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Relationship Between Serum Level of Matrix Metalloproteinase-9 and Vascular Risk Factors in Thrombolyzed Acute Stroke Patients

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Abstract

Serum levels of MMP-9 have been demonstrated to rise during the early stages of stroke in several studies. Stroke patients who were given tissue plasminogen activator (rTPA) as well as had elevated levels of MMP-9 in their blood had worse results. The present research intended to assess the correlation among serum level of Matrix Metalloproteinase-9 & vascular risk factor in individuals of acute ischemic stroke(AIS) treated with thrombolytic treatment. The research carried out on 100 acute ischemic stroke patients , separated into 2 groups, group 1(50 individuals) was eligible for treatment with rTPA and group 2(50 patients) who did not receive rTPA as a control group. The individuals included in the study were admitted to the Stroke Unit at Beni-Suef University Hospital between December 2020 and December 2021. Prior to commencing the investigation, written informed assent was obtained from every individual or their relatives after the research was thoroughly explained. The study received ethical sanction from the Faculty of Medicine's ethical committee at Beni-Suef University. The average value of MMP- 9 serum level for rTPA treated patients was 1138.5±412.5 pg/ml ,while for controls the MMP-9 serum level was 1131.06±367.4 pg/ml .There was no statistically significant variance among study groups regarding the serum level of MMP-9 (p-value>0.9). No statistically significant variance among study groups regarding the serum level of MMP-9 **.** In terms of vascular risk factors, no statistically significant distinction among the individuals & control units noted.

Keywords: Matrix metalloproteinase 9, cerebrovascular stroke, rTPA

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1. Introduction

Globally, stroke ranks second in terms of mortality, while in Western Europe & the US, it is the leading cause of disability within the elderly **(1)**. Matrix metalloproteinases (MMPs) are zinc-binding proteolytic enzymes that are structurally related as well as are extensively distributed in human tissues. They are well-known inflammatory mediators. Almost all extracellular matrix components are degraded by them in physiological & pathological processes (2) **.** MMPs serve numerous roles in the atherosclerosis process by stimulating smooth muscle cell migration as well as proliferation & also involved in the step of atherosclerotic plaques destabilization (3)**.** The adverse effects of rTPA were found to be mediated by the upregulation and activation of MMP-9 and then it pertains to stroke patients who have undergone treatment with tissue plasminogen activator (rTPA) (4).

2. Subjects and Methods

A case-control investigation was undertaken involving fifty individuals who presented with AIS within the therapeutic window for rTPA therapy (within the first 4.5 hours) (group I). The control group consisted of fifty individuals who presented with AIS within the 1st 4.5 hours of onset but had contraindications for rTPA (group II). Participants were enrolled between December 2020-December 2021 at Beni-Suef University Hospital's Stroke Unit. Before beginning the investigation, we made sure all participants understood the purpose of the research & obtained their or their legal guardians' signed informed permission. The medical school's ethical review board at Beni-Suef University gave its clearance.

2.1. Inclusion Criteria

1-Group I (Patient group): 50 individuals experiencing an acute ischemic stroke within the rTPA therapy window (first 4.5 hours). Individuals given rTPA (0.9 mg/kg) intravenously. 2-Group II (Control group): 50 individuals presented with AIS within the 1st 4.5 hours of onset but were ineligible for rTPA.

2.2. Exclusion criteria

- Patients with structural brain lesion other than the acute infarction.
- Individuals who are experiencing a transient ischemic attack.
- Individuals have past history of cerebrovascular stroke.
- Individuals afflicted with neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, or any form of dementia.
- Individuals have hemorrhagic blood diseases or malignancy.

3. Methods

3.1. All patients were subjected to the following

3.1.1. Clinical assessment history taking

Including risk elements: Age, smoking status, diabetes mellitus, hypertension, alcohol consumption, substance abuse, cardiac disease history, recurrent stroke history, as well as familial stroke predisposition. Medical examination: Involving body mass index, & heart evaluation in addition to vital signs. TOAST categorization of stroke (5) National Institute of Health Stroke Scale (NIHSS) were performed for all included individuals following admission, Modified Rankin Scale was performed for both included groups after 3 months from stroke onset. (6).

3.1.2. Radiological assessment

Computed Tomography (CT Brain): performed prior to commencing intravenous rTPA treatment for the patient group, and within the initial 4.5 hours of the stroke for the control group, to verify the absence of hemorrhage or any structural lesion other than infarction. Additionally, a CT Brain scan was performed twenty four hours after rTPA administration.

- Echocardiography: was performed on all of the individuals who participate in the study in order to evaluate the existence of valvular disease, ejection fraction, left atrium dilatation, & cardiomyopathy.
- Carotid and vertebrobasilar duplex: was performed to diagnose stenosis or atherosclerosis in the carotid and vertebrobasilar arteries.

3.1.3. Laboratory investigations

- Routine laboratory investigations as CBC, FBS, PPBS, lipid profile, Liver and Kidney functions test, PT, PC, INR.
- Serum level of MMP 9

Blood samples will be collected 24hours after infusion of rTPA in patients group and 24 hours after stroke onset in control group . venous blood will be collected in EDTA- containing tubes. Serum samples will be segregated as well as promptly frozen at -80C in the clinical laboratories of the participating hospitals. Concentrations of MMP-9 in serum will be determined utilizing an ELISA reagent that is commercially available.

3.1.4. Ethics approval and consent to participate

For this investigation, Ethic Committee of Beni-Suef University will grant approval for the utilization of human blood samples. All participants will be duly apprised of the objectives of this study, as well as will be required to provide written informed consent. Approval no: FMBSUREC/01102019/Sayed

3.1.5. Statistical methods

- The data will be entered & coded: utilizing SPSS version 22 (Statistical Package for the Social Sciences).
- The following parameters will utilized;
  - Descriptive analysis of the findings: For quantitative data, the distribution of frequencies will be utilized to summarize the information; for qualitative data, the minimum, maximum, mean, & standard deviation will be applied.
  - student t-test: is utilized to contrast the means of both groups of quantitative variables.
  - chi-square test is: utilized to compare the frequencies of events or categorical data between two groups.
  - P: The probability/significance value

P value at least 0.05: Not significant P value not over 0.05: Significant.

4. Results

This is case control research performed on 100 ischemic stroke individuals, admitted in the hospital in the first 4.5h from the onset of stroke.

- The demographic data of both groups are shown in (Table 1).
- No statistically significant variance (p-value more than 0.05) was realized in relation to vascular risk factors among both groups under investigation.
- According to the echocardiographic findings, In relation to the following, there was no statistically significant distinction detected among both groups: pulmonary hypertension, dilation of the left atrium, cardiomyopathy, & valvular heart disease (table 3).
- A statistically significant distinction was detected in the ejection fraction among the cases & the control group (P value equal 0.034) (table 3).

Table 1. Demographic characters in different study groups

Variables	**Group I** (N=50)		**Group II** (N=50)		**P-value**	**Sig.**
Age (years)						
Mean /SD	59.9	11.8	58.7	14.01	0.6	NS
Sex						
Male	27	54%	26	52%	0.9	NS
Female	23	46%	24	48%		

SD: standard deviation, NS: non-significant, n: number

Table 2. Medical history in different study groups

Variables	Group I (N=50)		Group II (N=50)		P-value	Sig.	
	No.	%	No.	%			
BMI (kg/m2)[Mean (SD)]	27.84(6.11)		28.78 (5.30)		0.582	NS	
Smoking							
No	32	64%	34	68%	0.8	NS	
Yes	18	36%	16	32%			
Diabetes mellitus							
No	33	66%	30	60%	0.7	NS	
Yes	17	34%	20	40%			
Hypertension							
No	23	46%	15	30%	0.1	NS	
Yes	27	54%	35	70%			
AF							
No	25	50%	22	44%	0.7	NS	
Yes	25	50%	28	56%			
Drug abuse							
N0	37	73.1%	36	(71.4%)	0.900	NS	
YES	13	26.9%	14	(28.6%)			
Family history							
NO	42	84.6%	45	90.5%	0.549	NS	
YES	8	15.4%)	5	9.5%			

Table 3. Relationship among echocardiographic findings & mean MMP-9 serum level

Variables	MMP-9(pg/ml)		P-value	Sig.
	Mean	SD		
Valvular heart disease				
Yes	1199.6	410.2	0.2	NS
No	1054.1	410.5		
Cardiomayopathy				
Yes	1237.9	515.2	0.3	NS
No	1107.1	377.1		
Left atrium				
Dilated	1047.9	430.3	0.2	NS
Not dilated	1215.6	388.1		
Diastolic dysfunction				
Yes	1118.8	377.3	0.7	NS
No	1158.1	452		
Pulmonary hypertension				
Yes	1101.4	389.5	0.7	NS
No	1155.9	427.5		
Ejection fraction				
<45%	1054	509.3	0.6	NS
>45%	1159.6	389.4		

n: number

Table 4. Impact of mean MMP-9 in different carotid duplex findings among cases

Carotid duplex	MMP-9(pg/ml)		P-value	Sig.
	Mean	SD		
Stenosis 0-50%	1027.2	475.2	0.3	NS
stenosis 50-69%%	1176.6	390.4		
stenosis 70-99%	1077.3	477.4		

n:number, NS:non significant

Table 5. Comparisons of MMP9 in different study groups

Variables	MMP9		P-value	Sig.
	Mean	SD		
Group I	1138.5	412.5	0.9	NS
Group II	1131.06	367.4		

SD: standard deviation, NS: non-significant, pg/ml: Picogram per milliliter, MMP-9: matrix metalloproteinase

4.1. Carotid artery duplex findings and MMP-9 level

The mean value for MMP-9 level for all included patients with diffuse atherosclerosis was1027.2 ±475.2 and for all included patients with mild stenosis < 70% was 1176.6±390.4 and in patients with severe stenosis > 70% was 1077.3±477.4. There was no statistically significant variance among cases (P-value equal 0.3) regarding the average level of MMP-9 (Table 4).

4.2. MMP -9 serum level in different study groups

There was no statistically significant variance with p-value over 0.05 among study groups concerning MMP9 level (Table 5).

5. Discussion

In this investigation, higher serum level (no statistically significant variance), of mmp-9 was noticed in female cases than in male cases. Our findings agree with Collazos et al.,2015 (7) who found that no strong associations between MMP-9 and gender. On the other hand, conflicting results were found, significantly elevated serum level of MMP-9 was detected in plasma in males in contrast to females (Ramachandran et al.,2004) (8). While Thrailkill et al.,2010 (9) & Gu et al.,2017 (10), found that Plasma MMP-9 was significantly greater in women in contrast to men. The present study found that the mean value for MMP-9 level in hypertensive cases was greater than in non-hypertensive individuals, but there was no statistically significant variance among serum level of MMP-9 in both groups.

In accordance with our results, Valente et al.,2020 (11), found no significant variance in MMP-9 levels among normotensive & controlled hypertensive groups, potentially as a result of hypertensive individuals' antihypertensive medication use. Our results disagree with Onal et al.,2009(12) who found that Elevated MMP-9 activity may contribute to elastin degradation in hypertensive cases, potentially causing it to lose its elasticity in comparison to collagen. Conversely, reduced TIMP-1 activity may result in the accumulation of fibril degradation products that are immature, poorly cross-linked, or unstable. Such products may subsequently misdirect the deposition of collagen**.** While Vilela et al.,2018 (13) found that MMP-9 concentration was significantly greater in the prehypertensive (PH) in contrast to controlled hypertensive (CHT) group. Subjects with prehypertension exhibit elevated levels of arterial stiffness & MMP-9 in comparison to those with normotension. In our research, there was no statistically significant variance between the mean value for MMP-9 level in diabetic & non-diabetic patients**.** While the controlled diabetics had lower seum level of MMP-9 than uncontrolled, but again this does not reach any statistically significant difference. According to Our Investigations, Castellanos et al.,2003 (14) discovered that no association among MMP-9 level and serum glucose level. Our result disagrees with Amit etal.,2022 (15), who reported correlation of MMP-9 with stroke patients who were diabetic was identified, whereas no such association with hypertension was identified. Furthermore, Gul et al., 2016 (16), and Setyopranoto et al.,2018(17) found that the upregulation of MMP-9 expression & activity in endothelial cells in response to hyperglycemia suggests a previously unidentified mechanism through which hyperglycemia may detrimentally impact the progression of atherosclerotic lesions. According to the results of the present investigation, the median MMP-9 serum level did not differ significantly among smokers & nonsmokers**.** Our findings are consistent with those of Ali et al., 2015 (18), who discovered that compared to controls, smokers and COPD patients had greater concentrations of MMP-9 & TIMP-1. Our study disagrees with Kang et al., 2003 (19) who found that Smoking & airflow restriction are both related with MMP-9 expression in human lung parenchyma, which suggests that MMP-9 may have a role in the pathogenesis of the airflow obstruction caused by cigarette smoke, a hallmark of COPD. In our research, there was no statistically significant variance among the serum level of MMP-9 in cases with normal and abnormal echocardiographic findings (regarding pulmonary hypertension, diastolic dysfunction, ejection fraction, left atrial dilatation, & valvular heart disease).

Our study agrees with Chu et al.,2013 (20), found that regarding the echocardiographic findings, there was no statistically significant variance among the diastolic dysfunction findings & the serum level of MMP-9. The present research found that there was no statistically significant variance among the serum level of MMP-9 in both groups regarding the carotid artery duplex findings including patients with mild, severe stenosis or diffuse atherosclerosis. Our study disagrees with Zhou et al., 2014 (22) who found that MMP-9 concentrations were significantly higher in cases with mild stenosis than the other groups. Also, As contrasted with

our study, Davorin et al.,2021 (23) observed that the serum levels of MMP-9 were significantly higher in cases with unstable atherosclerotic plaque contrasted with cases with stable atherosclerotic plaque and control group.

Compounds that can therefore decrease MMP-9 expression by inhibiting signaling pathways or directly inhibit its activity should be utilized in the development of novel therapeutics. TIMP-1 and other endogenous inhibitors of MMP-9 should be investigated for their potential beneficial effects. Combination therapies involving TPA and MMP-9 inhibitors may prove beneficial in the future for reducing the risk and severity associated with thrombolytic therapy for human stroke.

6. Conclusions

- No statistically significant distinction was observed among the study groups with regard to the serum concentration of MMP-9**.**
- When comparing the average MMP-9 levels of men & women, there was not a statistically significant distinction (P equal 0.8).
- The median MMP-9 levels of diabetic & non-diabetic subjects were not significantly different.
- Patients with & without additional vascular risk factors (AF, smoking, drug misuse, & family history) showed no significant variation in blood MMP-9 levels.

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