is a member of the inhibitors of apoptosis protein (IAP) family. It plays an anti-apoptotic role by activating nuclear factor kB and inhibiting caspase. In the presence of apoptosis-inducing factors, intact Omi/HtrA2 can be transformed into a mature form across the mitochondrial membrane. Mature Omi/HtrA2 does not contain the N-terminal 133 amino acid sequence, which prevents IAPs from inhibiting caspase activity [20, 21]. (2) Non-caspase dependent pathway: No caspase is involved in this pathway, and Omi/HtrA2 can bind to IAPs under its own enzyme action and directly shear XIAP, thereby conquering the inhibitory effect of IAPs on apoptosis [22].

Oxidative stress occurring after CH can trigger the production of excessive reactive oxygen species after CH. This leads to the release of Omi/HtrA2 from the intermembrane space of mitochondria. Subsequently, Omi/HtrA2 binds to IAP through amino-terminal Reaper-associated sequences, initiating apoptosis and activating caspase activity [23]. In this study, serum GSH-Px and BDNF levels were higher and MDA, 8-OhdG, Cor, NPY, NSE, and S100B levels were lower in the minimally invasive group than in the craniotomy group. This indicates that the oxidative stress in brain tissue of patients with CH was significantly reduced after MIIH removal, and the apoptosis of nerve cells was improved, which effectively improved the nerve function. In addition, it was found that the total response rate, hematoma clearance rate, SSS score, and BI score were higher, and surgery-related indices were better in the minimally invasive group than those in the craniotomy group. These results further demonstrate that MIIH has advantages of high efficacy, high hematoma clearance rate, and short treatment time. MIIH can reduce the neurological deficits and improve performance in activities of daily living. The YL-1 disposable needle used in MIIH has an external diameter of only 3 mm, and the patient only needs to undergo one puncture, resulting small trauma and less intraoperative blood loss [24]. Moreover, by employing biochemical enzyme technology and the principle of fluid dynamics, the powdered hematoma can be effectively flushed out using the side holes of the needle body. This technique facilitates the rapid clearance of semi-solid and liquid hematomas, improving the rate of clearance. The constant intracranial pressure during treatment shortens the time needed to clear the hematoma, which protects the brain tissue and reduces nerve damage and oxidative stress [25]. From a safety perspective, the overall incidence rate of complication in the minimally invasive group (5.26%) was lower than that in craniotomy group (27.27%). Cui et al. [26] found that the complication rate in the minimally invasive group (11.62%) was lower than that in the small bone window intracranial hematoma group (30.23%). This is similar to our study results, further indicating that MIIH has a high safety.

In summary, mitochondrial Omi/HtrA2 signaling pathway has a close correlation with the neuronal apoptosis in brain tissue after human CH and may play a role in the progression of neuronal apoptosis associated with CH. MIIH exhibited advantages of high efficacy, high hematoma clearance rate, and few complications, which can reduce nerve damage, regulate oxidative stress, and improve the performance of activities of daily living. However, there are some limitations in this study. First, only limited investigations were conducted to elucidate the specific conduction pathway of the mitochondrial Omi/HtrA2 signaling pathway in brain tissue after CH, without revealing the expression of its downstream signaling molecules, which needs to be verified by cellular or animal experiments in future research. Second, the sample size was small, which may lead to a certain bias in the results. Third, the timing of minimally inva-

sive surgery was not discussed. In the next study, we will further expand the number of cases.

Disclosure of conflict of interest

None.

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