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White Matter Hyperintensity Volume Correlates with Matrix Metalloproteinase-2 in Acute Ischemic Stroke

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Abstract

Background—White matter hyperintensity (WMH), a common radiographic finding associated with stroke risk and outcome, has been linked to matrix metalloproteinase (MMP) activity and increased levels of oxidative stress in non-stroke populations. We sought to determine whether WMH severity is associated with plasma levels of MMPs and oxidative stress (F2-isoprostane) in subjects with acute ischemic stroke (AIS).

Methods—We measured plasma biomarker levels at baseline and 48 hours in consecutive AIS subjects. WMH volume (WMHv) was quantified on admission MRI using a validated semi-

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Conflicts of Interest / Disclosures
None.

automated protocol, and Spearman correlation coefficients were derived for all measured biomarkers.

Results—We enrolled 405 AIS subjects (mean age 70±15 years; 58% male; median WMHv 3.4cm^3, IQR 1.4-9.5). WMHv and age were strongly correlated (ρ=0.57, p<0.0001). WMHv and MMP-2 levels were correlated at baseline (ρ= 0.23, p<0.0001) and at 48-hours post-stroke (ρ=0.19, p=0.002). In multivariate analysis, 48-hour MMP-2 levels were independently associated with WMHv (β=0.12, p=0.04). MMP-9 and F2-isioprostane levels did not correlate with WMHv.

Conclusion—In AIS patients, MMP-2 levels are associated with the pre-existing burden of WMH. If validated, these findings may further elucidate the role of MMP-2 in pathophysiology of chronic cerebrovascular injury such as WMH and in brain susceptibility to acute ischemia.

Search terms

leukoaraiosis; white matter hyperintensity; stroke; F2-isoprostane(s); matrix metalloproteinase 2; oxidative stress; matrix metalloproteinase 9

INTRODUCTION

The extent of cerebral injury resulting from acute ischemic stroke (AIS) and post-stroke outcomes are independently associated with pre-existing burden of cerebrovascular disease seen on T2-weighted brain MRI as white matter hyperintensity (WMH).[1-3] Pathophysiology of WMH is complex and poorly understood.[4-6] One proposed cause is primary blood brain barrier (BBB) dysfunction in the setting of brain matrix metalloproteinases (MMPs) upregulation.[4, 7-9] Because oxidative stress has also been shown to upregulate MMPs in the brain, [10] the link between WMH severity and biomarkers of oxidative stress in humans[11] have been used to support this hypothesis, including the association with decreased levels of the antioxidants lycopene and α-tocopherol,[12] as well as F2-isoprostane (F2-isoP), a product of free radicals and arachidonic acid that had demonstrated particular utility as a biomarker of oxidative stress in humans.[13]. MMP-2 and MMP-9, also known as gelatinase A and B, play an important role in central nervous system (CNS) injury and repair.[14] Inhibiting MMP-2 activity or deleting MMP-2 in a knockout mutation decreases white matter damage, glial activation, and BBB destruction in rodent models of cerebral hypoperfusion.[15, 16] In patients with vascular cognitive impairment and WMH,[17] there is evidence of a reduced cerebrospinal fluid (CSF)-to-plasma ratio of MMP-2.

In this paper, we examine the associations between MMPs, oxidative stress, and WMH severity in AIS patients. We hypothesized that in AIS patients, the volume of WMH is a marker of chronic oxidative stress and BBB disruption. If true, these factors may amplify the acute response to injury in ischemic stroke and may serve as future targets for intervention. To test this hypothesis, we measured plasma levels of MMP-2, MMP-9, and F2-isoP at the time of AIS and assessed their correlations with WMH volume (WMHv) quantified on brain MRI using validated, semi-automatic method.

METHODS

Subject Selection, Clinical Characteristics, and MRI analysis

We analyzed the relationship between WMHv and biomarkers of oxidative stress in AIS as part of a larger, prospective, observational NIH Specialized Program Of Translational Research In Acute Stroke (SPOTRIAS) study.[18] Consecutive patients presenting to the two participating academic medical centers within 9 hours of AIS symptom onset, who had plasma biomarkers collected as well as brain MRI obtained and available for WMHv assessment were included in this analysis.

For each patient, we recorded baseline demographics [age, sex, race, ethnicity] and vascular risk factors [hypertension (HTN), body mass index (BMI), alcohol use status, hyperlipidemia (HL), atrial fibrillation (AF), smoking status, diabetes mellitus (DM), prior stroke or transient ischemic attack (TIA), coronary artery disease (CAD)]. Stroke severity measured as NIH stroke scale (NIHSS) score on admission and at 48 hours, as well as acute treatment with intravenous tissue plasminogen activator (IV tPA) were also recorded.

All MRIs were performed within 96 hours from symptom onset on 1.5 Tesla scanners (GE Medical Systems, Milwaukee, WI). Axial T2 FLAIR, sagittal T1, and DWI sequences were used to quantify WMHv using a previously described and validated semi-automated, multistep protocol developed specifically for analysis of clinical brain MRI scans.[19, 20] This volumetric algorithm derives WMH maps from an overlap between automated signal intensity thresholding and supratentorial region-of-interest (ROI) outlines, which is followed by detailed manual editing. We exclude the cerebral structures that are prone to T2-hyperintensity artifact, such as basal ganglia and thalamus (calcifications), as well as the mesial temporal areas, cortico-medullary junction line, and ventricular (ependymal) lining from this analysis. Furthermore, hyperintense signal from prior cerebral infarcts are not considered WMH and the corresponding brain regions are masked during the process, as are those with motion artifact. To avoid confounding by hyperintensity signal resulting from acute cerebral ischemia in this WMH protocol, the total WMHv is derived by doubling the WMHv obtained from the hemisphere contralateral to AIS.[20] Similarly, this protocol was adopted to measure acute infarct volume on diffusion-weighted (DWI) MRI, using the apparent diffusion coefficient (ADC) sequences for quality assurance.

All participating subjects or their healthcare proxys provided informed consent to be enrolled as part of the ongoing prospective hospital-based cohort studies at the participating institutions. All aspects of this study have been approved by the Institutional Review Board.

Biological Samples—Plasma samples were collected twice by the SPOTRIAS research staff in the acute phase (less than 9 hours after stroke onset) and at 48 hours (36 - 60 hours after stroke onset), and analyzed by an investigator blinded to the clinical information.

Quantification of F2-isoprostane: To assay F2-isoP, acute phase and 48-hour plasma samples were frozen at -80 degrees Celsius prior to processing. F2-isoP was quantified using an 8-Isoprostane Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI) by the

Antioxidants Research Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University.

Quantification of the MMPs: To assay MMP-9 and MMP-2, samples were drawn in ethylene-diaminetetraacetic acid (EDTA) tubes, using a butterfly needle to reduce shear stress, placed on ice and immediately centrifuged at 3000 revolutions per minutes for 15 minutes. Plasma was subsequently isolated and frozen at -80 °C for later measurement. Levels of MMPs were analyzed using a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D systems) and expressed in nanograms per milliliter (ng/mL). ELISA was performed according to the manufacturer's instruction.

Statistical Analysis—Statistical analysis was performed using SAS statistical software (SAS Institute, Cary, NC) and PASW (SPSS Inc., Chicago, IL). WMHv was adjusted for head size and natural log-transformed (lnWMHv) prior to analysis. Univariate analyses to evaluate the relation between lnWMHv and dichotomous variables were tested by Student's *t* test or Mann-Whitney test. Because age, oxidative stress biomarker values, and MMP levels were not normally distributed, Spearman correlation coefficients were obtained between lnWMHv and age, F2-isoP, MMP-2, and MMP-9. Wilcoxon signed rank test was used to assess for significant differences between median values.

To account for strong effects of age on WMH, each variable was examined in bivariate, age-adjusted analysis prior to consideration for multivariable analysis. Those variables associated with lnWMHv at $p<0.2$ in age-adjusted analyses were included into two multivariable linear regression models constructed to examine the association between lnWMHv and MMP-2 levels. Multicollinearity was tested in the multiple linear regression analyses to confirm adequate model fit. We determined that NIHSS score was a strong predictor of acute infarct size (DWI) volume; thus, Model 1 included acute AIS phase variables: gender, prior stroke, HTN, HL, IV tPA, and admission NIHSS. Model 2 included 48-hour phase variables: gender, prior stroke, HTN, HL, IV tPA, and 48h NIHSS.

RESULTS

Table 1 summarizes the clinical characteristics of the study cohort with univariate and age-adjusted associations with lnWMHv. There were 405 participants (mean age 70 years, 42.5% female, and 92% white). Of these, 72% presented with hypertension, 47% with hyperlipidemia, 41% with reported current alcohol use, and 29% with obesity. Mean lnWMHv was 1.34 (±1.32) (Figure 1). As expected, lnWMHv strongly correlated with age ($\rho=0.6$, $p<0.0001$). History of HTN($p<0.001$), AF($p<0.001$), prior stroke ($p=0.001$), CAD ($p=0.008$), NIHSS at baseline ($p=0.01$) and 48 hours ($p<0.001$) were also correlated with lnWMHv. In age-adjusted analysis, only prior stroke remained significantly associated with lnWMHv ($p=0.006$).

Distributions of the study biomarkers measured after stroke are specified in Table 2. Data for each biomarker were available for subsets of the total population, ranging from 92% available for MMPs measured at baseline to 60% available for F2-isoP measured at 48 hours. Statistically significant differences were found between the levels of the MMPs

(p<0.0001 for both MMP-2 and MMP-9) and F2-isoP (p=0.001) measured at baseline and those measured at 48 hours.

Correlations between lnWMHv, MMPs, and F2-isoP measured at baseline and 48 hours are listed in Table 3. In this analysis, lnWMHv correlated with baseline (ρ=0.23, p=<0.0001) and 48 hour (ρ=0.19, p=0.002) plasma MMP-2 but not with MMP-9 concentrations (Figure 2). There was an inverse relationship between lnWMHv and the change in MMP-2 levels from baseline to 48h (ρ =-0.18, p=0.005). In multivariable analysis, 48-hour but not baseline plasma MMP-2 levels were independently associated with lnWMHv (β=0.12, p=0.047) (Table 4).

DISCUSSION

This is the first study to examine the association between WMH severity quantitatively assessed on MRI using a validated volumetric protocol and the plasma levels of MMP-2 in patients with AIS. Because MMP-2 is believed to play a role in chronic remodeling of the BBB by increasing its permeability, it may be increased in the plasma of patients with increased severity of WMH.[16] Our findings demonstrate that in patients with ischemic stroke, higher MMP-2 plasma levels are associated with larger volumes of WMH on MRI, likely reflecting increased capillary remodeling and angiogenesis in the region of chronically ischemic white matter. MMP-2 activity is likely to increase BBB permeability that contributes to the progression of WMH and evolution of AIS.[4, 8, 14] Evidence from animal and human studies suggests a link between MMP-2 and white matter disease. In a rat model of hypoperfusion, MMP-2, but not MMP-9 was associated with optic nerve and corpus callosum demyelination with increased MMP-2 expression seen in the microglia and later in the capillary endothelium.[21] In a rat model using a selective MMP-2 inhibitor, and a murine MMP-2 knockout MMP-2 was shown to mediate white matter injury in cerebral hypoperfusion.[16] A study in humans reported higher levels of MMP-2 in patients with small vessel stroke subtype.[22]

In this analysis, pre-existing cerebrovascular disease burden detected as WMHv on brain MRI of AIS subjects was associated with plasma levels of MMP-2 drawn at baseline and 48 hours after stroke onset. However, only the 48-hour plasma MMP-2 levels were independently associated with WMH severity. MMP-2 CSF levels rise shortly after the onset of hypoxia-ischemia in AIS and peak by 24 hours.[23] Thus, the baseline (hyperacute) values may be confounded by the chronic level of MMP-2 activation coupled with the MMP-2 response to acute ischemia. Therefore, the 48-hour value may be a better reflection of the relationship between MMP-2 and WMHv. Furthermore, the association of MMP-2 levels at 48 hours post-stroke with WMH burden independent of NIHSS score (as a measure of stroke severity) implies that plasma MMP-2 may also be specific for chronic BBB injury, even in the setting of acute ischemic injury.

One must interpret these findings with caution and in the context of limitations inherent to this study's design. Firstly, acute ischemic stroke is a complex and highly dynamic condition; thus, multiple confounders related to stroke severity, patient's premorbid condition, and therapeutic interventions may affect validity of plasma biomarker levels, and

consequently limit their utility. Statistical models including these confounders in a relatively small patient sample have limited power to uncover significant associations between the independent and the outcome variables; however, this conservative approach is unlikely to increase the chance of type I error.

Secondly, without pre-stroke biomarker levels as baseline and in the absence of longterm clinical post-stroke outcome measures – which are both limitations of many AIS studies – the relationship between WMHv and plasma MMP-2 levels is not fully elucidated. However, the major strength of this analysis, i.e. using a validated, quantitative, and reliable method of WMHv assessment, provides an outcome measure that reflects a pre-existing burden of cerebrovascular disease unlikely to be affected by the variables that arise acutely in the setting of cerebral ischemia.

Furthermore, although the relationship between systemic biomarkers and WMH would be least confounded by the effects of acute cerebral injury if measured prior to AIS, such design is impractical outside of the large, prospective population-based studies. If measured in convalescent subjects, chronic oxidative stress would most likely be more appropriate to assess at least 30-90 days post-stroke; however, variability due to major life style changes and medication-related effects post-stroke could independently alter the estimate of pre-stroke associations.

Finally, measuring systemic oxidative stress, as opposed to CSF biomarker level, may not be sensitive enough to reflect the degree of oxidative stress in the brain. This might in part explain why in our study of AIS subjects, WMHv did not correlate with F2-isoprostane, a known marker of oxidative stress. In addition to limitations related to timing of the biomarker assessment with respect to the acute *versus* chronic brain injury, limitations monitoring CNS processes using peripheral biomarkers are relevant, because altered access of the CNS biomarkers into systemic circulation proportional to the degree of injury and variability related to measurement of endothelial dysfunction in WMH may exist.[24]

Despite the limitations, this study serves as an essential proof-of-principle that the link exists between plasma MMP-2 levels and WMH burden detected on brain MRI of AIS subjects. The future research steps must include validation of these findings in a prospective cohort of stroke patients with convalescent plasma MMP-2 levels and, in parallel, testing of these biomarkers in a cohort of healthy adults with volumetric WMH assessment. If validated, plasma MMP-2 levels may provide a sensitive and specific estimate of pre-existing cerebrovascular disease burden in patients with stroke.

CONCLUSION

In patients with stroke, plasma MMP-2 levels correlate with pre-existing WMH burden. If validated, these findings may further elucidate the role of MMP-2 in pathophysiology of chronic cerebrovascular injury such as WMH and its role in susceptibility of the brain to acute ischemia.

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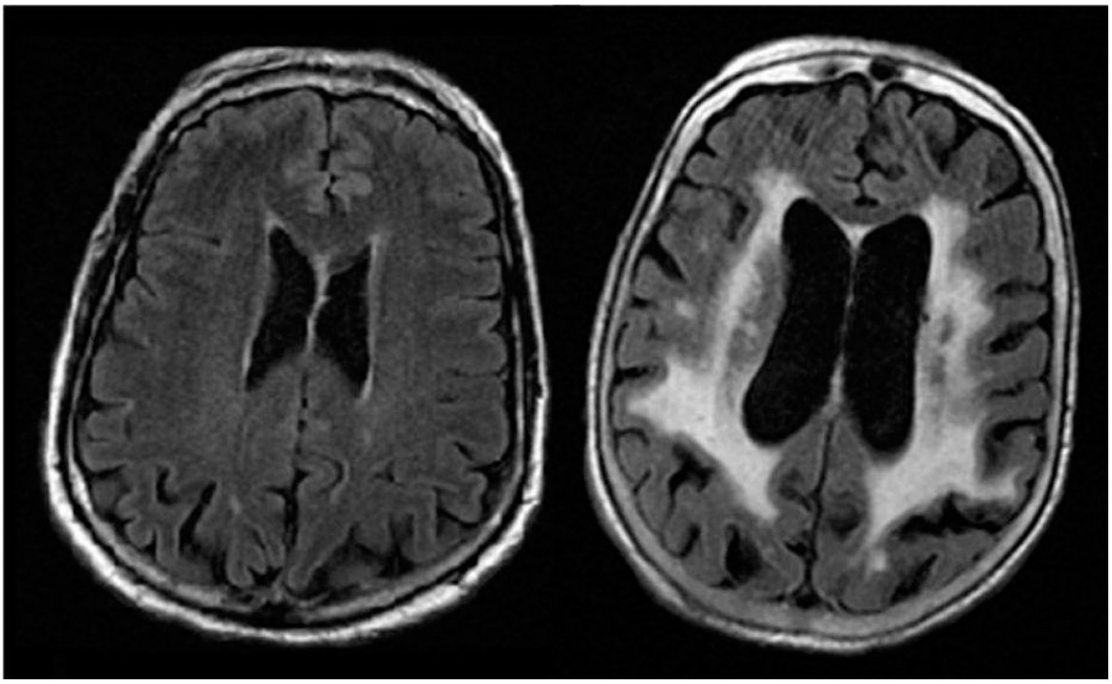

Figure 1.

Burden of white matter hyperintensity (WMH) identified on T2 fluid attenuated inversion recovery (FLAIR) MRI varies from mild (*left*) to severe (*right*) in patients with acute ischemic stroke

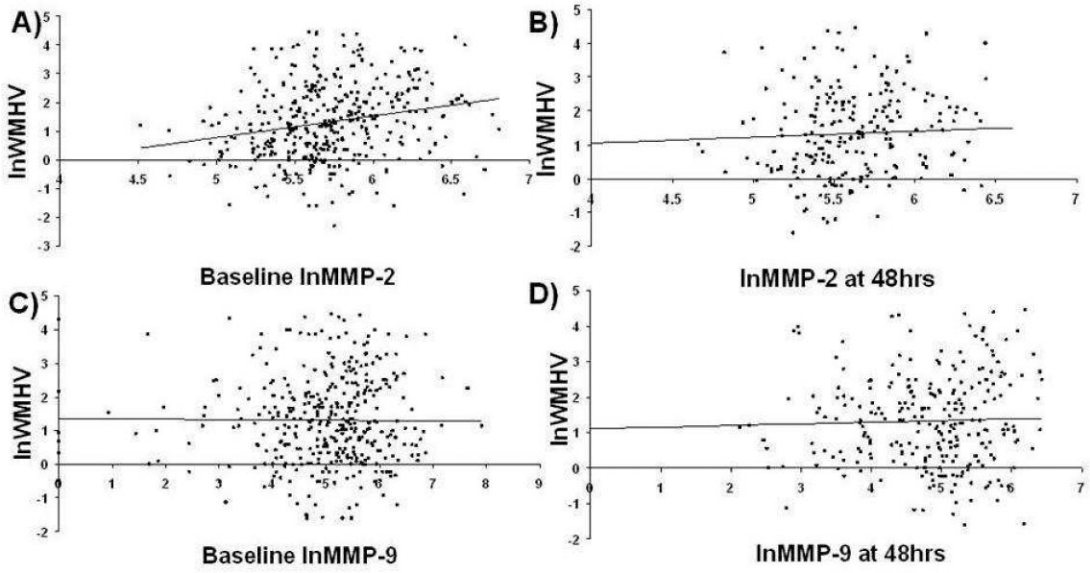

Figure 2.

Correlations between WMH volume and plasma MMP-2 and MMP-9 levels measured at baseline and at 48 hours after acute ischemic stroke.

Table 1

Clinical characteristics and univariate associations with WMH severity in acute ischemic stroke subjects (n=405).

Variable	Subjects	*p*[#]	Age-Adjusted *p*
Demographics and baseline measures			
Age (years), mean±SD	69.9±15.2	<0.001	…
BMI, mean±SD	27.8±6.0	0.12	0.90
Race (Caucasian), n (%)	371 (91.6)	0.55	0.40
Female sex, n (%)	172 (42.5)	0.05	0.13
NIHSS, median (IQR)	5 (3-12)	0.01	0.93
WMHv, median (IQR)	3.4 (1.4-9.5)	…	…
Risk factors, n (%)			
Hypertension	291 (71.9)	<0.001	0.06
Current alcohol use	165 (40.7)	0.24	0.72
Hyperlipidemia	191 (47.2)	0.25	0.18
Obese (BMI ≥ 30)	103 (29.1)	0.47	0.96
Atrial fibrillation	120 (29.6)	<0.001	0.95
Current smoking	80 (19.8)	0.32	0.95
Diabetes mellitus	87 (21.5)	0.28	0.63
Prior stroke	76 (18.8)	0.001	0.006
Coronary artery disease	105 (25.9)	0.008	0.82
Prior TIA	37 (9.1)	0.18	0.35
Treatments and outcomes			
IV t-PA, n (%)	158 (39.1)	0.28	0.17
48h NIHSS, median (IQR)	3 (1-9)	<0.001	0.78

[#]Spearman or Pearson correlations, and t-test or Mann Whitney test were used based on the distribution of the variables.

Abbreviations: BMI: body mass index; IQR: the 25^{th} to 75^{th} percentile interquartile range; IV tPA: intravenous tissue plasminogen activator; NIHSS: National Institutes of Health Stroke Scale; TIA: transient ischemic attack; WMH(v): white matter hyperintensity (volume)

Table 2

Distribution of plasma MMP and F2-isoP levels in subjects with acute ischemic stroke.

	Baseline			48 hours			*p*
	n	**Median**	**IQR**	**n**	**Median**	**IQR**	
F2-isoP (pg/mL)	346	55.5	36.8-78.1	241	45.1	28.7-71.4	**0.001**
MMP-2 (ng/mL)	374	296.8	239.0-376.7	258	266.3	219.5-338.8	<**0.0001**
MMP-9 (ng/mL)	374	176.3	91.6-309.0	258	144.8	77.4-229.6	<**0.0001**

*IQR = the 25th to 75th percentile interquartile range

Table 3

Correlations between lnWMHv, MMP and F2-isoP levels measured at baseline and 48 hours in subjects with acute ischemic stroke.

	Baseline			48 hours		
	n	**Correlation (ρ)***	***p***	**n**	**Correlation (ρ)***	***p***
F2-isoP	346	-0.06	0.30	241	-0.04	0.53
MMP-2	374	0.23	**<0.0001**	258	0.19	**0.002**
MMP-9	374	0.006	0.91	258	0.05	0.45

*Spearman's correlation coefficient

Abbreviations: F2-isoP: F2-isioprostane; IQR: the 25thto 75th percentile interquartile range; MMP: matrix metalloproteinase

Table 4

Multivariable analysis of WMHv in subjects with acute ischemic stroke at baseline and 48 hours post-stroke.

Model 1					
	B	**95%CI for B**		**β**	***p***
		Low	**High**		
Gender	0.238	-0.025	0.501	0.090	0.08
Prior stroke	0.368	0.042	0.694	0.110	**0.03**
Hypertension	0.768	0.473	1.064	0.266	**<0.001**
Hyperlipidemia	-0.030	-0.304	0.244	-0.218	0.83
IV t-PA	-0.267	-0.539	0.004	-0.100	0.05
NIHSS at baseline	0.028	0.008	0.049	0.143	**0.007**
MMP-2 at baseline	0.0002	-0.0001	0.001	0.055	0.27
Model 2					
Gender	0.362	0.052	0.671	0.138	**0.02**
Prior stroke	0.219	-0.184	0.621	0.064	0.29
Hypertension	0.640	0.294	0.985	0.231	**<0.001**
Hyperlipidemia	0.193	-0.127	0.514	0.075	0.24
IV t-PA	-0.160	-0.470	0.151	-0.061	0.31
NIHSS at 48 h	0.003	0.011	0.059	0.173	**0.005**
MMP-2 at 48 h	0.002	0.0001	0.003	0.121	**0.047**

Model 1 included all baseline variables associated with lnWMHv at p<0.2 in age-adjusted analyses
Model 2 includes all 48h variables associated with lnWMHv at p<0.2 in age-adjusted analyses

Abbreviations: IV tPA: intravenous tissue plasminogen activator; NIHSS: National Institutes of Health Stroke Scale; MMP: matrix metalloproteinase; lnWMHv: natural log of white matter hyperintensity volume