International Journal of Healthcare and Medical Sciences
ISSN(e): 2414-2999, ISSN(p): 2415-5233
Vol. 5, Issue. 8, pp: 37-41, 2019
URL: https://arpgweb.com/journal/journal/13
DOI: https://doi.org/10.32861/ijhms.58.37.41

Academic Research Publishing Group

Original Research Open Access

A Comparative Study of Serum Matrix Metalloproteinase-9 in Tuberculous Meningitis With and Without Stroke

Rajat Jhamb*
Professor, Department of Medicine, University College of Medical Sciences and GTBH, Delhi-110095, India

Amit Kumar Gauraw
Post Graduate Student, Department of Medicine, University College of Medical Sciences and GTBH, Delhi -110095, India

Rajarshi Kar
Assistant Professor, Department of Biochemistry, University College of Medical Sciences and GTBH, Delhi-110095, India

Priyanka Gogoi
Professor, Department of Pathology University, College of Medical Sciences and GTBH, Delhi-110095, India

Rajnish Avasthi
Professor and Head, Department of Medicine, University College of Medical Sciences and GTBH, Delhi-110095, India

Abstract

Background: Tuberculous meningitis is defined as an inflammatory response to mycobacterial bacterial infection of the pia, arachnoid and CSF of the subarachnoid space. It is a dangerous form of extrapulmonary tuberculosis because it can cause permanent neurological disabilities and even death. Stroke is a devastating complication which further increase the morbidity and mortality in the disease. Matrix metalloproteinases are endopeptidases which degrade all the components of the extracellular matrix and thus have potential to disrupt blood brain barrier and cause CNS damage. Matrix metalloproteinases have been associated with pathophysiology of ischemic stroke. MMP levels in serum and CSF have also been seen to rise with advancing stage of TBM. So it is postulated that MMP may have role in the pathophysiology of stroke in TBM and may serve as a biomarker to predict stroke in TBM. Aims: To compare Serum Matrix metalloproteinase-9 in patients with Tuberculous Meningitis with and without Stroke and correlate it with various clinical, biochemical and radiological features of TBM. Methods: 40 Patients of probable or definite TBM and 40 age and sex matched patients of TBM with clinical stroke were enrolled in the study and formed two groups i.e. cases and controls. The two groups were compared for various clinical parameters, biochemical parameters (CSF cytology, glucose and protein), neuroimaging parameters and serum MMP-9 levels. Serum MMP-9 was estimated by ELISA method. Results: Serum MMP-9 levels were (224 ± 261.627 ng/ml) in cases and (157.23 ± 197.155 ng/ml) controls, which though higher in cases but no difference was statistically significant (p value 0.157) between two groups. Also there was no correlation between the serum MMP-9 levels and various clinical features (duration of illness, fever, headache, vomiting, weight loss, seizure, hemiparesis), CSF characteristics (protein, sugar and cytology) and radiological findings (tuberculoma, and hydrocephalus). Conclusion: we conclude that MMP-9 levels is not correlated with occurrence of stroke in TBM. MMP-9 levels were not increased with severity of disease, complications and outcomes.

Keywords: Tuberculous meningitis; Matrix metalloproteinases 9; Cerebrospinal fluid; Endopeptidase; Ischemic stroke; ELISA.

1. Introduction

TBM is a dangerous form of EPTB because it can cause permanent neurological disabilities and even death. Permanent neurological sequelae occur in approximately half of the survivors. Also it has mortality rate of approximately 20%, which increases with delay in treatment and further progression of disease to as high as 55%. Thwaites, *et al.* [1] The estimated mortality due to tuberculous meningitis in India is 1.5 per 100,000 population. Chakraborty [2] Severe clinical manifestations of tuberculous meningitis occur due to robust inflammatory response generated in the brain against pathogenic bacilli [3]. During this process, microglial cells are activated to secrete proteases which have ability to degrade extracellular matrix and cause tissue destruction [4, 5]. Among these proteases, MMPs seem to have prominent role in tissue destruction. MMPs are endopeptidases which degrade all the components of the extracellular matrix and thus have potential to disrupt blood brain barrier and cause CNS damage [5]. Tuberculosis infection elicits the production of MMP-9 by direct interaction between cell wall components of bacilli and human monocytes & macrophages [6]. The higher increase of MMP-9 in pleural effusions of tuberculous meningitis patients than in patients with malignant pleural disease also signifies the role of MMP-9 in tuberculous infections and vice versa [7]. Besides, the synergistic effects of MMP-9 and Mycobacteria on each other, specific

*Corresponding Author

MMP-9 substrates type IV collagen and laminin, constitute essential structural components of the blood brain barrier reflecting its importance in tissue damage.

Investigation on the relation between MMP-9 and stroke is gaining much interest as it has been shown to be involved in stroke pathophysiology and its inhibition is of potential therapeutic role [8]. MMP-9 is also considered a promising biomarker of ischemic stroke[9].

It has also been shown that MMP-9 levels are raised in TBM [10-15] and few studies demonstrated correlation of the increasing levels with advancement of disease and poor outcome[10, 13, 15].

Hence, it is postulated that Matrix metalloproteinases (MMPs) may have role in pathophysiology of stroke in TBM and may serve as biomarker predicting stroke in TBM. So, this study was planned to compare the levels of S. MMP-9 in Tuberculous meningitis patients with and without stroke and to elicit its utility as a biomarker for occurrence of stroke in TBM. If same correlation can be established between the level of MMP-9 in TBM with occurrence of stroke it may have clinical significance and antiplatelet drugs such as aspirin and dipyridamole can be started in early stage of TBM patients, which may reduce and prevent the occurrence of stroke in patient with TBM. The study was conducted with the following objectives:-

2. Objective

2.1. Primary Objective

1. To measure and compare Serum Matrix metalloproteinase-9 levels in patients of TBM with stroke and those without stroke.
2. To correlate Serum Matrix metalloproteinase-9 levels with stroke in TBM patients.

2.2. Secondary Objective

1. To correlate the Serum Matrix metalloproteinase-9 levels in patients of Tuberculous meningitis with clinical features, CSF cytology (TLC, DLC), CSF protein and glucose & neuroimaging features.

3. Methods

Study Setting: The study was conducted in department of Medicine (OPDs and IPD), Biochemistry Department, Pathology Department and central laboratory at UCMS and Guru Teg Bahadur Hospital, Delhi. Subjects were recruited during time period of November 2017-April 2019.

3.1. Study Design: Case Control Study

40 Patients of probable or definite TBM [16] and 40 age and sex matched patients of TBM with clinical stroke were enrolled in the study and formed two groups. The two groups were compared for various clinical parameters(duration of illness, fever, headache,vomiting, weight loss, seizures, GCS, meningeal sign, hemiparesis, cranial nerve palsies), biochemical parameters (CSF cytology, glucose and protein), neuroimaging parameters (meningeal enhancement, tuberculomas & hydrocephalus) and serum MMP-9 levels.

3.2. Inclusion Criteria

1. Patients aged 14-60 years who were suspected to have meningitis clinically and fulfilling the diagnostic criteria for probable or definite tuberculous meningitis [16] with clinically and radiologically proven stroke (as defined by WHO) [17] were taken as cases.
2. Patients aged 14-60 years who were suspected to have meningitis clinically and fulfilling the criteria for probable or definite Tuberculous Meningitis [16] but without any evidence of stroke clinically were taken as control**.**

3.3. Exclusion Criteria

1. Patient who had once or more times taken treatment for tuberculosis in the past.
2. Patients with any chronic systemic disease like DM, HTN, chronic renal failure, hepatic failure, malignancy, congestive heart failure, Autoimmune disorder, collagen vascular disease or immunodeficiency.
3. Patient taking drugs which may affect MMP-9 level like Minocycline, Doxycycline, NSAIDs etc.

3.4. Estimation of Serum Mmp-9 Levels

Serum MMP-9 levels were done in all patients by human MMP-9 Elisa Kit by Elabscience, USA and their levels were compared between cases and controls.

3.5. Statistical Analysis

Data was entered into Microsoft Excel spreadsheet and after cleaning was analyzed using SPSS software v 20.0. Distributed variables were summarized as mean and SD. Both the groups with normal distribution curve were compared to each other using unpaired Student's *t*-test for quantitative variables and chi-square/fisher-exact test for qualitative variables and were categorized as significant or insignificant keeping the p-value <0.05. Correlation was done using spearman's rho correlation coefficient. Multiple logistic regression was done using SPSS software.

4. Results

The results obtained are summarized below:

4.1. Demographic Data

Mean age of cases (32.40±12.10 years) was similar to mean age of controls (28.93 ±12.85 years) with no statistically significant difference between the two groups (p value = 0.217).Study group comprised of 20 males and 20 females. Control group comprised of 22 females and 18 males. There was no statistically significant difference of sex between cases and controls (p=0.654).

4.2. Comparison of Clinical Profile with Occurrence of Stroke in TBM

On comparision between cases and controls only nuchal rigidity (p=0.014), cranial nerve palsy (p=0.034), poor GCS (p<0.001) and stage III TBM (p<0.001) were associated with occurrence of stroke in TBM. The two groups didn't differ statistically as per the presenting symptoms (duration of illness, fever, headache, vomiting, weight loss, seizures and hemiparesis)

4.3. Comparison of CSF Characteristics and Neuroimaging Features between two Groups

There was no significant difference in CSF characteristics (protein, sugar, TLC and DLC) and neuroimaging features (Tuberculomas, hydrocephalus) between cases and controls.

4.4. Serum MMP-9 Levels in Study Group and Control Group

Table-1.

Variable	Control	Cases	P value	Significance
Serum MMP-9 (ng/ml)	157.23±197.155	224.53±261.627	0.157	Non Significant

Serum MMP-9 levels were (224 ± 261.627 ng/ml) in cases and (157.23±197.155 ng/ml) controls which is statistically not significant (p value 0.157).

4.5. Univariate Comparison of Serum MMP-9 Levels with Clinical Features, CSF Characteristics and Neuroimaging Characteristics

Serum MMP-9 levels did not significantly correlate with clinical features (duration of illness, Fever, Headache, Vomiting, Weight loss, Seizures, Nuchal Rigidity, C. N. Palsy, GCS Hemiparesis & stage of TBM), CSF characteristics (protein, sugar, TLC & DLC) and neuroimaging features (tuberculomas & hydrocephalus)

5. Discussion

In our study the mean serum MMP-9 levels were 157.23±197.155 ng/ml in patients of TBM without stroke and 224.53±261.627 ng/ml in patients of TBM with stoke. The serum MMP-9 levels were measured by ELISA method in our study. MMP-9 levels were higher in patients of TBM with stroke than with TBM without stroke but difference was insignificant. So, no correlation was found between MMP-9 levels and stroke in TBM.

Some studies have shown that increased MMP-9 levels are associated with poor outcome and prognosis in patients of TBM. Price, *et al.* [14], reported that CSF MMP-9 concentration per leukocyte was significantly high in tuberculous meningitis patients. Price, *et al.* [14] In the year 2000, Matsurra et al reported that CSF MMP-9 levels correlated with severe disease in TBM. Matsuura, *et al.* [13] In year 2004 lee et al confirmed that MMP-9 levels were significantly high even after 7 days of treatment and were associated with the development of neurological complications. Lee, *et al.* [10], However these studies did not follow the standard definition of poor outcome. Also these studies were limited by small sample sizes and insufficient number of patients experiencing unfavorable outcome.

The results of our study are consistent with few studies showing no correlation of MMP-9 levels with the severity of the disease or the outcome of the disease. The similar observations were made by Thwaites et al and Green et al who have also documented lack of association between MMP-9 levels and outcome or decrease in MMP-9 levels improving the BBB permeability as measured by albumin index. Green, *et al.* [18]; Thwaites, *et al.* [3] Similarly, in a recent study by Sharada Mailankody et al in 2017 the Pretreatment MMP9 levels were not associated with treatment outcome. Mailankody, *et al.* [19] Very recently, a study of 36 HIV-negative tuberculous meningitis patients has been reported from northern India by D Rai et al, where in also MMP-9 level did not correlate with severity and outcome of the tuberculous meningitis patients [15]

Thus, taken together, available evidence on the relation between MMP-9 levels and outcome in tuberculous meningitis is equivocal. Further, the available studies are limited by small sample sizes and insufficient number of patients experiencing unfavorable outcome.

In our study the two groups didn't differ statistically as per the presenting symptoms (duration of illness, fever, headache, vomiting, weight loss, seizures and hemiparesis) are concerned but TBM patients with stroke were more likely to present with poor GCS (p=0.001), advanced stage (stage 3 BMRC) of TBM (p<0.001) and to exhibit nuchal rigidity (p=0.014), and cranial nerve palsy (p=0.034) on presentation.

On analysis of the CSF characteristics between the two groups CSF protein, CSF sugar, TLC and DLC did not differ statistically in TBM with infarct as compared to TBM without infarct. The presence of Hydrocephalus and tuberculoma had no significant association with stroke in TBM.

No correlation was found between serum MMP-9 levels and various clinical parameters, CSF characteristics and neuroimaging features (CSF characteristics protein, sugar and cytology). Also there was no correlation between serum MMP-9 levels and neuroimaging findings like tuberculoma and hydrocephalus.

The various studies have documented variable results for association of MMP-9 levels with various clinical / biochemical/ radiological features [3, 10, 11, 13-15, 19, 20]. The variation in these association can be explained as in all of the above studies, the number of subject recruited were low and few of those studied developed the complications, therefore further reducing the power of study. Also difference in the time at which MMP-9 level were measured may have effect on the above correlation as MMP-9 levels are shown to vary with time and treatment. Also, the method of measurement by ELISA vs Zymography method also may have influence on the above result as ELISA, as used in our study, measures pro-MMP-9 and active or degraded form whereas zymography measures only active form of MMP-9 levels. Snoek-Van, *et al.* [21] So, further large scale studies are required before any conclusion regarding correlation of MMP-9 levels with the above features can be made.

6. Conclusion

We conclude that the features such as nuchal rigidity, cranial nerve palsy, poor GCS and BMRC stage III at presentation are predictor of stroke in TBM and MMP-9 levels is not correlated with occurrence of stroke in TBM. Moreover we did not find any correlation between serum MMP-9 levels and clinical features, CSF characteristics & neuroimaging features but further prospective studies are needed to delineate the role of above parameters in predicting stroke in TBM.

References

[1] Thwaites, G. E., Nguyen, D. B., Nguyen, H. D., Hoang, T. Q., and Do, T. T., 2004. "Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults." *N. Engl. J. Med.,* vol. 351, pp. 1741-5.

[2] Chakraborty, A. K., 2000. "Estimating mortality from tuberculous meningitis in a community: use of available epidemiological parameters in the Indian context." *Indian J. Tub.,* vol. 47, pp. 9-13.

[3] Thwaites, G. E., Simmons, C. P., Than, H. Q. N., and Thi, H. C. T., 2003. "Pathophysiology and prognosis in vietnamese adults with tuberculous meningitis." *J. Infect Dis.,* vol. 188, pp. 1105–15.

[4] Rivera-Marrero, C. A., Schuyler, W., Roser, S., Ritzenthaler, J. D., Newburn, S. A., and Roman, J. M., 2002. "Tuberculosis induction of matrix metalloproteinase-9: the role of mannose and receptor-mediated mechanisms." *Am. J. Physiol. Lung Cell Mol. Physiol.,* vol. 282, pp. L546–55.

[5] Spanos, J. P., Hsu, N. J., and Jacobs, M., 2015. "Microglia are crucial regulators of neuro-immunity during central nervous system tuberculosis." *Front Cell Neurosci.,* vol. 9, p. 182.

[6] Chang, J. C. W. A., Tchou-Wong, K. M., Moskowitz, N., Zhang, Y., and Rom, W. N., 1996. "Effect of mycobacterium tuberculosis and its components on macrophages and the release of matrix metalloproteinases." *Thorax,* vol. 51, pp. 306–11.

[7] Sheen, P., O'Kane, C. M., Chaudhary, K., Tovar, M., Santillan, C., Sosa, J., and Caviedes, L., 2009. "High mmp-9 activity characterises pleural tuberculosis correlating with granuloma formation." *Eur. Respir J.,* vol. 33, pp. 134-41.

[8] Kurzepa, J., Kurzepa, J., Golab, P., Czerska, S., and Bielewicz, J., 2014. "The significance of matrix metalloproteinase (MMP)-2 and MMP-9 in the ischemic stroke." *Int. J. Neurosci.,* vol. 124, pp. 707-16.

[9] Provatopoulou, X., Gounaris, A., Kalogera, E., Zagouri, F., Flessas, I., and Goussetis, E., 2009. "Circulating levels of matrix metalloproteinase-9 (MMP-9), neutrophil gelatinase-associated lipocalin (NGAL) and their complex MMP-9/NGAL in breast cancer disease." *BMC Cancer,* vol. 9, p. 390.

[10] Lee, K. Y., Kim, E. H., Yang, W. S., Ryu, H., Cho, S. N., and Lee, B. I., 2004. "Persistent increase of matrix metalloproteinases in cerebrospinal fluid of tuberculous meningitis." *J. Neurol Sci.,* vol. 220, pp. 73-78.

[11] Li, Y. J., Wilkinson, K. A., Wilkinson, R. J., Figaji, A. A., and Rohlwink, U. K., 2019. "Elevated Matrix Metalloproteinase Concentrations Offer Novel Insight Into Their Role in Pediatric Tuberculous Meningitis." *J. Pediatric Infect Dis Soc.,* Available: https://academic.oup.com/jpids/article/doi/10.1093/jpids/piy141/5316464/

[12] Majeed, S., Singh, P., Sharma, N., and Sharma, S., 2016. "Role of matrix metalloproteinase -9 in progression of tuberculous meningitis: a pilot study in patients at different stages of the disease." *BMC Infect Dis.,* vol. 16, p. 722.

[13] Matsuura, E., Umehara, F., Hashiguchi, T., Fujimoto, N., Okada, Y., and Osame, M., 2000. "Marked increase of matrix metalloproteinase 9 in cerebrospinal fluid of patients with fungal or tuberculous meningoencephalitis." *J. Neurol. Sci.,* vol. 173, pp. 45-52.

[14] Price, N. M., Farrar, J., Tran, T. T., Nguyen, T. H., Tran, T. H., and Friedland, J. S., 2001. "Identification of a matrix-degrading phenotype in human tuberculosis in vitro and in vivo." *J. Immunol.,* vol. 166, pp. 4223-4230.

[15] Rai, D., Garg, R. K., Mahdi, A. A., Jain, A., Verma, R., and Tripathi, A. K., 2014. "Cerebrospinal fluid cytokines and matrix metalloproteinases in human immunodeficiency seropositive and seronegative patients of tuberculous meningitis." *Ann. Indian Acad. Neurol.*, vol. 17, pp. 171-178.

[16] Marias, S., Thwaites, G., and Shoeman, J. F., 2010. "Tuberculous meningitis: a uniform clinical case definition." *Lacet Infect Dis.*, vol. 10, pp. 803-812.

[17] WHO, 1993. *International statistical classification of diseases, injuries and cause of death*. Geneva: WHO, 10th revision.

[18] Green, J. A., Tran, C. T., Farrar, J. J., Nguyen, M. T., Nguyen, P. H., and Dinh, S. X., 2009. "Dexamethasone, cerebrospinal fluid matrix metalloproteinase concentrations and clinical outcomes in tuberculous meningitis." *PLoS One*, vol. 4, p. e7277.

[19] Mailankody, S., Dangeti, G. V., Soundravally, R., Joseph, N. M., Mandal, J., and Dutta, T. K., 2017. "Cerebrospinal fluid matrix metalloproteinase 9 levels, blood-brain barrier permeability, and treatment outcome in tuberculous meningitis." *PLoS ONE*, vol. 12, p. e0181262.

[20] Feigin, V. L., Forouzanfar, M. H., Krishnamurthi, R., Mensah, G. A., Connor, M., and Bennett, D. A., 2014. "Global and regional burden of stroke during 1990–2010: Findings from the global burden of disease study 2010." *Lancet*, vol. 383, pp. 245–255.

[21] Snoek-Van, Beurden, P. A., Von den, and Hoff, J. W., 2005. "Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors." *Biotechniques*, vol. 38, pp. 73-83.