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The Association between Increased Serum Level of Matrix Metalloproteinase 9 and the Risk of Acute Ischemic Stroke

Wafeek E.K. Omar; Ali M. Soliman; Sabah M. Lotfy and Hala Ahmad Fathy
Department of Neurology, Faculty of Medicine, Zagazig University, Sharkia, Egypt
Corresponding author: Wafeek Esam El-Din Kamar Omar, Tel: +201008959527
E-mail: xperia3480@gmail.com

ABSTRACT

Background: Acute ischemic stroke (AIS) is a neurological impairment that lasts for 60 minutes or less and is caused by localized cerebral ischemia**.** Matrix Metalloproteinase-9(MMP-9) is edema development, activation of proinflammatory cytokines & destruction to the blood-brain barrier are all associated with the pathogenic events that take place during an ischemic stroke and destruction of myelin proteins.

Objective: We focused our study on assessing the role of elevated serum MMP-9 and the risk of ischemic stroke in our patients within 24 hours of the stroke's commencement and its connection to the severity of an ischemic stroke.

Methods: The Neurology Department's Intensive Care Unit & Stroke Unit at Zagazig University Hospitals conducted this cohort research **.** The study included 54 patients (31 females and 23 males), presented with a first-time AIS clinical diagnosis.

Results: There was a statistically significant inverse relationship between the blood MMP-9 level and the GCS upon admission. There was observed to be a statistically significant positive link between both NIHSS scores and infarction size. The best cutoff of MMP-9 in the prediction of severe stroke was ≥ 1091 ng/ml. Also, the best cutoff of MMP-9 in the prediction of large-size infarction was ≥ 1042.

Conclusion: Serum Matrix Metalloproteinase-9 may be a useful predictive indicator for initial risk categorization in individuals experiencing acute ischemic stroke.

Keywords: ischemic stroke, 9-Membrane Metalloproteinase, stroke severity.

INTRODUCTION

Stroke is a heterogeneous and multi-factorial disease that affects people globally. It is considered the primary factor in disability, and mortality worldwide **(1).** Stroke comprises of two types, ischemic and hemorrhagic, with the ischemic type more prevalent than hemorrhagic as it accounts for 85% of all strokes **(2).**

Only recombinant tissue plasminogen activator should be used to treat an acute ischemic stroke. However, recombinant tissue plasminogen activator is only appropriate for treating a small percentage of individuals. Investigating the etiology also metabolic alterations that occur inside the infarct region during an acute ischemic stroke is therefore urgently needed. These highlight how crucial it is to create blood-borne biochemical indicators to aid in the identification of acute ischemic stroke. **(3)**.

Enzymes that degrade the matrix include matrix metalloproteinases which are implicated in various pathophysiology processes such as systemic inflammation, atherosclerosis, and neurological disorders **(4)**.

These proteolytic enzymes, which ordinarily rebuild the extracellular matrix, are members of a family that binds zinc. The main constituents of the basal lamina surrounding cerebral blood vessels, collagen of type IV, laminin, also fibronectin, are selectively attacked by matrix MMP-9 (5).

It contributes to the pathogenic actions that take place during an ischemic stroke, for example, the distraction of the blood-brain barrier, the formation of edema, the activation of pro-inflammatory cytokines (with tumor necrosis factor-a also interleukin-1b), and the degradation of myelin proteins (6).

Infarct extension and neurologic impairments are directly correlated with high levels of matrix metalloproteinase-9. **(7)** In addition to hemorrhagic transformation in individuals experiencing AIS **(8)** & its inhibition is of potential therapeutic roles**,** making it a potential ischemic stroke biomarker **(9, 10).**

The purpose of this research is to determine if and how elevated MMP-9 serum levels are connected with an increased risk of ischemic stroke as well as so, how these levels relate to stroke severity within the first 24 hours after stroke onset.

METHODS AND PATIENTS

The subject of this cross-sectional descriptive trial was fifty-four 1ST ever ischemic stroke patients from (November 2019 to March 2021). They were hospitalized in the intensive care unit & stroke unit of Zagazig University Hospital's Neurology department.

Inclusion standards: Older than 18 years old, on first neurological examination, a focal neurological impairment lasting longer than 24 hours, first-ever ischemic stroke, and acute cerebral ischemia can be shown in the brain's MRI or CT scan.

Exclusion standards:

Hemorrhagic stroke, severe systemic illness, or any other potentially harmful medical problems Matrix Metalloproteinase-9 level, regular drug use, which might have an impact Matrix Metalloproteinase-9 level,

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such as minocycline or tetracycline derivatives doxycycline, non-steroidal anti-inflammatory medications, or statins such atorvastatin or pravastatin, recent myocardial infarction, unstable angina, acute heart failure and pregnancy and postpartum.

Methods:

Clinical assessment which includes thorough neurological evaluation, comprehensive general examination, and complete medical history. The state of awareness also stroke severity were evaluated using the following scales, Glasgow Coma Scale (GCS), Trial of Organisation 10172 in Acute Stroke Treatment (TOAST), and National Institutes of Health Stroke Scale. Comprehensive standard laboratory testing, including complete blood count, liver function tests, renal function tests, random plasma glucose level, lipid profile as well as coagulation profile. Measurement level of serum matrix metalloproteinase-9 using enzyme-linked immunosorbent assay. Cardiovascular examinations, such as 12-Lead ECG and echocardiography, and carotid Doppler ultrasonography. Radiological tests, such as a straightforward brain CT scan, measurement of CT brain infarct size, and MRI of the brain.

All hospitalized patients underwent care following the Neurology Department's Intensive Care Unit and stroke unit protocols. Everyone was checked for their blood pressure, temperature, glucose levels, as well as blood gases on the first day after suffering a stroke.

Statistical analysis

SPSS 22.0 for Windows (IBM 2013) was used to analyze all of the data. The mean, standard deviation, also median was used to convey continuous information, while a number (%) was used to express categorical variables. We compared % of categorical parameters by using the Chi-square (2) test .

We consider the (+) sign to indicate a direct correlation, where an increase in the frequency of the independent causes the dependent to increase in frequency & the (-) sign to indicate an inverse correlation, where an increase in the frequency of the independent causes the dependent to reduce in frequency. We also consider values close to 1 to indicate a strong correlation and values close to 0 to indicate a weak correlation. Forward stepwise logistic regression analysis was done to detect predictors of post-stroke deterioration.

Every test had two sides. P-values between 0.05 and 0.01 were classified as statistically significant (S), highly statistically significant (HS), and non-significant (NS), respectively.

Ethical Approval:

The study was approved by the Ethics Board of Zagazig University and the patients were given all the information they need about the trial. Informed written consent was taken from each participant in the study. This work has been carried out following The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

RESULTS

This study included 54 patients, 57.4% of them were women. The mean age was 64.2 years, with a range of 50 to 85. Concerning associated risk factors, 57.4%, 79.6%, 40.7%, 35.2% and 18.5% had diabetes, hypertension, cardiac disease, dyslipidemia, and migraine respectively. about 38.7% of studied females were on contraceptive pills. Smokers and obese represented 37% and 46.3% of studied patients. Mean MMP-9 was 1085.93 with a range from 485 to 1544 ng/ml (**Table 1**).

Table 1: lists the demographic, clinical, and MMP-9 levels of the patients having ischemic stroke who were subjected to the present study

	N=54	**%**
Sex:		
Female	31	57.4%
Male	23	42.6%
Age (year):		
Mean ± SD	64.2 ± 7.96	
Range	50 – 85	
Diabetes	31	57.4%
Hypertension	43	79.6%
SBP (mmHg)Mean ± SD	165.19 ± 25.68	
DBP (mmHg)Mean ± SD	95.91 ± 12.2	
Smoker	20	37%
Cardiac	22	40.7%
Obesity	25	46.3%
Dyslipidemia	19	35.2%
Migraine	10	18.5%
Oral contraceptive pills	(12/31)	38.7%
MMP-9 (ng/ml)		
Mean ± SD	1085.93 ± 248.29	
Range	485 – 1544	

PS: Pulse rateMatrix MMP-9 Nine metalloproteinases Systolic blood pressure, or SBP Diastolic blood pressure, or DBP The standard deviation is SD.

Mean random blood glucose, hemoglobin, total leucocytic count, and platelet count were 205.93 mg/dl, 11.87 g/dl, and 9.97 (10^3/mm^3).

Mean ALT and AST were 20.12 and 22.62 U/L respectively. Mean serum creatinine, total, and direct bilirubin were 0.98, 0.63, and 0.21 mg/dl respectively. Mean triglycerides, total, LDL, and HDL cholesterol were 136.36, 175.27, 107.97, and 32.18 mg/dl respectively. mean INR was 1.14 and the mean partial thromboplastin time was 34.26 seconds (**Table 2**).

Table (2): Laboratory data of the studied ischemic stroke patients

	Mean ± SD
RBG (mg/dl)	205.93 ± 9.34
Hemoglobin (g/dl)	11.87 ± 1.52
TLC(10^3/mm^3)	9.97 ± 2.24
Platelet (10^3/mm^3)	263.29 ± 9.37
Serum creatinine (mg/dl)	0.98 ± 0.2
ALT (U/L)	20.12 ± 2.7
AST(U/L)	22.62 ± 3.07
Total bilirubin(mg/dl)	0.63 ± 0.19
Direct bilirubin (mg/dl)	0.21 ± 0.01
Serum triglycerides (mg/dl)	136.36 ± 6.25
Total cholesterol(mg/dl)	175.27 ± 5.46
LDL cholesterol(mg/dl)	107.97 ± 4.29
HDL cholesterol(mg/dl)	32.81 ± 1.24
INR	1.14 ± 0.13
PTT(seconds)	34.26 ± 1.62

INR international normalized ratio TLC total leucocytic count RBG random blood glucose TLC total leucocytic count, PTT partial thromboplastin time, ALT Alanine aminotransferase, AST Aspartate aminotransferase
LDL low-density lipoprotein, HDL high-density lipoprotein

Regarding the weakness side, 53.7% of patients had right-side weakness and 46.3% had left-side weakness. Cardioembolic stroke occurs in 37% and 26% had lacunar infarction. About 39% of patients had large-size infarctions and 33.3% of patients had small-size infarctions. Early signs of infarction were present in 35.2%. Basal ganglia were the commonest site of stroke (35.2%) (**Table 3**).

Table 3: Shows the characteristics of patients who were subjected to the study.

		N=54	%
Side of weakness:	Left	25	46.3%
	Right	29	53.7%
TOAST classification:	Cardioembolic	20	37%
	Lacunar	14	26%
	Large artery atherosclerosis	10	18.5%
	Undetermined	10	18.5%
Size:	Large	21	38.9%
	Moderate	15	27.8%
	Small	18	33.3%
Early sign:	Absent	35	64.8%
	Present	19	35.2%
Site of infarction:	Basal ganglia	19	35.2%
	Brainstem	2	3.7%
	Cerebellar	1	1.9%
	Frontal	1	1.9%
	Fronto-parietal	12	22.2%
	Parietal	7	13%
	Temporal	4	7.4%
	Tempo-parietal	6	11.1%
	Tempo-parieto-occipital	2	3.7%

TOAST: ORG 10172 trial for acute stroke therapy

Both differences were statistically significant mean scores of NIHSS and GCS at admission; 46.3% had a severe stroke at admission (**Table 4**).

Table (4): Severity of stroke as determined by GCS and NIHSS at admission

	On admission Mean ± SD	Test of significance	p
-NIHSS	13.43±5.46	t = 9.046	<0.001**
-GCS	11.96 ± 2.46	t = -6.777	<0.001**
-Severity			
Mild (1-4)	13 (24.1%)		
Moderate (5-15)	16 (29.6%)	Wx = -4.379	<0.001**
Severe (16-31)	25 (46.3%)		

Glasgow Coma Scale (GCS)The statistically significant value for the paired sample t-test using the Wilcoxon signed rank method for the NIHSS (National Institutes of Health Stroke Scale) is **p0.001.

According to the NIHSS's assessment of stroke severity upon admission, there is a statistically substantial positive connection between blood levels of MMP-9 as well as stroke severity. A statistically substantial positive link was established between MMP-9 also infarction size & a statistically substantial negative correlation between blood MMP-9 levels & GCS upon admission. (**Table 5**).

Table (5): Correlation amongst serum Matrix Metalloproteinase-9levels and infarction size, NIHSS admission & GCS

	r	P
NIHSS (at admission)	0.783	<0.001**
GCS (at admission)	-0.872	<0.001**
Size of brain infarction	0.609	<0.001**

Statistics show that the r Pearson correlation coefficient **p0.001 is extremely significant. GCS Edinburgh coma scale, MMP-9 Matrix of the National Institutes of Health's Stroke Scale Metalloproteinase-9

The optimal MMP-9 threshold for predicting a major stroke is 1091ng/ml, according to Table 6; this value has an area under the curve of 0.878, a sensitivity of 84%, a specificity of 63.6%, a positive predictive value of 75%, a negative predictive value of 84.6%, also an overall accuracy of 79.6% (p 0.001). The best MMP-9 threshold for predicting major infarctions is 1042 ng/ml, with an area under the curve of 0.764, a sensitivity of 81%, specificity of 63.6%, and a positive predictive value of 58.6%, negative predictive value of 84% & overall accuracy of 70.4% (p 0.001).

Table (6): MMP-9's prognostic significance in the detection of severe stroke and size of infarction among the studied patients

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
Stroke severity							
≥1091	0.878	84%	75.9%	75%	84.6%	79.6%	0.001**
Large infarction							
≥1042	0.764	81%	63.6%	58.6%	84%	70.4%	0.001**

**p≤0.001 is a statistically highly significant Area Under Curve (AUC)

PPV: Positive predictive value, or NPP: negative predictive power, MMP-9: Matrix Metalloproteinase-9

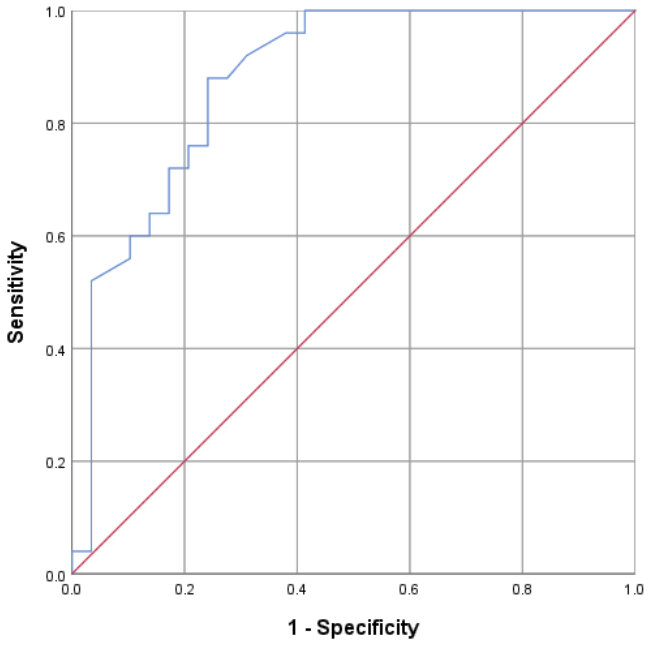

Figure (1) ROC curve displaying MMP-9's efficiency in the detection of severe stroke on admission among the studied individuals.

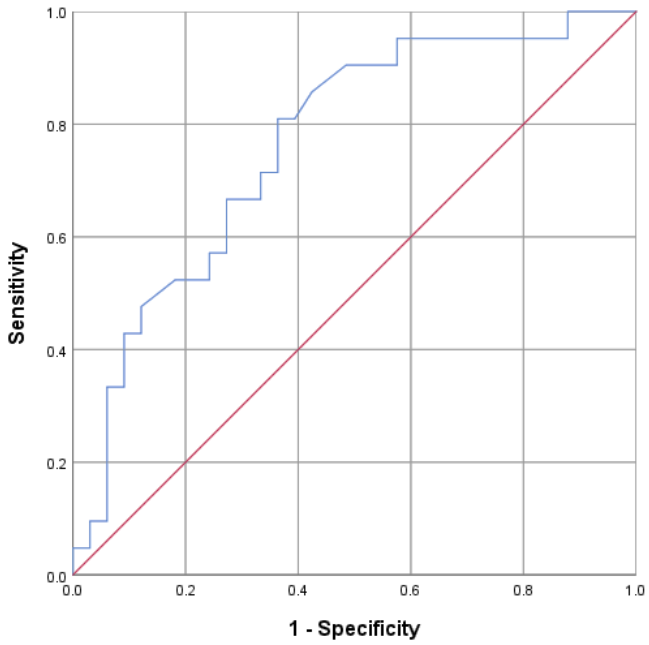

Figure (2) ROC curve displaying MMP-9's efficiency in the detection of large infarction among the studied patients

DISCUSSION

Stroke is one of the main causes of mortality besides morbidity worldwide, with enormous clinical & economic consequences. Age, stroke type, lesional site, level of awareness, degree of neurological impairment, medical risk factors (HTN also DM), premorbid diseases, fever, also prior stroke history are only a few of the variables that influence early death and disability following a stroke [(11)].

Several central nervous system development and regeneration processes are modulated by MMP-9. During tissue remodeling, it contributes to the disintegration of the extracellular matrix. It affects the extracellular matrix around the blood arteries in addition to neurons after a stroke, promoting the death of brain cells. In the initial stages after cerebral ischemia, MMP-9 disrupts the blood-brain barrier, and leukocyte infiltration, causing leakage, brain edema, as well as hemorrhage [(12)].

This cross-sectional descriptive research was carried out at the Neurology Department, Emergency Room & Intensive Care Unit of Zagazig University Hospitals. The study included 54 individuals experiencing their first acute ischemic stroke from November 2019 to March 2021).

We noticed that 50 to 85 years old, with a mean age of 64.2. This is consistent with **Idrovo *et al*. [(14)]** who quoted mean ages between 31 and 88 years.

Regarding sex distribution among studied patients, we noticed that 57.4% of hospitalizations for acute ischemic stroke in this research were females and 42.6% were males. **Weimar *et al*. [(15)]** found in their study that 846 (43.1%) were women. On the other hand, **Barrett *et al*. [(16)]** have frequently said that males experience incidence rates that are 25% to 30% higher.

In agreement to this finding, the Rothwell Rochester trial a study from the United States discovered that there were 16255 female strokes and 14149 male strokes, meaning that women outnumbered men by a ratio of 1.15. Women are more likely than males to have had a stroke in their parents. The beneficial effects of estrogen on cerebral circulation [(17)] may provide another explanation.

In our study, the mean MMP-9 was 1085.93 with a range from 485 to 1544 ng/ml. **Zhong *et al*. [(18)]** investigated the link between serum Matrix Metalloproteinase-9 levels and the probability of having an acute ischemic stroke and the outcome of such an occurrence. The median serum MMP-9 concentration (interquartile range) was 671.8 ng/mL. 414.7–1,025.8 ng/mL). Our study this observation concurred with that of **Abdelnaseer, [(19)]** who reported that Patients had considerably greater mean blood levels of Matrix Metalloproteinase-9 than controls.

In studying stroke characteristics among our patients, right-sided weakness was reported in 53.7% of patients and 46.3% had left-side weakness. As regards ischemic stroke subtypes, cardioembolic stroke occurred in 37% of patients, 26% had lacunar infarction and 18.5% had large artery atherothrombotic stroke. About 38.9% had large-size infarctions and 33.3% of patients had small-size infarctions. Early signs of infarction were present in 35.2% and basal ganglia were the commonest site of ischemic stroke (35.2%). **Weimar *et al*. [(15)]** found Patients who experience a deterioration of their neurologic condition have statistically substantially higher internal carotid artery (ICA) as well as middle cerebral artery (MCA) (M1) occlusions.

In our study, on assessing stroke severity, the mean baseline patients' NIHSS score was 13.43 ± 5.46. Results by **Abdelnaseer *et al*. [(19)]**, who examined the relationship between the blood level of matrix metalloproteinase-9 within one day of acute ischemic stroke start as well as clinical severity, confirmed this conclusion. They discovered that individuals' NIHSS scores ranged from 4 to 20. In addition, **Poudel *et al*. [(20)]** demonstrated that NIHSS score was linked with stroke patients' three-month prognosis

In our study, 46.3% had a severe stroke at admission. Similarly, **Abdelnaseer *et al*. [(19)]** found that Five patients (16.67%) had light strokes, 14 (46.67%) had moderate strokes, and 11 (36.67%) had severe strokes.

Results of the current research showed also that According to the NIHSS's assessment of stroke severity upon admission, there was a statistically substantial positive connection between blood matrix metalloproteinase-9 levels as well as stroke severity. However, there was a statistically substantial inverse relationship between serum MMP-9 levels also GCS at admission. These findings backed up MMP-9's position as a standalone predictor of ischemic stroke's clinical severity in its acute stage.

According to **Demir *et al*. [(7)]**, MMP-9 may be harmful to stroke patients during the acute stage because it is implicated in neurotoxicity also the breakdown of the blood-brain barrier during this stage of cerebrovascular stroke. Additionally, **Abdelnaseer *et al*. [(19)]** discovered that patients with normal MMP-9 serum levels had mean NIHSSs that were lower than those with high MMP-9 serum levels. The first stroke severity as determined by the NIHSS score and the blood level of MMP-9 showed a statistically significant positive connection.

A statistically significant positive connection between matrix metalloproteinase-9 and infarction size was found in the current investigation. Circulating MMP-9 has been linked in human research to the size of the infarct and the severity of the stroke, along with being a reliable indicator of cerebral edema also hemorrhagic transformation, particularly in patients receiving recombinant tissue plasminogen activator [(21)].

The current study's findings demonstrated that an MMP-9 threshold of 1091 ng/ml was the most accurate for predicting major stroke. Additionally, MMP-9's best

threshold for predicting large-sized infarction was 1042. From the receiver operating characteristic curve, **Zhong *et al*.** [18] determined the ideal MMP-9 cut point level (812.2 ng/mL), with a linear relationship between MMP-9 levels and the combined result and significant disability at 3 months. The combined result of AIS death or substantial disability may now be predicted more accurately by adding serum MMP-9 to traditional risk variables.

CONCLUSION

The finding serum MMP-9 level was elevated on the first day following AIS, as stated by the results of the current investigation. It is possible to assume that the initial stroke severity and the blood level of matrix metalloproteinase-9 on the 1ST day following an acute ischemic stroke are positively correlated. So, we can conclude having an elevated serum MMP-9 level is associated with a higher risk of ischemic stroke, a finding that makes it a potential biomarker for the first risk classification of ischemic stroke cases.

DECLARATIONS

- **Consent for publication:** I attest that all authors have agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** no conflicts of interest.

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