

## Relationship between plasma matrix metalloproteinase-9 and acute cerebrovascular stroke

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**Abstract: Background:** Acute cerebrovascular stroke is a leading cause of death. Hence, early prediction of complications is of great importance in management. **Objective:** The aim of this work was to study plasma level of matrix metalloproteinase-9 (MMP-9) within 24 h of acute cerebrovascular stroke onset and its relation with acute stroke complications during hospital stay. **Patients and methods:** Fourty patients with acute ischemic stroke and twenty patients with intracerebral hemorrhage were subjected to measurement of plasma MMP-9 within 24 h of stroke onset and clinical assessment of stroke severity. Twenty healthy volunteers of matched age and sex were included as controls. **Results:** Thirty male and thirty female patients with a mean age of  $60.93 \pm 8.088$  years were studied. The mean National Institutes of Health Stroke Scale (NIHSS) score on admission was  $13.37 \pm 7.422$ . The mean plasma level of MMP-9 in ischemic group was  $672.18 \pm 181.869$  ng/ml, and in hemorrhagic group was  $576.00 \pm 170.233$  ng/ml, which was significantly higher compared with the plasma level of MMP-9 in controls ( $P = 0.000$ ). There was a significant positive correlation between plasma level of MMP-9 and NIHSS score (sig.= 0.005).

**Conclusion:** Plasma MMP-9 level was found to be high in acute cerebrovascular stroke patients and correlated with complications.

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**Keywords:** Acute stroke, matrix metalloproteinase-9, NIHSS, hemorrhagic transformation.

### 1. Introduction

Acute ischemic stroke is the most common form of stroke and is majorly caused by thrombosis or embolism in the cerebral arteries. Blockage of blood flow (ischemia) results in oxygen deprivation, glucose deficiency in the affected region, and infarction in the brain. Cerebral ischemia initiates cascades of pathological events, including vasogenic edema, disruption of the blood-brain barrier (BBB), intracerebral hemorrhage (ICH), astroglial activation, and neuronal death(1).

Intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes. It is a particularly severe stroke subtype that is associated with a mortality rate of 30–50 %. Moreover, 74% of the survivors remain functionally dependent 12 months after the ictus(2).

The expression of matrix metalloproteinases (MMPs) in the adult brain is very low to undetectable, but clinical and experimental studies have shown that several MMPs are upregulated and activated after ischemic stroke. MMPs disrupt the BBB by degrading the tight junction (TJ) proteins and basal lamina proteins, thereby leading to BBB leakage, leukocyte infiltration, brain edema and hemorrhage (3).

Among MMPs, MMP-9 is the most widely studied enzyme in acute ischemic stroke. In particular, MMP-9 activity is significantly elevated in human brain tissue and serum after stroke as well as in animal stroke models. High plasma MMP-9 concentrations in

the acute phase of a cerebral infarction  $> 140$  ng/ml is considered to be an independent predictor of hemorrhagic transformation in all stroke subtypes(3).

MMP-9 has been shown to degrade tight junction (TJ) proteins (claudin-5, occludin, ZO-1) in cultured brain endothelial cells and in animal models of focal cerebral ischemia. Aberrant MMP-9 proteolytic activity degrades not only TJ proteins but also basal membrane proteins (e.g., fibronectin, laminin, collagen, and others). This degradation is associated with an increase in BBB permeability, resulting in brain infarction, edema and hemorrhagic transformation in both animal models and in human stroke patients (3).

MMPs play a central role in neuroinflammatory processes by degrading elements of the extracellular matrix. The potential role of MMPs in ICH has been examined in patients by examining whether MMP expression correlates with outcome or markers of injury and in animals by examining the effects of MMP inhibition or genetic deletion on ICH(4).

Thus, several studies have shown that MMP-9 expression correlates with hematoma expansion, perihematomal edema and neurological deterioration in ICH patients. **Li et al (2013)** recently found that increased plasma MMP-9 levels were associated with worse outcome in ICH patients(4), (5).

In humans, MMP-9 activity increases early, with a peak at 6 to 8 hours after stroke and a return toward

baseline by 24 to 26 hours. MMP-9 mRNA levels are also increased in human peripheral leukocytes at 3 and 5 hours after stroke with a return toward baseline by 24 hours(6).

The aim of this study was to assess the relationship between plasma MMP-9 concentration within 24 hours of acute stroke onset (cerebral infarction and cerebral hemorrhage) and subsequent complications during hospital stay.

## 2. Patients and methods

This case-control study was conducted on 60 patients (30 males and 30 females) with acute cerebrovascular stroke within 24 h from stroke onset. Their mean age was  $60.93 \pm 8.088$  years. Twenty normal volunteers of matched age (mean age:  $58.9 \pm 8.045$  years) and sex (10 males and 10 females) served as controls. There was no significant difference between the patient and control groups as regards diabetes, serum levels of cholesterol, triglycerides, liver function tests, renal function tests, PT, INR. They were recruited from the Neurology Inpatient Ward, Al-Azhar University Hospitals (Cairo, Egypt). Twenty healthy volunteers of matched age and sex were included as controls. All participants provided informed consent. The study was approved by the local ethics committee, in accordance with the Declaration of Helsinki, and all patients signed informed consent before inclusion.

Patients were selected according to the following inclusion criteria: All patients with an acute cerebrovascular stroke (ischemic and hemorrhagic), admitted within 24 hours from stroke onset. Stroke was defined, according to the WHO definition, as 'rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 h or leading to death, with no apparent cause other than that of vascular origin'. The time of onset of the stroke was defined as the time when the patient or observer first became aware of the symptoms, or the last time the patient was symptom free (if the patient was conscious after stroke). Exclusion criteria were as follows: Onset > 24 hours, patients with known cancer and severe renal or liver diseases, traumatic causes of cerebrovascular stroke.

Patients were subjected to thorough medical and neurological examination. Stroke severity was evaluated using the National Institutes of Health Stroke Scale. The assessment was carried out within 24 h of stroke onset. Stroke severity was categorized on the basis of NIHSS score as mild (0–5), moderate (6–13), or severe ( $\geq 14$ ), as described by Bruno *et al.* (9).

Brain CT was performed for all patients in the radiology department of Al-Azhar university hospitals. Axial CT cuts were performed with patients in the

supine position with 15–20° tilt with 1 cm slice thickness, in addition to posterior fossa cuts every 0.5 cm. MRI was performed when further needed.

### Laboratory investigations:

#### Sampling:

7 ml of venous blood were obtained from each subject in the study (after written consent) under complete aseptic conditions and drawn into the following tubes:

- 1- Citrate vacutainer for Prothrombin assay.
- 2- EDTA vacutainer for CBC.
- 3- Plain vacutainer with a clot activator. The separated serum was used for assay of liver function tests, Kidney function tests and random blood sugar. After 12 hours fasting another venous sample was drawn for assay of Lipid profile.
- 4- Heparin vacutainer tubes then the separated plasma were stored at -70C for assay of Plasma MMP-9.

#### Analytical procedures:

- 1- PT, INR and Prothrombin concentration were assayed by coagulometer Stago (DIGNOSTICA STAGO, Inc).
- 2- CBC was assayed by Cell counter Sysmex KX-21N (Roch Diagnostics).
- 3- Liver function tests (ALT and AST), Kidney function tests (Urea and Creatinine), random blood sugar and lipid profile (Total Cholesterol, Triglycerides, HDL and LDL) all were assay by fully automated chemistry analyzer Cobas c 311 (Roch Diagnostics).
- 4- Assay of plasma MMP-9 by ELISA Kit provided by eBioscience (Vienna, Austria) using Stat fax - 2600 (ChroMate) ELISA reader (supplied by Gamma Trade).

#### Principle of the assay:

This assay employs an antibody specific for Human MMP-9 coated on a 96- well plate. Standards and samples are pipetted into the wells and MMP-9 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human MMP-9 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MMP-9 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

#### Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS ver.20 Chicago, IL, USA). The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, which revealed that the data are not normally distributed. Quantitative data were described using: Mean: to measure a central value for a group of data. Standard

deviation: to measure the variations within a group. Qualitative data were described using number and percent. Correlation between MMP-9 and other quantitative variables was done using Pearson correlation test. Comparing quantitative variables between three groups was conducted using One-way ANOVA test, and if the results were significant, a post hoc test was used. Pearson chi square test was used to compare two categorical variables. In all statistical tests, level of significance of 0.05 used, below which the results considered to be statistically significant.

**3. Results**

The mean plasma level of MMP-9 in patients was significantly higher than that in controls ( $P = 0.000$ ). There was no significant difference between the two groups as regards age, sex, diabetes, hypertension, RBS, and serum cholesterol and triglycerides.

There was a statistically significant positive correlation between plasma level of MMP-9 and initial stroke severity as measured with NIHSS score ( $P = 0.000$ ). This correlation remained significant after adjustment for age, sex, diabetes, hypertension, RBS, serum triglycerides, serum cholesterol. There was no significant correlation between age of the patients and plasma MMP-9 level ( $P = 0.076$ ).

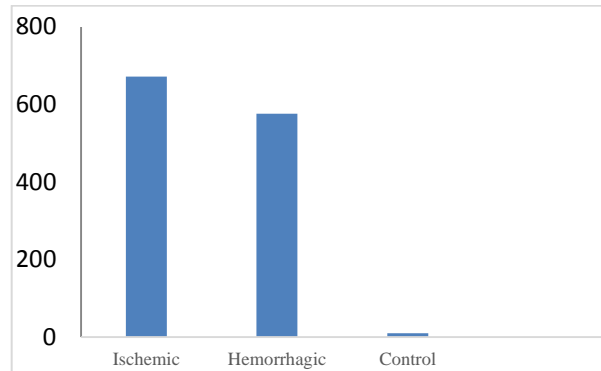
**Table (1): Clinical and laboratory findings**

	Mean	Std	Min	Max
Age	60.43	8.075	45	81
Platelets	218.41	79.313	65	426
SGPT	30.98	13.112	6	70
SGOT	31.38	14.182	8	78
Urea	29.63	14.976	8	65
Creat	1.03	.389	0	2
Cholst	184.98	51.682	92	320
TGL	97.24	35.473	35	256
RBS	127.58	46.597	57	285
PT	12.59	.977	12	16
INR	1.01	.112	1	2
MMP-9	482.61	316.592	2	992
Time to blood sampling	11.88	7.093	2	23
NIH	13.37	7.422	2	35

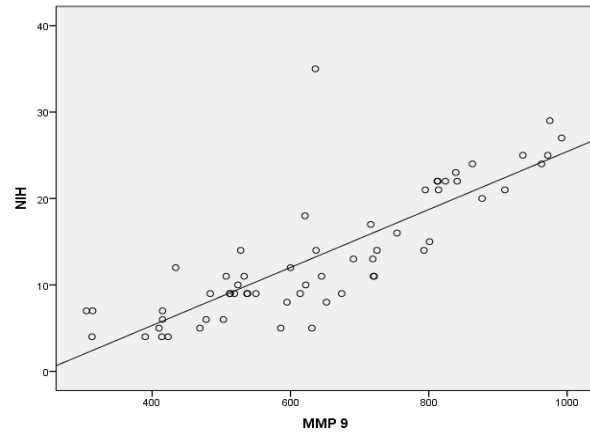
There was a difference in plasma level of MMP-9 between patients who developed hemorrhagic transformation and patients who showed no hemorrhagic transformation.

There was a statistically significant difference between levels of MMP-9 and NIHSS in patients having complete middle cerebral artery occlusion (complete MCA) and patients having non-complete middle cerebral artery occlusion (non-MCA). Also this

difference was found between patients who developed seizures, DVT, chest infection and death compared to patients who did not develop these complications.



**Figure (1): Mean of MMP-9 level in the three groups**



**Figure (2): Scatterplot demonstrating the relationship between MMP-9 level and NIHSS**

**Table (2): Correlation between MMP-9 and NIH stroke scale**

		MMP-9
NIH	Pearson Correlation	0.824
	P. value	0.000

**Table (3): Comparison between patients having HT and other patients**

	HT (7)	Non-HT (33)	P. value
MMP-9			
Median	812	525	0.04
(Min-Max)	(612-972)	(305-792)	
NIH			
Median	19	8	0.03
(Min-Max)	(13-25)	(4-17)	

**Table (4): Comparison between patients having complete MCA occlusion and other patients**

	MCA (4)	Non-MCA (36)	P. value
MMP-9 Median (Min-Max)	675 (613-992)	513 (305-775)	0.04
NIH Median (Min-Max)	22 (17-27)	11 (4-18)	0.03

**Table (5): Comparison between patients developed DVT and other patients**

	DVT (8)	Non-DVT (52)	P. value
MMP-9 Median (Min-Max)	852 (812-975)	597 (305-992)	0.001
NIH Median (Min-Max)	23 (21-29)	10 (21-29)	0.001

**Table (6): Comparison between patients who had seizures and other patients**

	Seizures (13)	No seizures (47)	P. value
MMP-9 Median (Min-Max)	824 (507-992)	586 (305-972)	0.001
NIH Median (Min-Max)	22 (11-35)	9 (4-25)	0.001

**Table (7): Comparison between mortality patients and other patients who had survived**

	Mortality (10)	No Mortality (50)	P. value
MMP-9 Median (Min-Max)	808.5 (621-992)	590.5 (305-975)	0.003
NIH Median (Min-Max)	22 (13-35)	9 (4-29)	0.001

**Table (8): Comparison between patients developed chest infection and other patients**

	Chest infection (11)	No chest infection (49)	P. value
MMP-9 Median (Min-Max)	812 (528-975)	600 (305-992)	0.004
NIH Median (Min-Max)	20 (9-29)	10 (4-35)	0.003

#### 4. Discussion

Stroke is one of the leading causes of persistent disability in Western countries. It induces acute deficits of motion, sensation, cognition, and emotion. In the majority of patients, stroke results from an interruption of cerebral blood supply and subsequent ischemic brain damage, while >25% of patients suffer from intracranial hemorrhage (7).

MMPs are normally expressed at very low levels under normal conditions with localized expression induced when remodeling of the extracellular matrix (ECM) is required (8).

In ischemic stroke patients, a correlation between plasma MMP-9 levels and National Institute of Health Stroke Scale (NIHSS) score has been observed. MMP-9 expression correlated with stroke severity and poor outcome, as assessed by the NIHSS (8).

A relationship between baseline MMP-9 levels and hemorrhagic transformation (HT) had been established, where high baseline levels were predictive of hemorrhagic events, further supported by the observation that MMP-9 levels were higher in patients who developed hemorrhagic transformation of their infarct. Furthermore, MMP-9 levels were significantly higher in those patients with hemorrhagic transformation, compared to those without (8).

This study found that MMP-9 level is elevated in ischemic and hemorrhagic groups compared with the control groups (4), (5), (8).

Routine laboratory investigations showed no statistically significant difference between patients and controls, thus allowing MMP-9 level and NIHSS the only changing parameter.

In this study, there was an elevation in plasma level of MMP-9 in patients having intracerebral hemorrhage and was markedly elevated in those patients having a higher NIHSS.

In this study, there was a statistically significant difference between levels of MMP-9 and NIHSS of patients who developed DVT, complete middle cerebral artery occlusion, seizures, chest infection, mortality compared to patients who did not develop these complications (10), (11), (12), (13), (14), (15).

#### Conclusion

Plasma level of MMP-9 in cases of acute cerebrovascular stroke is a non-invasive tool that can add important information regarding severity and clinical outcome.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

**References**

1. Jiankun Cui, Shanyan Chen, Chunyang Zhang, Fanjun Meng, Wei Wu. Inhibition of MMP-9 by a selective gelatinase inhibitor protects neurovasculature from embolic focal cerebral ischemia. *Molecular Neurodegeneration* 2012;7-21.
2. Eva Mracsko and Roland Veltkamp. Neuroinflammation after intracerebral hemorrhage. *Frontiers in Cellular Neuroscience* 2014.
3. Shaheen E Lakhan, Annette Kirchgessner, Deborah Tepper and Aidan Leonard. Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Fneur* 2013; doi: 10.3389/00032.
4. Richard F Keep, Ningna Zhou, Jianming Xiang, Anuska V Andjelkovic, Ya Hua and Guohua Xi. Vascular disruption and blood-brain barrier dysfunction in intracerebral hemorrhage. *Fluids and Barriers of the CNS* 2014; 11-18.
5. Na Li, Yan Fang Liu, Li Ma, Hans Worthmann, Yi Long Wang, Yong Jun Wang, et al. Association of Molecular Markers With Perihematomal Edema and Clinical Outcome in Intracerebral Hemorrhage. *Stroke* 2013;44:658-63.
6. Glen C Jickling, DaZhi Liu, Boryana Stamova, Bradley P Ander, Xinhua Zhan, Aigang Lu, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *Journal of Cerebral Blood Flow & Metabolism* 2014;34:185-99.
7. Rüdiger J Seitz and Geoffrey A Donnan. Recovery Potential After Acute Stroke. *Frontiers in Neurology* 2015;6: Article 238.
8. Renée J Turner and Frank R Sharp. Implications of MMP-9 for Blood Brain Barrier Disruption and Hemorrhagic Transformation Following Ischemic Stroke. *Front. Cell. Neurosci.* 2016;10:56.
9. Bruno A, Saha C, Williams LS. Percent change on the national institutes of health stroke scale: a useful acute stroke outcome measure. *J Stroke Cerebrovasc Dis* 2009; 18:56-59.
10. Sotgiu S, Zanda B, Marchetti B, Fois ML, Arru G, Pes GM, et al. Inflammatory biomarkers in blood of patients with acute brain ischemia. *Eur. J. Neurol.* 2006;13:505-13.
11. Vukasovic I, Tesija-Kuna A, Topic E, Supanc V, Demarin V, and Petrovcic M. Matrix metalloproteinases and their inhibitors in different acute stroke subtypes. *Clin. Chem. Lab. Med.* 2006;44:428-34.
12. Ning M, Furie KL, Koroshetz WJ, Lee H, Barron M, Lederer M, et al. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. *Neurology* 2006; 66:1550-55.
13. Ya-Jun Li, Zheng-Hai Wang, Bei Zhang, Xiao Zhe, Ming-Jue Wang, Shao Ting Shi, et al. Disruption of the blood-brain barrier after generalized tonic-clonic seizures correlates with cerebrospinal fluid MMP-9 levels. *Journal of Neuroinflammation* 2013;10-80.
14. Rodríguez-Yáñez M, Castellanos M, Blanco M, García MM, Nombela F, Serena J, et al. New-onset hypertension and inflammatory response / poor outcome in acute ischemic stroke. *Neurology* 2006;67:1973-78.
15. Yia-Ting Li, Yao-Chen Wang, Hsiang-Lin Lee, Min-Chi Lu and Shun-Fa Yang. Elevated Plasma Matrix Metalloproteinase-9 and Its Correlations with Severity of Disease in Patients with Ventilator-Associated Pneumonia. *International Journal of Medical Sciences* 2016;13:8:638-45.

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