JOURNAL OF RECENT ADVANCES IN APPLIED SCIENCES (JRAAS) 29:07-12, 2014
ISSN 0970 1990, ST CODE 08 ,www.jraas.org. E MAIL: mhcdr@rediff.com

SERUM LEVEL OF MATRIX METALLOPROTEINASE-3 IN IDIOPATHIC PARKINSON'S DISEASE PATIENTS

VINEETA GUPTA[1], MANISH KUMAR SINGH[1], RAVINDRA KUMAR GARG[2], RAJESH VERMA[2], KAMLESH KUMAR PANT[1], GIRDHAR GOPAL AGARWAL[3], SANJAY KHATTRI[14]

[1]Department of Pharmacology & Therapeutics, CSM Medical University, Lucknow, Uttar Pradesh, India. [2]Department of Neurology, CSM Medical University, Lucknow, Uttar Pradesh, India. [3]Department of Statistics, Lucknow University, Lucknow, India.
*Corresponding author: E-mail address: pharmacsmmu@gmail.com; Tel.: +91 9415015700; Fax: +91 522 2257448

ABSTRACT

Background: Due to the lack of definitive biomarkers, Parkinson's disease (PD) sometime becomes difficult to diagnose specially in early stages. Till date, its diagnosis is based on neurological examination. So, there is an unmet need to find out specific biomarkers. The matrix metalloproteinase-3 (MMP-3) found in the peripheral blood system may be involved in the pathophysiology of PD. The objective of the present study was to measure the level of serum MMP-3 in PD patients and controls.
Method: Case-control study was designed to assess the level of serum MMP-3 in 59 idiopathic PD patients (early PD=45, late PD=14) and compared with 16 neurologically healthy controls. The level of serum MMP-3 was examined and quantified using ELISA.
Results: The mean age of PD patients (early PD+late PD) was 56.78 years and controls was 53.44 years. Majority of the participants were male (early PD, late PD, Control: 73.3%, 85.7%, 68.8% respectively). The serum level of MMP-3 in Parkinson's patients (early PD= 74.38$\pm$6.84 ng/ml, late PD= 70.35$\pm$9.64 ng/ml) was significantly ($p<0.0001$) elevated in comparison to controls (45.89 $\pm$ 6.68 ng/ml). Significantly poor correlation was found between the serum level of MMP-3 and age in all the groups (early PD $r= 0.39$, $p<0.01$; late PD $r=-0.26$, $p>0.05$; controls $r= 0.18$, $p>0.05$).
Conclusion: Our study demonstrated the increased level of serum MMP-3 in Parkinson's patients that may provide clinical evidence for a supportive role of MMP-3 in the pathogenesis of Parkinson's disease.

KEY WORDS: Parkinson's disease, Early PD, Late PD, matrix metalloproteinase-

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder occurs due to the massive loss of dopaminergic neurons in the substantia nigra pars compacta (1). Resting tremor, rigidity, akinesia (bradykinesia), and postural instability are the primary symptoms of PD. The patients with PD are diagnosed by the combination of cardinal motor features, associated and exclusionary symptoms, and response to levodopa (2). Usually, the diagnosis of PD is based on clinical signs and symptoms but sometimes it can be mistaken by other forms of parkinsonism, specially in early stage (3). Due to symptoms overlap with other diseases e.g. essential tremor, multiple system atrophy, and progressive supranuclear palsy (4), only 75% of clinically diagnosed patients of PD are confirmed to be of idiopathic etiology at autopsy (5). Currently, no blood test or laboratory test has proven to help in diagnosing sporadic PD (6). In view of the above prospective, specific biomarkers are needed that could be picked up by a blood test for the precise diagnosis of PD.

In the past few decades, matrix metalloproteinase-3 (MMP-3) is being focused in PD research. MMP-3 belongs to the family of matrix-metalloproteinase (MMPs), zinc dependant endopeptidases, involved in the remodeling of extracellular macromolecules (7). Experimental studies have shown that MMP-3 plays a crucial role in degeneration of dopaminergic neurons in substantia nigra (8). MMP-3 also disrupts the Blood Brain Barrier (BBB) (9) which may allow infilteration of the blood immune cells to the damaged region. This BBB leakage is observed in PD patients (10). These findings suggest that MMP-3 may be associated with PD.

In order to find out the involvement of MMP-3 in PD, serum concentration of MMP-3 was measured in PD patients and controls. As far as our knowledge, present study is the first attempt

to estimate serum level of MMP-3 in PD patients.

MATERIAL AND METHODS

Participants

Case-control study was designed, including 59 patients with idiopathic PD (45 males, 14 females) and 16 neurologically healthy controls. PD patients were recruited from the outpatient clinic of the Department of Neurology, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India. PD patients were included from stage I to stage IV using Hoehn and Yahr (1967) scale and diagnoses were made according to the UK Parkinson's Disease Society Brain Bank criteria (11). Patients were divided into two groups according to disease severity. First group includes 45 patients of early PD (stage 1-2) and second group includes 14 patients of late PD (stage 2.5-4). Patients with secondary parkinsonism were excluded from the study.

Sixteen neurologically healthy controls (11 males, 5 females) were also recruited from local community. The control subjects were neither related to one another nor to PD patients. None of the participants had confounding diseases, such as rheumatoid arthritis and systemic lupus erythematosus

The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from each participant.

Samples and Immunoassay of MMP-3

Five ml of blood was drawn from all participants. Each sample of blood was allowed to clot at room temperature and centrifuged at 2400 g for 10 minutes. Serum was aliquoted and stored at -80^0 C until assayed. Human proMMP-3 and active MMP-3 forms were measured in serum as per manufacturer's protocol (RayBio® Human MMP-3 ELISA Kit #ELH-MMP3-001) with sensitivity of 0.3 ng/ml. Antibody specific for human MMP-3 was coated on a 96-well plate. Standards and samples were pipetted into the wells and MMP-3 present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human MMP-3 antibody was added. After washing away unbound biotinylated antibody, horseradish peroxidase enzyme (HRP)-conjugated streptavidin was pipetted into the wells. The wells were again washed, a tetramethyl benzidine (TMB) substrate solution was added to the wells and color developed in proportion to the amount of MMP-3 bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm.

Serum samples of PD patients and controls were analyzed together in batch runs. All measurements were repeated twice and the average value was determined. All samples taken from recruited PD patients and controls underwent same laboratory test to assess the level of serum MMP-3.

Data Analysis

The summary statistics of continuous variables are presented as mean $\pm$ SD and categorical variables are presented in proportion. Kruskal Wallis test was used to asses the significance of difference among groups. Chi-square test was used to test the difference between categorical variables. All statistical tests were evaluated at an alpha level of 0.05. The software used for statistical analysis was SPSS version-16 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Subjects of all the groups were not statistically different in terms of age and sex ($P>0.05$) with male predominance. However, the disease duration (years) of PD patients was significantly higher in late PD patients (4.24 ± 1.80) as compared to early PD patients (1.88 ± 1.32). More than half (60%) of early PD and 21.4% of late PD patients were not on treatment (Table 1).

Age, sex and disease duration:

In the correlation analysis, age was significantly poorly correlated with the serum MMP-3 level in either controls ($r= 0.18$, $p>0.05$) or PD patients (early PD $r= 0.39$, $p<0.01$; late PD $r=-0.26$, $p>0.05$). Mean level of MMP-3 in males vs females of early PD patient was 75.01 ± 7.15 vs 72.63 ± 5.77, in late PD patients was 69.07 ± 8.85 vs 77.94 ± 14.54 and controls was 48.07 ± 5.91 vs 41.07 ± 6.15 ($P<0.0001$; Early vs Control, late vs controls). Disease duration was also not showed significant correlation with the serum level of MMP-3 in PD patients (early PD $r=0.05$, $p= 0.05$; late PD $r=-0.04$, $p>0.05$) (Table not shown).

Level of serum MMP-3 in participants:

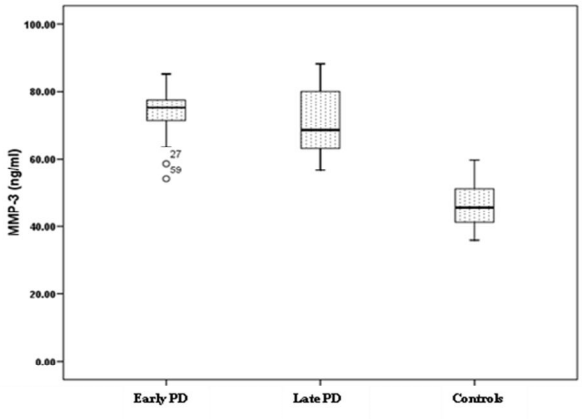
Serum MMP-3 level in early PD (74.38 ± 6.84 ng/ml) and late PD (70.35 ± 9.64 ng/ml) was significantly elevated as compared to controls (45.89 ± 6.68 ng/ml) (Figure 1).

Table 1: Baseline characteristics of PD patients and controls

Characteristics	Early PD patients (n=45) H/Y Stage 1-2	Late PD patients (n=14) H/Y Stage 2.5-4	controls (n=16)	p value
Gender:				
Male	33 (73.3%)	12 (85.7%)	11 (68.8%)	0.54
Female	12 (26.7%)	2 (14.3%)	5 (31.3%)	
Age (years) at blood sampling	56.62 ±9.42	57.29±10.67	53.44 ±5.87	0.42
Age (Years) at PD onset	55.30±9.50	52.79 ±10.39	-	0.40
Weight (Kg)	54.60±12.05	51.86±9.39	60.44 ± 6.65	0.011*
Duration of PD (years)	1.88±1.32	4.24±1.80		<0.0001*
Patients: with treatment	18 (40.0%)	11 (78.6%)	-	0.01*
without treatment	27 (60.0%)	3 (21.4%)	-	

PD= Parkinson's disease, n= Number, H/Y stage= Hoehn & Yahr stage * = Significant

Fig. 1: Serum MMP-3 level in PD and controls



Median and quartiles (box plots) for serum MMP-3 level in controls (n=16), early PD (n=45) and late PD (n=14).Serum level of MMP-3 in PD patients was significantly elevated as compared with control group (Kruskal Wallis Test, $p<0.0001$).

DISCUSSION:

According to our investigation, serum level of MMP-3 in control subjects came out 45.89 ± 6.68 ng/ml. In previous studies, MMP-3 level was reported 61.85±35.35 ng/ml (12) and second one showed 79.66±26.62 ng/ml (13) in Indian control subjects. On the other hand, Mona *et al* reported the concentration of MMP-3 in the serum of healthy controls which was 15.1 ± 7.07 ng/ml (14) and this value agreed with another study done by Chen et al (15.45 ng/ml) (15). Recently, Steven et al reported the mean level of MMP-3 (23.4 ng/ml) in healthy controls serum (16) and it was close to 22.6 ng/ml (17). These different concentrations of MMP-3 indicated that results might vary according to ethnicity. In the present study, MMP-3 level in males (48.07±5.91 ng/ml) was higher than females (41.07±6.15 ng/ml) which was contrary to the finding of Manjari et al (12).

A study reported the altered levels of MMPs expression in the different parts of postmortem brains from PD patients (18). According to our study, increased level of serum MMP-3 in PD patients indicates that altered expression of MMPs are not restricted only to brain sites and

may also contribute to peripheral dysfunction. MPTP mouse model of PD have also been showed elevated levels of MMPs. Treatment with an MMP inhibitor showed the reduction in dopamine depletion and dopaminergic cell loss in these animal models (19). Kim et al observed the catalytic activity of MMP-3 responsible for the microglial activation because pharmacological inhibition of MMP-3 blocks the response, and the catalytic domain of MMP-3 applied by itself causes similar responses (7). In a further study, it was found that microglial activation following treatment with the neurotoxin MPTP is abrogated in MMP-3 knockout mice, and this is accompanied by a lower level of superoxide production compared to their wild type (20).

It is well documented that neuronal degeneration occurs in PD but the reason behind it is unknown. It is supposed that several molecular and cellular events can take place in the course of PD, including oxidative stress, neuroinflammation, mitochondrial dysfunction, proapoptotic mechanisms, and accumulation of altered proteins (21). Possibly the activation of MMPs and oxidative stress may be correlated to each other. Effect of oxidative stress on MMP-3 has been studied *in vitro* which showed that oxidative stress increased MMP-3 release (22). Neuroinflammation and apoptosis are also one of the causes that may contribute to PD pathogenesis (23). MMP-3 may be involved in both the events. The release of MMP-3 from neurons causes activation of resting microglial cells (7) and inhibition of MMP-3 leads to suppression of proinflammatory cytokine production (24). On the other hand, cytokines and free redicals in microglial cells induce MMP-3 (25). Thus, there may be a complex mechanism where extracellular MMP-3 leads to the production of cytokines and free redicals and this in turn further increase MMP-3 in microglial cells and subsequent release (26). According to *in vitro* studies, inhibition of MMP-3 via pharmacologically, gene knockdown, and gene depletion all lead to neuroprotection against oxidative stress and ER-stress triggered apoptosis (8, 27). MMP-3 involvement in the abnormal protein aggregation has also seen as it increased the tendency of alpha-synuclein to aggregate (28).

One of the limitation of the present study was few number of controls' samples because of non-consent of participants at the time of experiment. In conclusion, elevated level of serum MMP-3 in Parkinson's patients may provide clinical evidence for a supportive role of MMP-3 in the pathogenesis of Parkinson's disease. However, further investigations are needed to elucidate the role of MMP-3 in PD pathogenesis in large number of participants.

ACKNOWLEDGEMENT:
We would like to thank all the participants who participated in the study. This research work was supported by a Grant from the Council of Science and Technology, Uttar Pradesh, Lucknow, India.

REFERENCES

1. Ledger S, Galvin R, Lynch D, Stokes EK (2008). A randomised controlled trial evaluating the effect of an individual auditory cueing device on freezing and gait speed in people with Parkinson's disease. BMC Neurol 8:46.
2. Jankovic J (2008). Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79(4):368-376.
3. Li QX, Mok SS, Laughton KM, McLean CA, Cappai R, Masters CL, Culvenor JG, Horne MK (2007). Plasma alpha-synuclein is decreased in subjects with Parkinson's disease. Exp Neurol 204:583-588.
4. Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Leverenz JB, Baird G, Montine TJ, Hancock AM, Hwang H, Pan C, Bradner J, Kang UJ, Jensen PH, Zhang J (2010). DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain 133:713-726.
5. Parkinson's Disease Diagnosis. Parkinson's Disease Diagnosis. (http://www.news-medical.net/health/Parkinsons-Disease-Diagnosis.aspx). Accessed on September 24, 2012.
6. National Institute of Health. National institute of neurological disorders and stroke. Parkinson's Disease: Hope Through Research. ; (http://www.ninds.nih.gov/disorders/parkinsons_disease/detail_parkinsons_disease.htm) Accessed on September 24, 2012.
7. Kim YS, Kim SS, Cho JJ, Choi DH, Hwang O, Shin DH, Chun HS, Beal MF,

Joh TH (2005). Matrix metalloproteinase-3: a novel signaling proteinase from apoptotic neuronal cells that activates microglia. J Neurosci 25(14):3701-3711.
8. Choi DH, Kim EM, Son HJ, Joh TH, Kim YS, Kim D, Flint Beal M, Hwang O (2008). A novel intracellular role of matrix metalloproteinase-3 during apoptosis of dopaminergic cells. J Neurochem 106(1):405-415.
9. Gurney KJ, Estrada EY, Rosenberg GA (2006). Blood-brain barrier disruption by stromelysin-1 facilitates neutrophil infiltration in neuroinflammation. Neurobiol Dis 23(1):87-96.
10. Kortekaas R, Leenders KL, van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, Hendrikse NH (2005). Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann **Neurol 57(2):176-179.**
11. Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55(3):181-184.
12. K. Sri Manjari, Pratibha Nallari, A. Vidyasagar, A. Jyothy, A. Venkateshwari (2012). Plasma TGF-□1, MMP-1 and MMP-3 Levels in Chronic Pancreatitis . Ind J Clin Biochem 27(2):152-156.
13. Bassiouni HM, El-Deeb M, Kenawy N, Abdul-Azim E, Khairy M (2011). Phonoarthrography, musculoskeletal ultrasonography, and biochemical biomarkers for the evaluation of knee cartilage in osteoarthritis. Mod Rheumatol 21(5):500-508.
14. Al-Sebaie MA, Al-Yasaky AZ, Assaf NY, Mahdy MM, Elwan N (2003). Serum and synovial fluid levels of mmp-3 and timp-1 in rheumatoid arthritis and osteoarthritis. Egypt Rheumatol Rehab 30:841-860
15. Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen HA, Liang TH, Liao HT, Tsai CY, Wei JC, Chou CT (2006). Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. Rheumatology (Oxford) 45(4):414-420.
16. Luckman SP, Gilhus NE, Romi F (2011). Matrix metalloproteinase-3 in myasthenia gravis compared to other neurological disorders and healthy controls. Autoimmune Dis. 2011:151258.
17. Green MJ, Gough AK, Devlin J, Smith J, Astin P, Taylor D, Emery P (2003). Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. Rheumatology (Oxford) 42(1):83-88.
18. Lorenzl S, Albers DS, Narr S, Chirichigno J, Beal MF (2002). Expression of MMP-2, MMP-9, and MMP-1 and their endogenous counterregulators TIMP-1 and TIMP-2 in postmortem brain tissue of Parkinson's disease. Exp Neurol 78(1):13-20
19. Lorenzl S, Calingasan N, Yang L, Albers DS, Shugama S, Gregorio J, Krell HW, Chirichigno J, Joh T, Beal MF (2004). Matrix metalloproteinase-9 is elevated in 1-methyl-4-phenyl-1,2,3,6-etrahydropyridine-induced parkinsonism in mice. Neuromolecular Med 5(2):119-132.
20. Kim YS, Choi DH, Block ML, Lorenzl S, Yang L, Kim YJ, Sugama S, Cho BP, Hwang O, Browne SE, Kim SY, Hong JS, Beal MF, Joh TH (2007). A pivotal role of matrix metalloproteinase-3 activity in dopaminergic neuronal degeneration via microglial activation. FASEB J 21(1):179-187
21. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M (2009). Peripheral cytokines profile in Parkinson's disease. Brain Behav Immun 23(1):55-63.
22. Alge-Priglinger CS, Kreutzer T, Obholzer K, Wolf A, Mempel M, Kernt M, Kampik A, Priglinger SG (2009). Oxidative stress-mediated induction of MMP-1 and MMP-3 in human RPE cells. Invest Ophthalmol Vis Sci 50(11):5495-5503.
23. Nagatsu T, Sawada M (2005). Inflammatory process in Parkinson's disease: role for cytokines. Curr Pharm Des 11(8):999-1016.
24. Woo MS, Park JS, Choi IY, Kim WK, Kim HS (2008). Inhibition of MMP-3 or -9 suppresses lipopolysaccharide-induced expression of proinflammatory

cytokines and iNOS in microglia. J Neurochem 106(2):770-780.
25. Jian Liu K, Rosenberg GA (2005). Matrix metalloproteinases and free radicals in cerebral ischemia. Free Radic Biol Med 39(1):71-80.
26. Kim EM, Hwang O (2011). Role of matrix metalloproteinase-3 in neurodegeneration. J Neurochem 116(1):22-32.
27. Kim EM, Shin EJ, Choi JH, Son HJ, Park IS, Joh TH, Hwang O (2010). Matrix metalloproteinase-3 is increased and participates in neuronal apoptotic signaling downstream of caspase-12 during endoplasmic reticulum stress. J Biol Chem 285(22):16444-16452
28. Levin J, Giese A, Boetzel K, Israel L, Högen T, Nübling G, Kretzschmar H, Lorenzl S (2009). Increased alpha-synuclein aggregation following limited cleavage by certain matrix metalloproteinases. Exp Neurol 215(1):201-208.