Int J Clin Exp Med 2017;10(8):12336-12342
www.ijcem.com /ISSN:1940-5901/IJCEM0046606

Original Article

Serum levels of matrix metalloproteinase-2 and matrix metalloproteinase-9 have clinical significance for the evaluation of the prognosis of patients with ruptured intracranial aneurysms after interventional therapy

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Received December 14, 2016; Accepted March 30, 2017; Epub August 15, 2017; Published August 30, 2017

Abstract: The present study is to investigate changes of serum levels of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) before and after interventional therapy of ruptured intracranial aneurysm (IA), as well as their correlation with prognosis. A total of 200 patients with ruptured IAs who received interventional treatments at our hospital between December 2014 and November 2015 were enrolled in the present study. Peripheral venous blood was collected before the therapy, and on day 3, in the end of week 1 and in the end of month 1 after therapy. In the meantime, 150 healthy subjects were included as control. Serum levels of MMP-2 and MMP-9 were determined using enzyme-linked immunosorbent assay. The clinical effects of all patients were evaluated using Glasgow outcome scale (GOS) after discharging. Univariate analysis was performed using Chi-square test for the identification of factors that might mainly affect the prognosis. Multivariate logistic regression analysis was performed to investigate the factors with statistical significance. The prognostic value was determined by the area under the receiver operating characteristic curve. Serum levels of MMP-2 and MMP-9 in patients with ruptured IAs were different before and after interventional therapy. The majority of patients with ruptured IAs had good prognosis after interventional therapy. Prognosis of patients with ruptured IAs was correlated with cerebral angiospasm, Hunt-Hess classification and rehaemorrhagia. Serum levels of MMP-2 and MMP-9 were distinct in patients with different grades of Hunt-Hess classification or different severity of cerebral angiospasm. Serum levels of MMP-2 and MMP-9, Hunt-Hess classification and cerebral angiospasm severity had diagnostic significance in the evaluation of the efficacy of interventional therapy on ruptured IAs. The present study demonstrates that serum levels of MMP-2 and MMP-9 are of clinical significance for the evaluation of the prognosis of patients with ruptured IAs after interventional therapy.

Keywords: Intracranial aneurysm, matrix metalloproteinase-2, matrix metalloproteinase-9, prognosis

Introduction

Intracranial aneurysm (IA), a kind of abnormal swelling on the wall of the cerebral artery, is a common cerebrovascular disease with an incidence of 1-2% in adults [1]. IA occurs usually because brain vessels are thinner and weaker than peripheral blood vessels and are lacking of branches. IA rupture usually leads to subarachnoid hemorrhage (SAH), which is the main cause of deaths. The incidence of IA rupture is the third highest in China, being only lower than that of hypertensive cerebral hemorrhage and cerebral thrombosis [2, 3]. Recurrent IA hemorrhage accounts for 14-44% of all cases of IA hemorrhage, and the mortality of patients with recurrent IA hemorrhage is 20-85% [4, 5]. The prognosis of IA hemorrhage is mainly dependent on the incidence of complications and treatment methods. As the development of medical imaging technology, computed tomography angiography, magnetic resonance angiography and digital subtraction angiography have been gradually improved and applied in clinical practice, leading to higher and higher detection rates of cerebral aneurysms.

Table 1. Clinical data of patients with ruptured intracranial aneurysms

Clinical data		Number	Percentage
Diameters	≤ 5 mm	96 patients	34.7%
	6-15 mm	134 patients	84.7%
	15-25 mm	40 patients	14.4%
	> 25 mm	6 patients	2.1%
Different locations	Anterior cerebral artery	28 intracranial aneurysms	10.1%
	Anterior communicating artery	47 intracranial aneurysms	17.0%
	Middle cerebral artery (left/right)	66/59 intracranial aneurysms	23.9%/21.3%
	Posterior communicating artery (left/right)	41/35 intracranial aneurysms	14.8%/12.6%

Early surgical treatments mainly include craniotomy and endovascular interventional therapy. Surgical treatment is very important in reducing the incidence of preoperative re-bleeding and improving prognosis. Interventional therapy has become one of the main methods for the treatment of ruptured IA due to its advantages such as simple approach, less trauma and fewer complications [6]. Cerebral angiospasm caused by cerebral aneurysm rupture has become a difficult problem for patients with cerebral aneurysm after surgery. Cerebral ischemia caused by cerebral angiospasm has ranked the second among all causes of cerebral ischemia [7]. The formation and development of IA rupture are closely related to matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9 [8]. Detection of MMP-2 and MMP-9 in peripheral blood can be of importance in the prediction of prognosis of IA rupture. Hunt-Hess classification is an important scale to evaluate the conditions of SAH patients, with higher grades corresponding to more severe symptoms and weaker tolerance to cerebral angiospasm [9]. In the present study, we retrospectively analyze changes in serum contents of MMP-2 and MMP-9 before and after interventional treatment of ruptured IA, and investigate the correlation between these changes and the prognosis of IA rupture.

Materials and methods

Patients

A total of 200 patients with ruptured IAs who received interventional treatments at our hospital between December 2014 and November 2015 were enrolled in the present study, including 92 males and 108 females (age range, 26-70 years; mean age, 56.72 ± 13.51 years). Peripheral venous blood was collected before therapy, and on day 3, in the end of week 1 and in the end of month 1 after therapy. In the meantime, 150 healthy subjects were included as control. The total number of confirmed ruptured IAs was 276. The sizes of the ruptured IAs ranged between 0.6 and 35 mm. The number of ruptured IAs with a diameter less than 5 mm was 96, that with a diameter between 5 and 15 mm was 134, that with a diameter between 15 and 25 mm was 40, and that with a diameter over 25 mm was 6. The neck widths of the IAs were between 1.0 and 9.9 mm (**Table 1**). All procedures were approved by the Ethics Committee of Nanjing Medical University. Written informed consents were obtained from all patients or their families.

Inclusion and exclusion criteria

The inclusion criteria were: i) the patient received computed tomography (CT) or magnetic resonance imaging (MRI) and classified into stage I or II according to Hunt-Hess classification scale; ii) the patient had at least one ruptured IAs; iii) the patient received endovascular treatment by embolization materials that were mainly Guglielmi detachable coils; iv) the patient had complete clinical data and was glad to cooperate in follow-ups. The exclusion criteria were: i) the patient had cerebral vascular diseases other than IAs; ii) the patient had severe heart, liver and kidney dysfunctions.

Surgical method

All patients were subjected to general anesthesia or heparinization. Under the supervision by DSA, femoral artery puncture was performed using Seldinger technique. After successful puncture, 6F guiding tube was placed in the internal carotid artery at the affected side in femoral artery. The micro-catheter reached the

Table 2. Serum levels of MMP-2 and MMP-9 in patients with ruptured intracranial aneurysms before and after interventional therapy (means ± standard deviations)

Groups		MMP-2 (ng/ml)	*P* value	MMP-9 (ng/ml)	*P* value
Control		88.16 ± 3.62	-	138.62 ± 3.04	-
Patients	Before therapy	153.68 ± 16.56	< 0.05	317.35 ± 36.72	< 0.05
	Day 3 after therapy	145.32 ± 5.63	< 0.05	369.35 ± 32.46	< 0.05
	Week 1 after therapy	162.35 ± 12.36	< 0.05	410.82 ± 40.32	< 0.05
	Month 1 after therapy	124.63 ± 10.63	< 0.05	352.36 ± 29.63	< 0.05

Note: MMP, matrix metalloproteinase.

1/3-1/2 near end of the ruptured IA under the guidance by a guiding wire. Treatment plan was made according to the sizes and shapes of IAs. Using suitable angles, the tip of the 6F guiding catheter was pushed to a suitable position. According to the location of the aneurysm, the tip of the micro-catheter was bent. Under the guidance of micro-guide wire, the micro-catheter head was pushed to the 1/3-1/2 in the IA. If the tip of the micro-catheter was stable, the micro-guide wire was retracted. Appropriate coils were selected and filled into a more flexible and smaller coil, in order to embolize the ruptured IAs. After embolization, conventional anticoagulant therapy was performed. If there is an obvious cerebral hemorrhage after surgery, lumbar puncture and drainage should be maintained for 3-7 days, together with treatment of cerebral angiospasm.

Enzyme-linked immunosorbent assay (ELISA)

Morning fasting venous blood (5 ml) was withdrawn from all patients at the time of onset, and 3 days, 1 week and 1 month after the onset. The blood samples were centrifuged at 3,000 r/min for 20 min for the collection of serum. Serum levels of MMP-2 and MMP-9 were tested using ELISA kits (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's manual.

Glasgow outcome scale

The clinical effects of all patients were analyzed using Glasgow outcome scale (GOS). Patients with 5 or 4 points were considered to have good prognosis, while those with 3, 2 or 1 points were predicted to have poor prognosis.

Statistical analysis

The results were analyzed using SPSS 17.0 software (IBM, Armonk, NY, USA). Measurement data were expressed as means ± standard deviations. Univariate analysis was performed using Chi-square test for the identification of factors that might mainly affect the prognosis, such as Hunt-Hess classification, cerebral angiospasm, rehaemorrhagia, hydrocephalus and epileptic seizure. Then, multivariate logistic regression analysis was performed to investigate the factors with statistical significance. The prognostic value was determined by the area under the receiver operating characteristic (ROC) curve (> 0.5 was considered effective). Differences with $P < 0.05$ were considered statistically significant.

Results

Serum levels of MMP-2 and MMP-9 in patients with ruptured IAs are different before and after interventional therapy

To determine the changes of MMP-2 and MMP-9 levels before and after interventional therapy, ELISA was performed. Before interventional therapy, the serum levels of MMP-2 and MMP-9 in patients with ruptured IAs were significantly higher than those in normal subjects ($P < 0.05$). On day 3 and in the end of week 1, the serum level of MMP-2 in the patients was significantly higher than that before therapy ($P < 0.05$). In the end of month 1, the serum level of MMP-2 in the patients was significantly lower than that before therapy ($P < 0.05$). On day 3 and in the end of week 1, the serum level of MMP-9 in the patients was significantly higher than that before therapy ($P < 0.05$). In the end of month 1, the serum level of MMP-9 in the patients was significantly lower than that in the end of week 1 ($P < 0.05$), but still higher than that before therapy ($P < 0.05$) (**Table 2**). The results suggest that the serum levels of MMP-2 and MMP-9 in patients with ruptured IAs are different before and after interventional therapy.

Figure 1. Cerebral angiography of a patient with spontaneous subarachnoid hemorrhage. A. Two-dimensional angiography showing the position of aneurysm. B. Three-dimensional angiography showing the neck of aneurysms and their sizes. Cystic dilatation was observed in the right posterior communicating artery, with a size of 3.1 × 3.2 mm and a neoplasia neck of 2.6 mm. Cerebral angiography showed dense aneurysm embolization, and tumor-bearing artery was unobstructed.

Table 3. Logistic regression analysis of preoperative risk factors and prognosis of ruptured intracranial aneurysms

Variables	Exp (B)	95% Exp (B)		*P* value
		Lower limit	Upper limit	
Age	0.94	0.65	0.83	0.28
Gender	0.71	0.62	1.94	0.17
Hydrocephalus	0.39	0.07	1.25	0.35
Cerebral angiospasm	8.7	3.4	9.1	0.04
Hunt-Hess classification (I and II)	0.96	0.17	8.98	0.01
Rehaemorrhagia	1.36	4.65	13.5	0.00
Epileptic seizure	8.34	12.65	24.8	0.43

The majority of patients with ruptured IAs have good prognosis after interventional therapy

To evaluate the clinical effect of interventional therapy on ruptured IAs, GOS was used. Imaging data of IAs showed that the IAs varied a lot before, in the middle of and after therapy, and disappeared after embolization. When the patients were discharged, GOS scores showed that 146 cases (73%) had good prognosis (4 or 5 points in GOS) and 54 cases (27%) had poor prognosis (1, 2 or 3 points in GOS) (**Figure 1**). The result indicates that the majority of patients with ruptured IAs have good prognosis after interventional therapy.

Prognosis of patients with ruptured IAs is correlated with cerebral angiospasm, Hunt-Hess classification and rehaemorrhagia

To analyze the preoperative risk factors that are related with prognosis, logistic regression analysis was performed. The data showed that preoperative cerebral angiospasm, Hunt-Hess classification (grades I and II) and rehaemorrhagia were significantly correlated with prognosis ($P < 0.05$), but age, gender, hydrocephalus and epileptic seizure were not significantly correlated with prognosis ($P > 0.05$) (**Table 3**). The result suggests that the prognosis of patients with ruptured IAs is correlated with cerebral angiospasm, Hunt-Hess classification and rehaemorrhagia.

Serum levels of MMP-2 and MMP-9 are distinct in patients with different grades of Hunt-Hess classification or different severity of cerebral angiospasm

To examine the serum levels of MMP-2 and MMP-9 after interventional therapy in patients with different grades of Hunt-Hess classification (grades I and II), different severity of cerebral angiospasm, and presence or absence of

Table 4. Postoperative serum levels of MMP-2 and MMP-9 in the presence of different relevant risk factors

		MMP-2 (× 10^3 ng/ml)	*P* value	MMP-9 (× 10^3 ng/ml)	*P* value
Hunt-Hess classification	I	141.3 ± 17.1	0.04	320.5 ± 37.4	0.05
	II	196.8 ± 15.6		416.5 ± 54.1	
Cerebral angiospasm	Severe	154.6 ± 19.8	0.01	415.7 ± 46.2	0.00
	Mild to moderate	115.5 ± 16.7		326.9 ± 34.4	
Rehaemorrhagia	Yes	136.7 ± 18.2	0.12	411.2 ± 29.8	0.28
	No	120.7 ± 12.1		314.0 ± 37.2	

Note: MMP, matrix metalloproteinase.

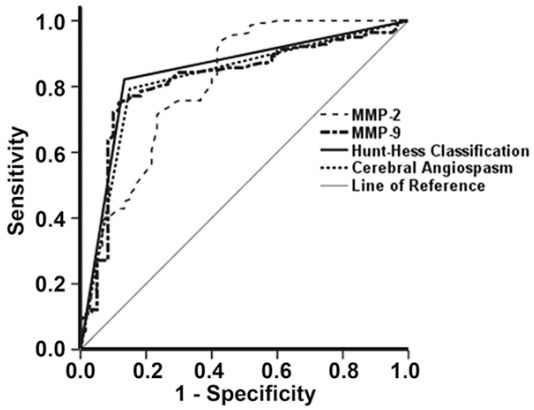

Figure 2. Receiver operating characteristic (ROC) curves for serum levels of MMP-2 and MMP-9, Hunt-Hess classification and cerebral angiospasm.

rehaemorrhagia, ELISA was employed. The data showed that the levels of MMP-2 and MMP-9 in patients with Hunt-Hess grade I were significantly different from those in patients with grade II ($P < 0.05$). In addition, the levels of MMP-2 and MMP-9 in patients with severe cerebral angiospasm were significantly different from those in patients with mild to moderate cerebral angiospasm ($P < 0.05$). However, the levels of MMP-2 and MMP-9 in patients with rehaemorrhagia were not significantly different from those in patients without rehaemorrhagia ($P > 0.05$) (**Table 4**). The results indicate that serum levels of MMP-2 and MMP-9 are distinct in patients with different grades of Hunt-Hess classification or different severity of cerebral angiospasm.

Serum levels of MMP-2 and MMP-9, Hunt-Hess classification and cerebral angiospasm severity have diagnostic significance in the evaluation of the efficacy of interventional therapy on ruptured IAs

To test the diagnostic values of serum levels of MMP-2 and MMP-9, Hunt-Hess classification and cerebral angiospasm severity, ROC curve was constructed. The areas under the ROC curves for MMP-2 level, MMP-9 level, Hunt-Hess classification and cerebral angiospasm were 89%, 80%, 75% and 72%, respectively, and their sensitivity and specificity were both higher than 90% (**Figure 2**). Of note, the diagnostic points for serum levels of MMP-2 and MMP-9 were 124 ng/ml and 321 ng/ml, respectively. The result suggests that serum levels of MMP-2 and MMP-9, Hunt-Hess classification and cerebral angiospasm severity have diagnostic significance in the evaluation of the efficacy of interventional therapy on ruptured IAs.

Discussion

Ruptured IA is a common cerebrovascular disease in clinical practice, and SAH accounts for a great percentage of all cases of ruptured IA [10]. Cerebral angiospasm is the most common and severe complication of ruptured IA that leads to high mortality rate and poor prognosis. The time point with the peak death and disability rates is within one week after rupture of IAs. Early treatment can effectively prevent the aneurysm from bleeding again [11]. The treatment time is earlier, the incidence of complications of patients is smaller [12]. In the present study, patients with ruptured IAs received endovascular interventional treatment, which has become one of the main methods for the treatment of ruptured IA. It is shown that the direct pathologic triggers for the development and rupture of aneurysms are hemodynamic changes induced by vascular endothelial cell lesions and extracellular matrix decomposition [13-15]. In clinical practice, inflammation-related factors MMP-2 and MMP-9 are usually used for auxiliary diagnosis and the evaluation of therapeutic effect. It is reported that expression of MMP-2 and MMP-9 in aneurysm wall tissues is significantly higher than that in normal arterial

blood vessels [16]. Changes in serum levels of MMP-2 and MMP-9 in patients with ruptured IAs before and after interventional therapy suggest that MMP-2 and MMP-9 play important roles in the occurrence of ruptured IAs.

A study shows that Hunt-Hess classification is correlated with the prognosis of disease, and patients with higher grades of Hunt-Hess classification usually have poorer prognosis and higher mortality rate [17]. The reason may be that higher grades usually lead to higher incidence of cerebral angiospasm. Cerebral angiography shows that about 70% patients with ruptured IAs have cerebral angiospasm, among which 30% patients present clinical manifestations. Pathological analysis shows that inflammatory responses play important roles in the development of cerebral angiospasm. MMP-2 and MMP-9 are mainly secreted by macrophages and neutrophils. In an inflammatory environment, microglia cells are stimulated and secrete abundant MMP-2 and MMP-9 [18]. In the present study, 73% patients have 4 or 5 points in GOS on discharging, suggesting that interventional therapy is effective in the treatment of ruptured IAs. Logistic regression analysis of preoperative prognostic risk factors shows that cerebral angiospasm, Hunt-Hess classification (I and II grades), and rehaemorrhagia have significant influence on prognosis. In addition, serum levels of MMP-2 and MMP-9 under different Hunt-Hess grades and different cerebral angiospasm severity were distinct, suggesting that MMP-2 and MMP-9 serum levels have great influence on the occurrence of ruptured IAs. ROC curves of MMP-2 and MMP-9 serum levels, Hunt-Hess classification and cerebral angiospasm demonstrate that these factors have diagnostic significance for evaluating the efficacy of interventional therapy for patients with ruptured IAs. In addition, the optimal diagnostic points of MMP-2 and MMP-9 serum levels are 124 ng/ml and 321 ng/ml, respectively, and have guiding significance for the evaluation of prognosis. In conclusion, MMP-2 and MMP-9 are highly specific factors for patients with ruptured IAs, and changes in serum levels of MMP-2 and MMP-9 can be used for the evaluation of clinical efficacy and prognosis of interventional therapy of ruptured IAs.

Acknowledgements

This work was supported by the Nanjing Medical University. We would also like to thank Dr. Gang Deng of Zhongda Hospital of Southeast University for his valuable help.

Disclosure of conflict of interest

None.

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