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Higher Serum Occludin Level after Intracerebral Haemorrhage is Associated with Early Neurological Deterioration

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ABSTRACT

Early neurological deterioration (END) significantly impacts the prognosis of patients with acute intracerebral haemorrhage (ICH). The present study aims to investigate the predictive value of serum occludin in END among patients with ICH. A total of 34 patients with ICH and 16 healthy individuals were prospectively enrolled in the present study. The control group comprised individuals without ICH matching the age and gender of the patients in the ICH group. The serum occludin levels were measured using enzyme-linked immunosorbent assays. Computed tomography was used to assess the boundaries of each layer of cerebral haematoma and cerebral oedema and to establish the cumulative area and thickness of all layers to determine the volume of cerebral haematoma and cerebral oedema. According to the Glasgow coma scale score, patients were divided into the END group and the non-END group. Furthermore, the relationship between the degree of cerebral oedema or haematoma and the level of serum obliterating protein was evaluated by the Pearson correlation coefficient. The receiver operating characteristic (ROC) curve analysis and logistic regression analysis were employed to assess the relationship between serum occludin and END in patients with ICH. The serum occludin levels were increased in patients with ICH following ICH, peaking at days 1 and 3 post-haemorrhage ($p < 0.001$) and thus aligning with cerebral haematoma and oedema trends. Among the patients with ICH, serum occludin levels exhibited a strong negative correlation with neurological function scores ($r = -0.600$, $p < 0.001$). On the first day after the onset of ICH, the occlusive protein level was significantly higher in the patients with ICH than in the individuals without ICH. On day 14 after the onset of ICH, the occlusive protein level returned to the level of the control group ($p < 0.001$). The ROC analysis demonstrated that serum occludin levels at day 1 post-haemorrhage had a significant predictive value for END (area under the curve: 0.85, $p < 0.001$), and the regression analysis identified serum occludin as an independent risk factor for END in patients with ICH (adjusted odds ratio: 2.61, $p < 0.001$). To conclude, serum occludin levels are closely associated with END following ICH and may serve as an independent predictive risk factor for END in patients with ICH.

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SS major role in the acquisition of data, drafted and revised the article, data collection and processing. CZ interpreted and analyzed the data, revised the article. JC design and conceptualized study, drafted and revised the article. All authors have approved the final manuscript.

Key words
Occludin, Brain edema, Blood–brain barrier, Cerebral hemorrhage, Blood

INTRODUCTION

Intracerebral haemorrhage (ICH) represents a prevalent and severe form of stroke. It contributes to 15%–20% of all stroke cases and exhibits a notably high incidence and mortality rates (Morawo and Gilmore, 2016). Furthermore, it instigates primary brain injury as blood accumulates in the brain's vicinity (Zheng *et al*., 2016); subsequently, secondary brain injury ensues, involving inflammatory responses, neuronal apoptosis, disruption of the blood–brain barrier and the onset of brain oedema, ultimately leading to neurological impairment (Zheng *et al*., 2016). Early neurological deterioration (END) occurs as a frequent complication, substantially elevating the risk of unfavourable outcomes (Godoy and Boccio, 2005). Presently, clinical assessments primarily rely on the ICH score and brain imaging, both of which present challenges. Although the ICH score is the most reliable measure of prognosis for ICH, it was not designed to determine the risk of END (Kim *et al*., 2016). Although there has been much research on the relationship between impact testing and END, imaging methods are time-consuming,

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expensive and thus unsuitable for the rapid determination of the patient's status. Although some serum biomarkers, such as glutamate and glycine, and the pro-inflammatory cytokine interleukin-6 have shown some clinical value in END judgment, their relevance to patient disease change requires further study (Gong *et al*., 2021; Su *et al*., 2022). Consequently, there is a pressing need for novel detection methodologies. Although serum markers have been extensively investigated, specific markers needed to fulfil this objective remain elusive (Petty and Lo, 2002).

Occludin, an essential constituent of tight junction proteins at the blood–brain barrier (BBB), assumes a pivotal role in upholding the barrier's structural integrity (Petty and Lo, 2002). Intracerebral haemorrhage results from blood vessel rupture and is often accompanied by BBB disruption, potentially culminating in vascular brain oedema, white blood cell extravasation and the infiltration of vascular and neurotoxic substances into brain tissue, thereby triggering a cascade of secondary brain injuries (Keep *et al*., 2018). Moreover, oxidative stress affects the expression, distribution and phosphorylation of tight junction proteins and increases BBB permeability (Zhou *et al*., 2015). Very early oxygen therapy may affect molecular pathways involved in BBB damage after ICH, including MMP-9 and occludin (Zhou *et al*., 2015). Nonetheless, it remains uncertain whether serum occludin holds relevance in the context of END in patients with ICH. This study endeavours to elucidate the connection between post-ICH serum occludin levels and the occurrence of END.

MATERIALS AND METHODS

Study design and participants

The present investigation was conducted as a prospective observational study. A total of 34 consecutive patients with ICH were randomly enrolled in the study between May 2020 and October 2022, while 16 healthy individuals were randomly selected as control participants at the Physical Examination Centre of the same hospital during the same period. The study was approved by the ethics committee of our hospital, and all patients provided signed informed consent.

The patients included for the study were admitted to the hospital within 24 h after the onset of ICH symptoms and were confirmed to have ICH via a CT scan, aged between 18 and 80 years.

The patient who had secondary haemorrhage as a result of a tumour, trauma, vascular malformation, aneurysm or blood coagulation abnormalities; did not undergo brain CT re-examination on time; had liver and kidney dysfunction, serious digestive system disease, peripheral vascular embolism, autoimmune disease, tumour and/or blood system disease; were unable to complete the project; and were using sedatives, anticoagulants and/or other medications.

The control were excluded from the study group had basic information of age and gender consistent with that of the ICH group; but did not have ICH. Other exclusion criteria were consistent with that of the study group.

Measurement of serum occludin levels

Peripheral venous blood samples (4 ml) were obtained from the patients at four distinct time points: 1, 3, 7 and 14 days following ICH. Similarly, blood samples from healthy individuals were acquired during their visits to the Physical Examination Centre. These blood specimens were subsequently subjected to centrifugation, with the resulting serum carefully transferred to Eppendorf tubes and then stored at −80°C for future use. Centrifugation was conducted at 3000 rpm with a force of 145,000 g for 10 minutes. The serum occludin levels were quantified using a commercially available enzyme-linked immunosorbent assay kit designed for human samples (Occludin: USCN, Wuhan, China).

Measure the volume of intracerebral haemorrhage and cerebral oedema

The ICH and cerebral oedema volumes were assessed via computed tomography (CT) on days 1, 3, 7 and 14 post-haemorrhage. All images underwent digital analysis using PACS workstations. A mouse was employed to delineate the boundaries of the cerebral haematoma and brain oedema zone for each layer, adjusting the window width and layer thickness as needed. Subsequently, the cumulative area of all layers was computed and multiplied by the thickness of each layer to ascertain the volumes of cerebral haematoma and brain oedema (Jack *et al*., 1990). Depending on the quantity of cerebral haematoma or brain oedema, the groups were categorised as mild (0–30 ml), toxic (31–50 ml) and severe (>50 ml) (Nogueira *et al*., 2018).

Neurological function deterioration

Neurological function deterioration was defined as a two-point or greater decrease in the GCS score from the time of presentation examination to 72 h post-haemorrhage that was not associated with sedation. According to the GCS score, the patients were divided into the non-END group and the END group.

Statistical analysis

Continuous data were presented as mean±standard deviation (SD). For normally distributed data, the student's t-test or ANOVA was conducted. Data not conforming to a normal distribution were analysed using the Mann–

Whitney test. Categorical data were expressed as n (%) and compared between groups using the chi-squared test or Fisher's exact test. Pearson's correlation coefficient was employed to assess the relationship between the extent of brain oedema or cerebral haematoma and serum occludin levels. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of serum occludin, logistic regression analysis was performed to assess the correlation between serum occludin levels and END, and statistical analysis was carried out using the SPSS 23.0 software (IBM Corporation, Armonk, NY, USA), with a significance level of $p < 0.05$.

RESULTS

Baseline clinical characteristics of the study participants

The present study included 34 patients with ICH in the ICH group and 16 healthy individuals in the control group. There were no significant differences between the two groups in terms of mean age (61.2±12.8 years vs. 61.2±11.0 years) or gender distribution (17.6% women vs. 18.8% women). Additional clinical data for the patients with ICH are presented in Table I. The basic medical history of hypertension, diabetes and coronary heart disease were further studied in two study cohorts. The results showed no significant difference in the patient baseline status. In addition, the bleeding locations of patients with ICH (prefrontal [n= 3, 8.8%], occipital lobe [n= 2, 5.9%] and the parietal lobe brain stem [n= 3, 8.8%; n= 2 with a low incidence, 5.9%]) were compared: the incidence of haemorrhage in the temporal lobe (n= 4, 11.8%), cerebellar (n= 5, 14.7%) and basal ganglia (n = 8, 23.5%) was higher than in the other locations (Table I).

Serum occludin levels, brain haematoma and brain oedema in patients with ICH

A significant increase in serum occludin levels was observed following ICH. Specifically, on days 1 and 3 post-haemorrhage, serum occludin levels were notably higher compared with the control group ($p < 0.05$). However, on days 7 and 14 post-haemorrhage, serum occludin levels in the patients exhibited a declining trend ($p < 0.05$), and no significant differences were observed in comparison with the healthy control group ($p > 0.05$). Additionally, the volume of cerebral haematoma was the most substantial on days 1 and 3 ($p < 0.05$); in comparison, a significant reduction in haematoma volume was observed on day 14 ($p < 0.05$). Furthermore, cerebral oedema was most severe on days 3 and 7 post-ICH, with a slight reduction by day 14; however, no statistically significant difference was detected when compared with days 1, 3 and 7 ($p > 0.05$; Fig. 1).

Table I. Baseline characteristics of ICH patients and healthy volunteers.

Characteristic	Patients with ICH (n = 34)	Healthy volunteers (n = 16)	P
Age (mean ± SD)	61.2 ± 12.8	61.2 ± 11.0	0.990
Female n (%)	6 (17.6%)	3 (18.8%)	1.000
History n (%)			
Hypertension	18 (52.9%)	6 (37.5%)	0.308
Diabetes	2 (5.9%)	1 (6.2%)	1.000
Coronary heart disease	43 (26.7%)	31 (12.5%)	1.000
Operation or not	11 (32.4%)	NA	NA
Location of cerebral hemorrhage			
Frontal lobe	3 (8.8%)	NA	
Temporal lobe	4 (11.8%)	NA	
Occipital lobe	3 (8.8%)	NA	
Parietal lobe	2 (5.9%)	NA	
Cerebellum	5 (14.7%)	NA	
Brain stem	2 (5.9%)	NA	
Basal ganglia	8 (23.5%)	NA	

Data are presented as mean±standard deviation or n (%). N/A, not applicable; ICH, acute cerebral hemorrhage.

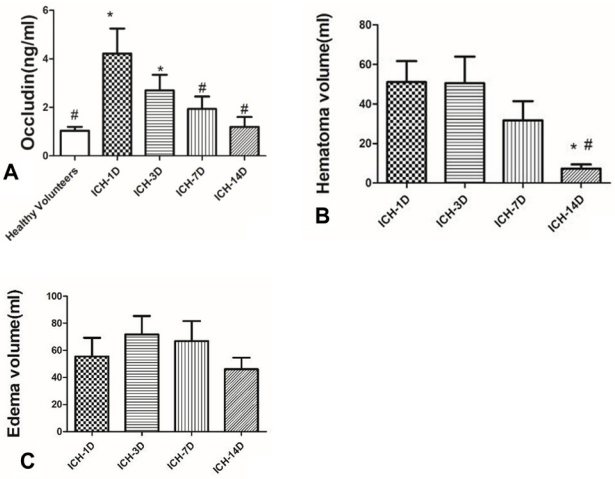

Fig. 1. Serum occludin levels (A), brain hematoma (B) and brain edema (C) in patients with ICH. (A) *P < 0.05 versus the healthy volunteer group. # P < 0.05 versus day 1. (B) *P < 0.05 versus day 1; # P < 0.05 versus day 3. (C) No significant differences were found at each time point. ICH, acute cerebral hemorrhage.

The relationship between the serum occludin at day 1 post-haemorrhage and the volume changes of cerebral haematoma and oedema in patients with ICH were further

observed. Pearson's correlation analysis demonstrated a significant positive correlation between serum occludin and the volume of haematoma ($r = 0.259$, $p < 0.05$). However, no significant correlation was observed between serum occludin and the volume of brain oedema ($r = 0.157$, $p = 0.292$). The level of occludin and GCS score ($r = -0.600$, $p < 0.001$) were highly correlated with each other in the present study population. Therefore, the serum occludin level was considered a reliable decision tool reflecting the ICH status.

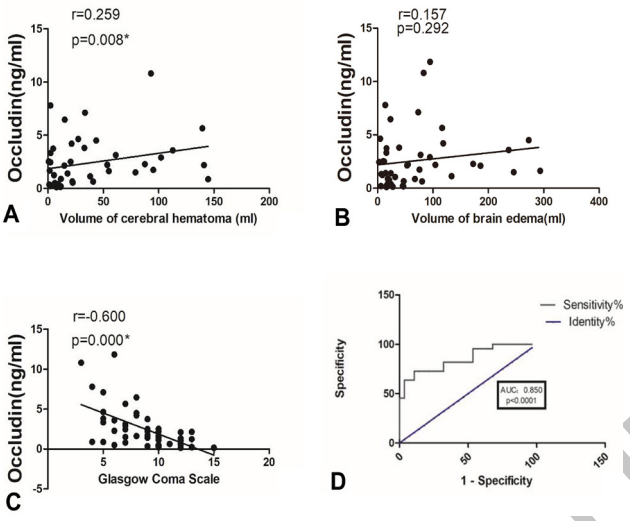

Fig. 2. The relationship between the serum occludin and the volume of brain edema, cerebral hematoma, and GCS score. (A-C) Pearson's correlation analysis showing relationship between the serum occludin at 1 day post-ICH with the volume of brain edema (A), cerebral hematoma (B), and GCS score (C). (D) shows receiver operating characteristic curve analysis of serum occludin level at 1 day post-ICH for END in ICH patients.
ICH, acute cerebral hemorrhage; GCS, Glasgow coma scale; END, early neurological deterioration.

The ROC curve analysis underscored the predictive capacity of the serum occludin level at day 1 post-haemorrhage for END, with an area under the curve (AUC) value of 0.850 ($p < 0.0001$; Fig. 2). The maximum point of the Youden index (sensitivity + specificity − 1) was taken as the best cut-off value, and the maximum sensitivity and specificity of occludin in ICH detection was calculated by the abscissa and ordinate of the corresponding ROC curve. The diagnostic value of plasma occludin in patients with ICH was evaluated by the size of the ROC AUC, the optimal cut-off value and the corresponding sensitivity and specificity (95% CI: 0.7424-0.9589, $p < 0.0001$).

Relationship between serum occludin and early neurological deterioration in patients with ICH

As shown in Table II, the univariate analysis showed that the risk factors significantly associated with END in patients with ICH included age, the serum occludin level at day 1 post-haemorrhage, the volume of cerebral haematoma and brain oedema and surgical operation ($p < 0.05$). Conversely, sex and past medical history showed no significance between the END group and the non-END group. A logistic multivariate regression model was developed with only one significant factor of serum occludin, showing that serum occludin at day 1 post-haemorrhage was an independent predictor for END in patients with ICH (unadjusted OR: 2.607, 95% CI: 1.043–4.755, $p = 0.002$; Table III).

Table II. Univariate analysis for variables associated with severity in patients with cerebral hemorrhage (n (%)).

Variable	END group (n=22)	Non-end group (n=28)	P
Age	73.00 ± 0.00	66.43 ± 8.85	0.000*
Male	3 (13.6)	5 (17.9)	1.000
Diabetes mellitus	0 (0)	4 (14.3)	0.121
Hypertension	12 (54.5)	12 (42.9)	0.412
Serum occludin level	0.49 ± 0.00	0.42 ± 0.19	0.011*
Hematoma volume	48.80 ± 46.92	21.420 ± 32.18	0.025*
Brain edema volume	95.70 ± 91.97	43.13 ± 43.89	0.021*
Surgical operation	12 (54.5)	7 (25.0)	0.033*

Data are presented as mean±standard deviation or n (%). * denotes statistical significance ($P < 0.05$). END, early neurological deterioration.

Table III. Logistic regression model for severity in patients with cerebral hemorrhage.

Variable	Wals	OR (95% CI)	P
Serum occludin level	9.772	2.607 (1.0430–4.755)	0.002*
Constant	10.118	0.112	0.001*

Adjusted for variables with P value < 0.1 in univariate analysis (age, the volume of cerebral hematoma and brain edema, and surgical operation). CI, confidence interval; * denotes statistical significance ($P < 0.05$).

DISCUSSION

The present study highlights that the serum occludin levels in patients with ICH were notably elevated compared with healthy individuals. The levels were also found to be

positively correlated with the peak volume of haematoma and brain oedema. These results suggest potential utility of serum occludin levels as a screening tool for assessing brain injury.

The need for new therapeutic approaches has led to the search for new markers to assess the extent of brain damage (Li *et al*., 2022). When blood vessels break down, products may be released into the cerebral spinal fluid (CSF); examining protein changes in the CSF can provide insight into the pathogenic status and prognosis of ICH (Kolias *et al*., 2009). Additionally, circulating biomarkers are practical, economical and easily measured, making them accessible in clinical settings. However, there are currently no ideal circulating markers to reflect the degree of brain injury or to predict the outcome of ICH (Senn *et al*., 2014). Some studies have shown higher serum amyloid A (SAA), homocysteine (Hcy) and plasma B-type brain natriuretic peptide (BNP) levels in patients with spontaneous ICH (SICH) (Bunevicius *et al*., 2015; Bian *et al*., 2019). High levels of SAA and Hcy had a negative impact on the vascular endothelial function of patients, which increased significantly with an increase in the degree of neurological deficits (Vassalli *et al*., 2020). Therefore, SAA, Hcy and BNP can be used to indicate the severity of patients with SICH (Lorente *et al*., 2022); however, none are capable of evaluating the severity of ICH well, and their specificity and sensitivity are not ideal.

Occludin is an important component of tight junctions that constitute the BBB. Previous studies reported that cerebral ischemia resulted in the activation of MMP-2 and MMP-9 in brain tissue, leading to the degradation of occludin and destruction of the BBB (Liu *et al*., 2012; Kazmierski *et al*., 2012). Furthermore, one study found that the number of tight junctions was significantly higher in patients with ICH than in individuals who had no BBB damage (Jiao *et al*., 2015), which indicated disassembly of the tight junctions and disruption of the integrity of the BBB. The present study has revealed an elevated serum occludin level in patients with ICH compared with healthy controls, suggesting that ICH induces damage to the BBB, potentially resulting in the loss of occludin from the BBB.

The present study observed a correlation between occludin levels and haematoma size. Larger haematoma volumes could potentially trigger a more robust systemic inflammatory response in both the central nervous system and periphery, resulting in elevated occludin levels shortly after ICH. Furthermore, an association between higher occludin levels and the extent of perihaematomal oedema (PHE) was found. However, likely due to the limited sample size, no statistically significant correlation between serum occludin and the presence of brain oedema surrounding the haematoma was identified. Notably, PHE (detected as a hypodense rim around the ICH on CT scans) serves as a neuroimaging indicator of secondary injury following ICH and has been linked to unfavourable outcomes (Urday *et al*., 2015, 2016). The early formation of PHE is believed to arise from osmotic fluid shifts and the early activation of immune cells in the perihaematomal region, triggered by oxidative stress and the coagulation cascade (Ziai, 2013; Grunwald *et al*., 2017). The present study suggests that occludin levels are independently associated with both the ICH volume and haematoma formation.

The present study revealed a strong correlation between serum occludin levels and neurological function scores. To evaluate the discriminative capacity of serum occludin levels in distinguishing END of ICH based on neurological function scores, an ROC curve analysis was employed, yielding an excellent AUC value of 0.850 that was indicative of high discriminatory power. Collectively, the present findings suggest that serum occludin levels hold promise as a novel biomarker for assessing END in patients with ICH. Notably, differences in neurological function could potentially be attributed to surgical factors. However, regression analysis did not reveal any significant correlations with surgical variables. This observation may be explained by the strong collinearity between surgery and patients' serum occludin levels, as surgery can also lead to considerable BBB disruption.

The GCS neurological function score 24 h after stroke is very important for estimating disease severity and prognosis (Grunwald *et al*., 2017; Zhao *et al*., 2018). This study is the first to establish a correlation between serum occludin levels and GCS scores following ICH.

Furthermore, it was observed in the present study that patients with relatively low GCS scores exhibited elevated serum occludin levels, suggesting that these levels may serve as indicators of the extent of neurological damage. Notably, the assessment of serum occludin offers a more objective, convenient and concise alternative to traditional neurological function scores. This is particularly advantageous for patients who may have difficulty cooperating with early neurological function tests, where assessing their condition can be challenging. In addition, the present results showed that patients had significantly elevated serum blocking protein levels on the first day of ICH occurrence. Interestingly, by day 14 of ICH, serum atretic protein levels in patients with ICH returned to those of healthy controls. The results revealed the important clinical significance of the serum atresia protein level in monitoring the progression of ICH. In addition, it was also found that the level of cerebral haematoma and the degree of cerebral oedema changed from day 1 to day 14 of ICH

and there were also changes in the atresia protein level. Overall, the serum occludin level is a potential biomarker of brain injury.

CONCLUSION

This study ascertains whether serum occludin levels are associated with stroke severity and the clinical outcome of ICH. Using multivariate analysis, it was revealed that rising serum occludin levels were dramatically related to the GCS score and haematoma volume. Hence, it is possible that serum occludin levels could be used to reflect the severity and clinical outcome after ICH.

DECLARATIONS

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Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of First Affiliated Hospital of Baotou Medical College, and all patients provided signed informed consent.

Availability of data and materials

The results of our study supporting the findings are included within this paper. In order to protect patient privacy, the personal data supporting the findings of this study are restricted by the Ethics Committee of First Affiliated Hospital of Baotou Medical College. Data are available to researchers who meet the criteria for access to confidential data from the corresponding author.

Consent for publication

Not applicable.

Statement of conflict of interest

The authors have declared no conflict of interest.

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