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Estimation Of MMP-2 And MMP-9 In Glioma And Its Correlation With Pathological Prognosticators

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ABSTRACT

Glial cells give rise to gliomas, a form of primary tumor of the central nervous system. A matrix Metalloproteinases (MMPs) are a group of secreted, zinc-dependent endopeptidases that play a role in tissue remodelling processes such as wound healing, embryo implantation, tumour invasion, metastasis and angiogenesis. MMPs are involved in angiogenesis, cell migration, and tissue morphogenesis, among other normal physiological activities. MMP-2 and MMP-9 levels in the blood were measured and correlated with other prognostic factors, such as menopausal state, age, and gender. The circulatory level of MMP-2 and MMP-9 were determined enzyme-link immunosorbent assay in a total of 48 Glioma patients and the same number of controls. Level of MMP-2 significantly elevated in controls than Glioma patients ($p<0.001$) while MMP-9 showed a non-significant elevation in controls than patients. The level of MMP-2 in the male of both categories was significant in Age ≤40 and age 40-60 and also the level of MMP-9 significant in age ≤40 than 40-60 and ≥60. Level of MMP-2 and MMP-9 was higher in premenopausal women than perimenopausal and, but it was not substantial. This very high and complex regulation of the expression of MMPs represents a host response to the tumor and neoplastic cell interaction with the tumor stromal component is fundamental to cancer invasion and metastasis.

KEYWORDS: MMP-2, MMP-9, ELISA, Gelatinase, Age, Gender, menopausal status.

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INTRODUCTION

The malignant transformation of astrocytes, oligodendrocytes, or their progenitor cells gives rise to tumors that are collectively called gliomas.[1] A glioma is a type of tumor that starts in the glial cells of the brain, or the spine.[2] Gliomas comprise about 30 percent of all brain tumors and central nervous system tumors, and 80 percent of all malignant brain tumors.[3] Malignant gliomas are notoriously resistant to currently available therapies because these cancer cells can't be induced to undergo apoptosis upon anticancer treatment.[4,5] The most common site of involvement of glioma is the brain, but they can also affect the spinal cord, or any other part of the CNS, such as the optic nerves. Astrocytes, ependymal, and oligodendroglia cells are all examples of glial cells that compose the supportive tissue of the brain. Gliomas comprise nearly one-half of primary brain tumors and one-fifth of all primary spinal cord tumors. Matrix metalloproteinases are a family of secreted, zinc-dependent endopeptidases and are involved in tissue-remodeling processes, including wound healing, embryo implantation, tumor invasion, metastasis, and angiogenesis. MMPs play a role in normal physiological processes, including tissue morphogenesis, cell migration, and angiogenesis. MMPs are also involved in pathophysiological processes including wound healing, inflammation, and cancer.[6]

Matrix metalloproteases MMP-9 and MMP-2 have been shown to participate in the processes of tumor growth, vascularization, and invasion of gliomas.7 MMP2 expression is higher in gliomas than in healthy brain tissue.[6,7,8] Furthermore, the brain tumors expressing higher levels of MMP2 are frequently associated with higher degrees of invasion, metastasis, and angiogenesis. The increase in MMP2 could be found in both tumor and vasculature cells, indicating multiple roles for MMP2 in tumor progression.[9, 10, 11] Physiologically MMP play a role in normal tissue remodeling as well as in angiogenesis. MMP belong to a family of 23 gene products. MMP-2 and MMP-9 belong to the group of gelatinases. Peptides used as prognosticators. In the present work, the prognostic significance of serum levels of gelatinase A (MMP-2) and EDTA plasma level of gelatinase B (MMP-9) in patients with glioma. The Circulatory level of MMP-2 and MMP-9 estimated by enzyme-link immunosorbent assay. Pretherapeutic Circulating levels of MMP-2 and MMP-9 evaluated in Total 48 Glioma patients and the same number of controls.

MATERIALS

48 glioma patients and 48 controls were enrolled in the current study, which was conducted on human brain tumour patients and health control patients at Gujarat Cancer Research Hospital (GCRI), Ahmedabad. Commercial ELISA kits were used to estimate the levels of MMP-2 and MMP-9 in the blood. purchased from the UK's GE Health Care.

Inclusion criteria
The patient should be pre-therapeutic. Patient with Negative HBsAg and HIV were enrolled. For healthy controls, no evidence of any health problem, Persons with negative HBsAg and HIV entered.

Exclusion criteria
The individuals in the present or past with diabetes or other growth hormone-related disorder were not enrolled. The patient who receives earlier treatments not enrolled.

Procedure for quantitative determination of MMP-2 (GE Health Care U.K)
The biotraktm MMP-2 ELISA provides a simple, specific, and precise quantitative determination of MMP-2 in serum. MMP-2 measured in the range 1.5-24 ng/ml and sensitivity of the assay was 0.37ng/ml. All reagent equilibrated to 20-27ºc before use. It does with the enzyme substrate TMB. Microtiter was set up with a plate with sufficient wells for running of all zero (blanks), standers and samples as required. 100µl of assay buffer was pipetted out into zero conventional wells. 100µl of each standard was pipetted out into the appropriate wells using a clean polypropylene pipette tip for each measure. 100µl of the unknown sample was pipetted out into the appropriate wells. The plate was covered with the lid provided and incubated at 20-27ºc for precisely 2 hours. Aspirated and washed all wells four times with wash buffer ensuring that the wells were filled and emptied at each wash. The plate has blotted on tissue paper, providing any residual volume was removed during the blotting procedure. 100µl was pipette out of peroxidase conjugate into all wells. The plate was covered with the lid and incubated at 20-27ºc for precisely 1 hour. Aspiration and washing have done according to the above sample. Immediately dispensed 100 µl of room temperature equilibrated TMB substrate into all wells. The plate was covered with the lid and stand for precisely 30 minutes at room temperature (20-27 ºc). The blue color has developed may be read at 630nm. The reaction was stopped by the addition of 100 µl 1m sulfuric acid to all wells and read the plate at 450nm within 30 minutes.

Procedure for quantitative determination of MMP-9 (GE Health Care U.K)
The MMP-9 ELISA provides a simple, specific, and precise quantitative determination of MMP-9 in plasma. The assay was based on two-site ELISA using two antibodies directed against different epitopes of MMP-9. During the first incubation step, MMP-9 present in the samples or the standards bound to a micro plate percolated with the antibody. During the second incubation step, detection antibody conjugated to horseradish peroxidase is added, which forms an immobilized complex.

The amount of peroxidase bound to each well, and is determined by the addition of tetramethylbenzidine (TMB) ‘ready to use' substrate. The reaction had stopped by the addition of acid solution and resultant color measured at 50nm in a micro plate spectrophotometer. The concentration of proMMP-9 in samples was determined by interpolation from a standard curve. MMP-9 may measure in the range 1-32 ng/ml for tissue culture, and the sensitivity of the assay was 0.6 ng/ml. Each pack contains sufficient material for 96 wells so; this permits the construction of one standard curve and measurement of 0 unknowns in duplicate, according to MMP-2.

Statistical analysis
SPSS 11 was used to perform statistical analysis of data. Students't' test was carried out to find significance. Mean values with $P<0.05$ considered statistically significant. For parametric statistic, Pearson's correlation test and for non-parametric correlation Spearman's test performed.

RESULT

Results were expressed as ng/ml for both MMP-2 and MMP-9. The pathological prognosticators such as sex, age, menopausal status were measured.

Table 1. Incidence of MMP-2 & MMP

	Total	MMP-2 ng/ml	MMP-9 ng/ml
		X ± SE	X ± SE
Patient	48	1087.91± 41.67*	34.85±1.99
Control	48	1642.33± 28.39*	40.11±3.48

The sample is found significant *P<0.001

Incidence of MMP-2 & MMP-9
Comparison between patients and control showed that MMP-2 levels in controls was significantly elevated than Glioma patients (p <0.001) while MMP-9 showed non-significant (P<0.3) elevation in controls than Glioma patients it shows in table-1.

Incidence in gender
17 males and 31 females in both Glioma patients and control group taken. In patients, MMP-2 was elevated in Males than Females, while MMP-9 levels increased in Females than Males. In the control group, both MMP-2 and MMP-9 were increased in Females than males show in table-2

Table 2. Incidence in gender

Category	Total	MMP-2 ng/ml	MMP-9 ng/ml
Patient	**N**	**X ± SE**	**X ± SE**
Male	17	1661.73±55.36*	25.52±3.64
Female	31	1631.69±32.38#	48.11±4.42
Control			
Male	17	1057.94± 68.03*	34.02±3.24
Female	31	1104.35±53.23#	35.30±2.55

*P<0.001, #P<0.001 (The sample is found significant *P<0.001)

Incidence in Age

Age has a Great influence in the development of any cancer the levels of MMP-2 and MMP-9 can also vary with respective to the age of the patients. In this incidence first correlate Male of both category it indicate level of MMP-2 is significant in Age ≤40 and age 40-60 and also level of MMP-9 significant in age≤40. Afterward Female of both category it indicate level of MMP-2 is significant in all range of age ≤40, 40-60 and ≥60. But not significant MMP-9. In male patient's age between □40 and □60. In this correlation level of MMP-2 (p<0.3) and MMP-9 (p<0.9) is not significant. In correlation with female patient age between □40 and 40-60, 40-60 and≥60 and □40-≥60 is not significant in both MMP-2 and MMP-9. In male of age between □40 and □60. In this correlation level of MMP-2 (p<0.01) is significant but in MMP-9 (p<0.3) is not significant. In correlation with female control age between □40and 40-60, 40-60 and≥60 and □40-≥60 is not significant in both MMP-2 and MMP-9. In this category patient's age show non significant & inverse correlation with MMP-2 both by parametric statistic [Pearson's correlation = - 0.072 (p=0.626, NS) and non parametric statistic [spearman's r = - 0.057(0.557, NS). In correlation of age and MMP-9 has not significant and inverse correlation by parametric statistic [Pearson's correlation=-0.014 (p=0.923, NS) and non parametric statistic [spearman's r=-0.044(0.0.766, NS)].

Table 3. Incidence in Age

Category	Patient		Control	
	Male	**Female**	**Male**	**Female**
MMP-2	**X ± SE**	**X ± SE**	**X ± SE**	**X ± SE**
≤40□	1099.00±100.52+♥	1106.40±62.58*	1699.07±54.3+	1638.50±46.17*
40-60	897.00±77.25#♥♣	1140.22±119.26●	1487.50±176.3#	1563.33±48.82●
≥60	1255.00±55.00♣	997.50±76.82♦	00.00	1811.25±47.71♦
MMP-9	X ± SE	X ± SE	X ± SE	X ± SE
≤40	37.90±4.10♠	36.18±3.10	24.31±4.05♠	41.24±6.60
40-60	25.20±5.99	32.72±4.84	31.16±9.13	57.79±6.59
≥60	36.75±6.25	38.87±9.81	00.00	44.87±10.7

+P<0.001, #<0.05, *P<0.001, ●P<0.01, ♦P<0.001, ♥P<0.01, ♣P<0.02, ♠P<0,05

The incidence in Menopausal status

Females Menopausal Status was an important parameter to decide prognosis. Menopausal status divided into Pre, and Post Menopausal depending upon the exposure to Menstrual Cycle (Table-4). In this study incidence from 31 Female patients, only 21 Female's Menopausal status known. These 21 Females divided into three categories Premenopausal, Peri menopausal, and Postmenopausal. The analysis showed that the level of MMP-2 and MMP- 9 was higher in premenopausal women, but it was not significant. In correlation of menopausal status and MMP- 2 was not significant and inverse correlation by parametric statistic [Pearson's correlation=-0.205(p=0.372, NS) and non-parametric statistic [spearman's r=-0.194(0.0.398, NS). patient's Menopausal status showed non- significant & inverse correlation with MMP-9 both by parametric statistic [Pearson's correlation=-0.148(p=0.523, NS) and non-parametric statistic [spearman's r=0.089(0.701, NS)].

Table 4. Incidence in Menopausal status

Menopause status	Percentage	MMP2	MMP9
Category	**%**	**Median**	**Median**
Patient	100	1200	39.00
PreM	61.90	1225	39
periM	00	00	00
PostM	38.10	1107.5	36.75

Control	100	1650.0	46
PreM	58.06	1643.75	47
PeriM	19.35	1567.5	46
PostM	22.58	1760	44.5

DISCUSSION

Substantial work over the past decade had shown that MMPs play a pivotal role in the process of malignant progression and that inhibition of MMP-9 expression and activity resulted in the reduction of tumor in studies. Pathology Laboratory, Nihon University Itabashi Hospital, 30-1 Ohyaguchi-kamimachi, Itabashi-ku, Tokyo 173- 8610, Japan.[12] In this study, 38% of the cases were positive for MMP-2, and Tumor cells were immunohistochemically positive for MMP-9 in 81% of the samples. The relationship between the expression of MMP-2 and -9 and the histological features of tissues from 21 cases of human glioma investigated. Immunohistochemical studies detected MMP-2 and -9 proteins. This study concluded that there was no significant relationship between the expression of MMP-2 protein and the biological nature of the tumors, including aggressiveness and Histologic classification.[13] Level of MMP2 was higher in male than the female & level of MMP9 was higher in control female than male. Which compare to Malekpourafshar et al., 2006 study had sex ratio was (male: female) 2:1. Which justify this study.[14, 15]. Mohammad Dilmaghani studied on 40 patient from this 40 patient has sex ratio was 4:1. It also reveals our considered.[16] The level of MMP2 was higher in age≥60, and level of MMP9 was more elevated in age ≤40 in male. In the female standard of MMP2 elevated in age 40- 60 and level of MMP9 was higher in age ≥60. The most frequent age group of occurrences was geriatric, which studied by MacLendon et al., 1985.[17]This study also justified that geriatric group age ≥60 had more chance of happening. In Malekpourafshar et al., 2006 study out of 120 patient, 82 cases were, and 38 were female. The age at diagnosis range from 3 to 80 years, and mean±SD was 52.7 ±17.6. The maximum range of patient age was 60 years old; there was not any significant difference in the average age at both groups of sex.[18]

Level of MMP2 in Premenopausal female was more significant than the degree of MMP9. Level of MMP2 and level of MMP9 was higher in premenopausal female than peri and postmenopausal female.

CONCLUSION

The current investigation involved the examination of 96 samples, of which 48 were patients with brain tumours and 48 were healthy controls. MMP2 and MMP9 enzyme-link immunosorbent assays were conducted using serum and EDTA plasma samples, respectively. Establish a correlation between their level and a distinct pathological variable, such as sex, age, menopausal status, tumour size (both clinical and pathological), and cancer grade. MMP2 expression is often low in tumour cells. MMP2 is strongly yet briefly induced in stromal cells.

This very high and complex regulation of the expression of MMPs represented a host response to the tumor and neoplastic cell interaction with the tumor stromal component was fundamental for cancer invasion and metastasis. Level of MMP2 and MMP9 was higher in age ≤40 and lower in age 40-60. From this, it has been concluded that the occurrence of brain tumor was more elevated in age 40-60. Level of MMP2 and MMP9 was higher in premenopausal female and less in postmenopausal and then perimenopausal female. From 21 patient, 13 female has premenopausal status, and 8 female has postmenopausal status. Level of MMP-2 in significantly higher in controls than Glioma patients ($p<0.001$) while MMP-9 showed a non-significant elevation in controls than patients. From this correlation, I concluded that the level of MMP2 was higher than the level of MMP9 in both categories of patient and healthy control.

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