Correlations of polymorphisms in matrix metalloproteinase-1, -2, and -7 promoters to susceptibility to malignant gliomas

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ABSTRACT

Background: Oligodendrogliomas are infiltrative astrocytic tumors. They constitute about 1-5% of intracranial tumors. These have been graded into benign and malignant grades. The single nucleotide polymorphisms (SNPs) in the promoter regions of MMP genes may influence tumor development and progression. This study was done to explore the correlations of the promoter SNPs in MMP-1, MMP-2 and MMP-7 genes susceptibility in development and progression of oligodendrogliomas.

Objectives: We aimed to investigate the association of MMP1 ($-1607A > G$), MMP-2 (-1306 C/T) and MMP-7($-181A > G$) gene polymorphism in oligodendrogliomas (grade I, II, III).

Materials and Methods: In the present case control study, we enrolled a total of 30 cases of oligodendrogliomas (grade I to III) confirmed by histopathology and 30 healthy cases as control. Polymorphism for MMP-1 gene ($-1607A > G$), MMP-2 (-1306 C/T), MMP-7($-181A > G$) were genotyped by restriction fragment length polymorphism.

Results: Frequencies of MMP-1 ($-1607A > G$) genotypes and 2G alleles were significantly associated with the cases of oligodendrogliomas (30%) in relation to healthy controls (13%). [OR = 6.89; $P = 0.02$; 95%CI= (1.33-35.62)] and [OR = 2.66; $P = 0.01$; 95% CI= (1.26-5.64)]. A significant association of MMP-2 (-1306C/T) polymorphism with oligodendroglioma ($P = 0.54$) was not found, suggesting that MMP-2 (-1306C/T) polymorphism is not associated with increased oligodendroglioma susceptibility. Frequencies of MMP-7($-181A > G$) genotypes and 2G alleles were significantly associated with the cases of oligodendrogliomas (33.33%) in relation to healthy controls (13.33%). [OR = 5.65; $P = 0.02$; 95%CI= (1.26-25.36)] and [OR = 2.49; $P = 0.01$; 95% CI= (1.17-5.27)].

Conclusions: MMP-1 (-1607 A > G), MMP-7($-181A > G$) genotypes and 2G alleles were significantly associated with oligodendroglioma (grade I, II, III), but MMP-2 (-1306C/T) polymorphism is not associated with increased oligodendroglioma susceptibility.

Key words: Oligodendrogliomas, polymerase chain reaction, polymorphism

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Introduction

Oligodendroglioma accounts for 2-3% of supratentorial tumors in children and 1-5% of tumors overall. When measured together with mixed glioma, its incidence reaches 9-30%. The grading system identifies benign and anaplastic forms (grade I, II, III), with the anaplastic being recurrent in adult population. It is known that benign form may extend along the CSF pathways. Five-year survival following treatment varies from 75 to 85%, but it is considerably lower in anaplastic form.[1] Matrix metalloproteinases (MMPs) are large family of zinc-dependent endopeptidases belonging to subfamily M10A, which are

capable of modifying various components of the extracellular matrix as well as collagens, laminin, fibronectin, vitronectin, and proteoglycan. These enzymes have been implicated in diversity of physiological and pathological conditions including embryogenesis, tumor invasion, and metastasis.[2-4]

The members of the MMP family have the capability to degrade macromolecules of the extracellular matrix and are accountable for tumor invasion and infiltration, limiting the efficiency of the neurosurgical resection of brain tumors. Amongst the glial tumors, the benign astrocytomas and oligidendrogliomas are the most significant tumor entities requiring comparatively different therapeutic approaches as they have better outcome.[5] MMPs are synthesized by stromal cells and formed entirely by cancer cells. They contribute directly in the process of metastasis in squamous cell carcinomas of the head, neck, and lung, and adenocarcinoma of the breast and prostate cancer.[6-8]

There are very few studies available in world literature to reveal the association of MMP-1, MMP-2, and MMP-7 in oligidendroglioma which is a type of glial tumor. The possibility of such association cannot be denied in view of previous studies revealing associations with gliomas.[9-11] In the present study, we have tried to see the association of MMP-1, MMP-2 and MMP-7 gene polymorphisms in genomic DNA in blood samples of histologically proven cases of oligidendrogliomas.

Aims and Objectives

To study the role of MMP-1 ($-$1607 1 G/2 G), MMP-2 (1306 C > T), and MMP-7 ($-$181 A > G) polymorphisms in cases of oligidendrogliomas (grade II and grade III) by genotyping method.

Materials and Methods

Study population

This prospective study was carried out in Neurosurgery Department of Sanjay Gandhi Post Graduate Institute of Medical Sciences between years 2006 and mid 2012. The cases included were histologically proven cases of oligidendrogliomas in which we tested blood samples, but not the tumor tissue. The lab work was carried out in basic lab of the same department. The blood samples were taken from the Department of Neurosurgery of Sanjay Gandhi Post Graduate Institute of medical Sciences and Chhatrapati Shahuji Maharaj University, Lucknow. All subjects were ethnic northern Indians. Thirty cases of histologically confirmed oligidendrogliomas were studied. The blood samples of 30 healthy volunteers matching in age gender and ethnicity were also tested. The samples collected from other tumor and mixed gliomas cases were excluded following their histopathology report. There were 24 cases of grade II and 6 cases of grade III oligidendrogliomas in the subject population. Reoperated

cases or the cases of malignant transformation from a lower grade to higher grade of oligodendroglioma were excluded from the study. The Institutional Ethics Committee granted approval for the study of gliomas. Written informed consent was obtained from all the study subjects. A brief clinical evaluation of each case was carried out by senior residents of Neurosurgery Department. All the variables, e.g. dietary habits, smoking habits, chewing habits, use of solvents, family history, use of immunosuppressive agents, occupation detail, and presence of any other disease, were recorded in each proforma.

DNA isolation (from blood samples)

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood by salting-out method (1 ml blood in EDTA → add 1 ml lysis buffer → centrifuge at 11,000 rpm for 5 min, discard supernatant → add 300 μl lysis buffer → centrifuge at 11,000 rpm for 2 min → discard supernatant → add 300 μl water → centrifuge 11,000 rpm for 2 min, discard supernatant → add 100 μl proteinase k buffer, add 10% sodium dodecyl dodamide sulfate (SDS) 5-6 ml → foaming with pipette → add 100 μl 5 M NaCl and mix by tapping 400 μl phenol chloroform (4:1) → mixed by inversion → centrifuge at 11,500 rpm for 10 min → transparent layer (200 μl + 1 ml alcohol) → discard supernatant, add 250/100 μl 70% ethanol → centrifuge at 11,500 rpm for 2 min, discard supernatant, add 50/100 μl RNA free water and keep at $-$20°C refrigerator for storage of DNA).[12] DNA samples of 100 ng/μl concentration were used for the detection of single nucleotide polymorphisms (SNPs) of the selected MMP genes.

Genotyping

MMP-1 ($-$1607 1 G/2 G), MMP-2 ($-$1306 C > T), and MMP-7 ($-$181 A > G) genotyping was done by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based on the methods described earlier.[13-15] The digested PCR fragments were separated on polyacrylamide gel and observed with UV light.

Statistical analysis

The sample size was calculated with QUANTO 1.1, using minor allele frequency data from Hap Map (http://hapmap.ncbi.nlm.nih.gov/). Statistical analysis was done using SPSS statistical analysis software, version 16.0 (SPSS, Chicago, IL, USA). Hardy–Weinberg equilibrium was checked in controls by χ^2 test. Logistic regression analysis was applied to calculate approximate association with oligodendroglioma susceptibility after adjusting for age and gender.

Results

Characteristics of oligodendroglioma patients and control subjects

The mean age ± SD (years) of patients and healthy controls were 34.80 ± 12.36 years and 34.87 ± 11.96 years, respectively. Characteristics of oligodendroglioma patients are shown in Table 1.

MMP-1 (−1607 1 G/2 G) polymorphisms in oligodendroglioma

The genotypic distributions of MMP-1 (−1607 1 G/2 G) polymorphisms in controls were in Hardy–Weinberg equilibrium. The genotype and allele frequencies of MMP-1 (−1607 A > G) polymorphism in healthy controls and patients with oligidendrogliomas are shown in Table 2. The individuals with MMP-1 (−1607 1 G/2 G) genotype and 1607, 2 G allele were significantly associated with oligidendrogliomas [(OR = 6.89; P = 0.02; 95% CI = 1.33–35.62) and (OR = 2.66; P = 0.01; 95% CI = 1.26–5.64), respectively] [Table 2 and Figure 1].

MMP-2 (−1306 C > T) polymorphism in oligodendroglioma

The genotype frequency was compared between patients and controls to analyze the association of MMP-2 (−1306 C/T) polymorphism with oligodendroglioma. The genotypic distribution of MMP-2 (1306 C/T) polymorphism demonstrated no association of oligodendroglioma susceptibility in patients with C/T (OR = 1.03; P = 0.95; 95% CI = 0.288-3.748) as well as with T/T (OR = 2.19; P = 0.54; 95% CI = 0.17-28.01) genotypes, compared to normal subjects. Patients with oligodendroglioma showed almost similar prevalence of T allele of 1306 MMP-2 gene polymorphism (OR = 2.66; P = 0.61; 95% CI = 1.26-5.64) compared to controls [Table 3 and Figure 2].

MMP-7 (−181 A > G) polymorphism in oligodendroglioma

The genotype distributions of MMP-7 (−181 A > G) polymorphisms in controls were in Hardy–Weinberg
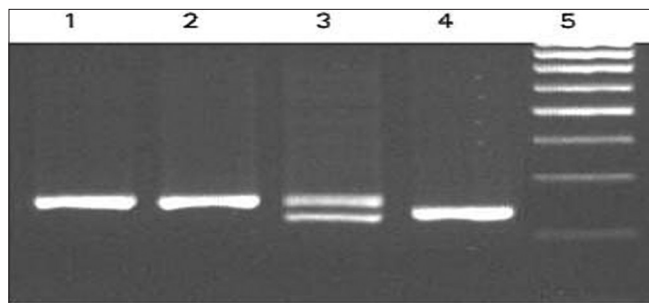

Figure 1: Representative gel picture showing MMP1(1607) A>G polymorphism, on 100 bp ladder. Lane 1 and 2 shows variant, lane 3 heterozygous and lane 4 shows wild type alleles
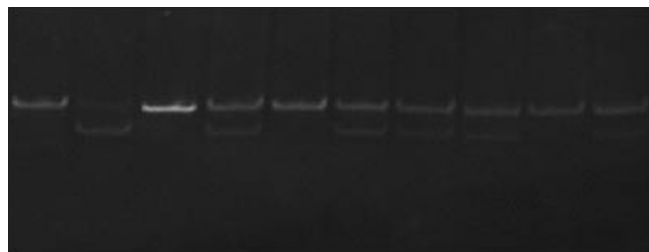

Figure 2: Representative gel picture showing MMP7(181) A>G polymorphism, on 100 bp ladder. lanes shows variant, heterozygous and wild type alleles

equilibrium. The genotype and allele frequencies of MMP-1 (−181 A > G) polymorphism in healthy controls and patients with oligodendroglioma are shown in Table 4. Individuals with MMP-7 (−181 A > G), GG genotype and −181 G allele were significantly associated with oligodendroglioma [(OR = 5.65;

Table 1: Demographic characteristics of the study subjects

Characteristics	Patients (n=30)	Controls (n=30)
Age in years (mean±SD)	34.80±12.36	34.87±11.96
Gender (%)		
Male	20 (66.7)	21 (70)
Female	10 (33.3)	9 (30)

Table 2: Influence of MMP-1 polymorphism in oligodendrogliomas

Gene polymorphism	Subjects (%) (n=30) (n=30)	Controls (%) (n=30) (n=84)	P value	OR # (95% CI)
MMP-1 (−1607) genotype				
GG	7 (23.33)	16 (53.33)	-	Ref
AG	14 (46.67)	10 (33.33)	0.045	3.58 (1.02-12.50)
GG	09 (30)	04 (13.33)	0.021	6.89 (1.33-35.62)
Allele				
A	28 (46.67)	42 (30)	-	Ref
G	32 (53.33)	18 (30)	0.010	2.66 (1.26-5.64)

MMP – Matrix metalloproteinase

Table 3: Influence of MMP-2 polymorphism in oligodendrogliomas

Gene polymorphism	Subjects (n=30) (%)	Controls (n=30) (%)	P value	OR # (95% CI)
MMP-2 (−1306 C>T) genotype				
TT	22 (73.33)	23 (76.67)	-	Ref
CT	06 (20)	06 (20)	0.953	1.03 (0.28-3.748)
CC	02 (6.67)	01 (3.33)	0.544	2.197 (0.17-28.01)
Allele				
T	50 (83.33)	52 (86.67)	-	Ref
C	10 (16.67)	08 (13.33)	0.610	1.30 (0.47-3.56)

MMP – Matrix metalloproteinase

Table 4: Influence of MMP-7 polymorphism in oligodendrogliomas

Gene polymorphism	Subjects (n=30) (%)	Controls (n=30) (%)(n=84)	P value	OR # (95% CI)
MMP-7 (181) genotype				
A	8 (26.67)	16 (53.33)	-	Ref
AG	12 (40)	10 (33.33)	0.153	2.45 (0.71-8.43)
GG	10 (33.33)	04 (13.33)	0.024	5.65 (1.26-25.36)
Allele				
A	28 (46.67)	42 (30)	-	Ref
G	32 (53.33)	18 (30)	0.017	2.49 (1.17-5.27)

MMP – Matrix metalloproteinase

$P = 0.02$; 95% CI = 1.26-25.36) and (OR = 2.49; $P = 0.01$; 95% CI = 1.17-5.27)] [Table 4].

Discussion

The importance of MMP-1 polymorphism in glioblastoma multiforme and most likely in astrocytomas as such was incompletely understood till the year 2005.[16] Until recently, it was thought that MMPs are associated with many types of cancers,[6-8] but recent studies have proven the association of MMP-1 in gliomas.[9-11] MMPs are responsible primarily for the deprivation of extracellular matrix components, but it has become clear that the MMP family has a wide range of other influences also on biological processes, including the generation of bioactive proteins.[3,17,18] MMP-1 is the most universally expressed and known as collagenase-1.[19-22] It is a member of the MMP family of zinc-dependent endopeptidases, which has the capability to cleave substrates in the extracellular matrix. Substrates for MMP-1 comprise collagen types I, II, III, VII, and X, gelatin, entactin, aggrecan, and tenascin. Sometime back it was thought that MMPs were responsible primarily for the degradation of extracellular matrix components. However, it has become clear that the MMP family has an extensive range of other influences on biological processes, as well as the generation of bioactive proteins.[3,17,23] The level of MMP-1 expression can be influenced by different SNPs in the promoter region. MMP-1 expression and its potential to mediate connective tissue degradation and tumor succession can be influenced by the genetic variation in the MMP-1 promoter. A functional SNP is known to occur at −1607 base pair (bp) position in the MMP-1 promoter. It consists of either the presence or absence of a guanine nucleotide adjacent to a pre-existing guanine nucleotide at −1606 bp position. These two allelic phenotypes are referred to as 2 G or 1 G allele, respectively.[24] Recently, MMP-1 has been described in a broad variety of highly developed cancers,[23,25-33] and in nearly all instances, there was a significant negative association between expression of MMP-1 and survival.

MMP-1 is less studied in human brain tumors, in comparison to other MMPs such as MMP-2 and MMP-9.[34-39] The synthesis of many MMPs is considered to be synchronized by growth factors, cytokines, and hormones. MMP-2 and MMP-9 have been well studied in gliomas particularly because these enzymes are easy to be determined by gelatine zymography. Some reports show upregulation of MMP-2 and MMP-9 in glioma specimens *in vitro* and *in vivo*.[40-44]

A significant difference in the MMP-1 promoter genotype in human glioblastomas was noted for the first time by McCready *et al.* in 2005.[16] The experiments described in a study showed the distribution of the MMP-1 (−1607 A > G) promoter polymorphism in the tissue samples with glioblastoma multiforme when compared this with normal healthy population. Our previous study has also proven the role of

MMP-1 gene polymorphism in malignant gliomas in adult population. Considering the role of MMP-1 polymorphism in glioblastomas and malignant gliomas, we thought of investigating the cases of oligodendrogliomas also.[9]

MMP-1 protein levels were significantly higher in glioblastoma multiforme (GBMs) when compared to normal brain,[45,46] in a study conducted by Nakano and Nakagawa *et al.*, where comparison of total MMP-1 levels in a subset of GBM samples of varying genotypes showed that the tumors with 1 G/2 G genotype expressed the highest levels. A study by Lu *et al.* focused on genotyping for MMP-1 (−1607 A > G) where SNPs were performed in 236 adult astrocytoma cases and 366 healthy controls. The results established that the general distribution of MMP-1 allelotype and genotype among astrocytoma cases and healthy controls was significantly altered ($P = 0.002$).[47] In a previous study by Malik *et al.*, they showed that MMP-1 (−1607 1 G/2 G) gene polymorphism was significantly associated with malignant GBM cases compared to healthy population ($P = 0.016$).[9] Other authors have shown that the frequency of 2 G allele was appreciably higher in gliomas as compared to healthy population (62.9% vs. 47.3%; $P = 0.002$). MMP-1 gene is located on chromosome 11q22 and expressed in a wide variety of normal cells such as stromal fibroblast cells, macrophages, endothelial and epithelial cells, and in various tumor cells.[22] In the present study on oligidendrogliomas, we found a significant association of MMP-1 (−1607 A > G) polymorphism in the cases of oligodendroglioma in northern Indian population. Hence, it signifies that MMPs have associations with probably all glial tumors.

MMP-2 also has prominent activity toward several other bioactive molecules such as growth factor-binding proteins and growth factor receptors, which are well recognized to have a tough consequence on stimulating cell proliferation and inhibiting apoptosis. These activities of MMP-2 are supposed to be linked to both cancer spread and progression.[3] However, like many MMPs, MMP-2 is not upregulated by gene amplification or activating mutations, and genetic alterations in the gene of the cancer cells are generally deficient. Therefore, germ-line polymorphisms vary constitutively on induced expression. The enzyme activity of MMP-2 may affect individual susceptibility to certain cancers.[48] The 1306 MMP-2 gene polymorphism has been revealed to be associated with various cancers such as esophageal, lung, breast, prostrate, bladder, and gastric cardia adenocarcinoma, as well as with brain cancer.[42,49-55] However, our previous study on malignant gliomas of adult population did not reveal the association of MMP-2 with these tumors ($P = 0.475$). The study showed that CC is the most frequent genotype of 1306 MMP-2 gene polymorphism in both GBM as well as in normal subjects (76.4% and 72.7%, respectively).[15] These data on genotype may be useful for supplementary studies in Indian population. This dissimilarity

in the study is most likely to be due to small sample size and also can be endorsed to racial and ethnic variations in different populations. Our present study also did not find the association of MMP-2 (−1306 C/T) gene polymorphism with oligodendrogliomas.

MMP-7, known as matrilysin or PUMP-1, is a protease with the lowest molecular weight and has broad substrate specificity. It degrades elastin, proteoglycan, fibronectin, and type IV collagen.[56-58] MMP-7 has the propensity to localize in epithelial cells. It has also been shown that MMP-7 is immunolocalized predominantly in the cytoplasm of cervical carcinoma cells, monocytes, and normal mucosa.[58] There is a positive correlation between MMP-7 and invasive potential predicted in several types of cancer.[59-62] study shows the overall genotypic distribution of MMP7 (181 A > G) SNP in astrocytomas in relationship to healthy controls ($P = 0.001$). When compared with the A/A genotype, both the G/G and the A/G genotypes significantly increased the susceptibility to astrocytoma.[63] A study by Wang *et al.* presented the association between A to G transition at the 181-bp position in the promoter of MMP-7 gene and susceptibility to adult astrocytoma.[63] The MMP-7 (−181 A/G) polymorphism was genotyped among 221 adult astrocytoma patients and 366 healthy controls in inhabitants of northern China. The results showed that the genotypes of MMP-7 (181 A > G) were significantly associated among astrocytoma patients in relation to healthy controls ($P < 0.001$).[64] MMP7 181 A>G polymorphism may influence the susceptibility to astrocytoma. Our present study also suggests that MMP-7 has a role ($P = 0.02$) in influencing the malignancy of oligodendroglioma, as documented in other glial tumors. The presence of G allele for the MMP-7 (−181 A > G) gene promoter sequence may be a facilitating factor for cancer cell escalation in oligodendroglioma cases.

It is to be understood that the normal brain may be immunologically quiescent, but immune activity in brain is under regulatory control, just as in other organs. The endogenous regulatory molecules play a major role in local and site-specific immune regulation in brain. Site specific proinflammatory cytokines such as tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), and other molecules that can increase the expression of T cells arresting adhesion of molecules on endothelial cells are secreted in the course of ongoing inflammatory or immune response (secondary to brain tumors). The B lymphocytes secrete immunoglobulin class antibodies that can be carried out in serum, cerebrospinal fluid, and other fluids, hence immune effector function in brain is subserved by both T and B lymphocytes under regulatory control.[65] Angiogenesis is one of the important factors in brain tumor development. In fact, it is a process by which tumor cells and endothelial cells communicate to give rise to new blood vessels. The language of this communication is growth factor receptors' interaction and signal transduction. Tumor cells produce a number of factors including vascular endothelial growth factors (VEGF), powerful angiogenic growth factors, interleukin-8 (IL-8), basic epidermal growth factor (bEGF), platelet-derived growth factor (PDGF), etc., Interplay of interwoven pathways controlling signal transduction, cell cycle control, apoptosis, angiogenesis, invasion, etc., plays a role in development of glioblastoma (the most malignant brain tumor).[66] MMPs can also be one of the factors.

A localized immune response to the inflammatory oncogenic process results in secretion of various cytokines and different types of growth factors, regulated spontaneously by immune mechanism. The secretions of cytokines, IL-3, and TNFα are also upregulated. As MMP gene polymorphism has been detected in tissues of brain tumors in studies,[67] it seems that due to broken blood barriers in brain tumors, some amounts of these factors are bound to escape into systemic circulation. These factors most probably affect the bone marrow also, Obviously through systemic circulation. Probably upregulation of MMP genes occurs in hematopoietic cell by systemic circulation route[22,68,69] from where these can be detected, as was done in our present and previous studies.

The expression of RNA and role of MMP gene polymorphism have been established in brain tumor tissues in previous studies.[10,11,22,70] Though studies are available in literature to show the association of MMPs with oligodendroglioma, these are very few to show the association of MMP genes through RNA expression. There are no studies available in literature through DNA Isolation from blood samples related to gene polymorphism. Ours is probably the first study on MMP gene polymorphism in oligidendrogliomas.

Conclusions

MMP-1 (-1607 1 G/2 G) and MMP-7 (−181 A > G) polymorphisms are significantly associated with oligodendroglioma (grade II and III), but there is no association of MMP-2 (−1306 C > T) polymorphism with increased oligodendroglioma susceptibility.

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