EVALUATION OF MICROSATELLITE INSTABILITY IN TUMORS OF CENTRAL NERVOUS SYSTEM: A PILOT STUDY

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ABSTRACT

Microsatellite instability (MSI) characterized by alterations at simple repetitive genomic sequences is a distinct mechanism in tumorigenesis. Central nervous system (CNS) tumors have been reported to exhibit MSI, indicator of defective mismatch repair system with controversies. The present study was undertaken to examine sixteen primary brain and two spinal tumors for MSI at six mono: BAT - 26, BAT - 40, BAX, TGF β RII, IGFIR and hMSH3 and four dinucleotide loci: D2S123, D9S1851, D9S283 and D18S58. Polymerase chain reaction (PCR) was done to amplify tumour and blood DNA, analyzed on 8% denaturing Polyacrylamide gel followed by autoradiography. Out of 18 CNS tumors examined, 39% exhibited MSI at BAT - 26, BAT - 40, D9S1851, D9S283 and D18S58 in tumoral DNA. However, no alteration was observed at BAX, TGF β RII, IGFIR, hMSH3 and D2S123 loci. Low incidence of MSI - high hypothesizes role of MSI in evolution of CNS tumors but not in cancer initiation or progression.

KEYWORDS

Brain and spinal tumors, Tumor development, Mono- and dinucleotide microsatellite markers, Mismatch repair.

INTRODUCTION

Genetic instability is manifested as an altered DNA repair property which is one of the major driving forces in the generation of cells with increased invasive and metastatic potential indicated by alteration at microsatellite sequences.

Microsatellites are the short repetitive nucleotide sequences dispersed through out the genome. The tandem nature of elemental units within simple repeats undergoes slipped strand mispairing and therefore, results in insertion and deletion mutagenesis (1). Microsatellite instability (MSI) is defined as a change in the sequences at repetitive

motifs, which can be either contraction or expansion. This altered pattern can be observed as a shift in electrophoretic mobility of microsatellite alleles (2). Since MSI is due to defect in replication or mismatch repair system, it is also called as replication error positive phenotype (RER +ve). MSI can be at one locus or at multiple loci according to which it has been classified as MSI - L (low) if it is at <30% of the loci studied and MSI - H (high) if it is at $\geq$ 30% of the loci examined (2, 3).

Microsatellite instability was first reported in 80 - 85% in Hereditary non polyposis colorectal cancer (HNPCC) associated with inactivating mutations in human mismatch repair (MMR) genes - hMSH2, hMLH1, hPMS1, hPMS2 and hMSH6. This defect is also seen in 10 - 15% of sporadic colorectal cancers and in many other cancers like endometrial, skin, pancreatic, lung, stomach, renal, esophageal, ovary, cervix, bladder, breast, testis, prostate, liver and brain with lot of variations (4).

Central nervous system (CNS) tumors exhibit wide range of both benign and malignant tumors that are clinically distinct and their different behavior could be attributed to genetic differences (5, 6). Several reports for the presence of microsatellite

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instability in brain tumors have appeared but the results are conflicting. First report published in 1994 demonstrated very low level of MSI (1.8%) of 54 tumors examined (7). Subsequently, many other studies confirmed MSI in different types of brain tumors varying from as high as 12.5% to as low as 0.15% (7, 8). Recently a study from Hungary reports very high prevalence of microsatellite instability that is 54.5% and 45.5% in primary and recurrent gliomas but no alteration was found in hMLH1 and hMSH2 genes (9). The present study has been initiated to evaluate the pattern of microsatellite alterations with the panel of six mono- and four dinucleotide markers situated on different chromosomes in 16 brain and 2 spinal tumors to look for the presence of genetic changes caused by defects in mismatch repair genes.

MATERIAL AND METHODS

Patient selection

Patients were enrolled in the Neurosurgery Department of our institute. The diagnosis was based on clinical and laboratory investigations according to standard Proforma. Sixteen of total eighteen cases presenting with intracranial mass lesions, irrespective of histological nature of masses were operated on account of their neurological indications while remaining 2 cases were spinal tumors which were operated because of their progressive neurological deficit. All masses were biopsied and tumor substance was subjected for MSI studies using blood as control of the same individual. Histological reports were obtained simultaneously before discharging the patients. Histopathology of the tumors/ masses turned out

Table 1. Clinical and histopathological features of brain and spinal tumors

Case no.	Tumor location	Histology	Age (years)	Gender
BT 1	Cerebello - pontine angle	Hemangiopericytoma	7	Male
BT 2	Multiple intracranial tuberculomas Including medulla	Tuberculoma in medulla	12	Male
BT 3	Posterior cranial fossa	Recurrent epidermoid	42	Male
BT 4	Right posterior frontal lobe	Aspergillosis with leukemic infiltration	11	Female
BT 5	Right cerebellar	Hemangioblastoma	30	Male
BT 6	Right temporal lobe	Glioma	16	Female
BT 7	Left lateral 1/3 rd sphenoid wing	Meningioma	50	Male
BT 8	Left intra ventricular	Meningioma	34	Female
BT 9	Anterior corpus callosum	Glioblastoma	47	Male
BT 10	D9 - 10 intradural extra medullary	Schwannoma	40	Male
BT 11	Right parietal lobe	Glioblastoma	55	Male
BT 12	D6 spine	Plasmacytoma	50	Male
BT 13	Left occipital and mastoid lobe	Paraganglioma	25	Male
BT 14	Medulla	Hemangioblastoma	24	Male
BT 15	Left temporal lobe	Glioblastoma	60	Male
BT 16	Left petrous apex	Hemangiopericytoma	63	Male
BT 17	Frontotemporal	Glioma (Grade III)	60	Male
BT 18	Pituitary	Pituitary macroadenoma	35	Male

to be single case of multiple intracranial tuberculoma, epidermoid, aspergillosis with leukemic infiltration, pituitary macroadenoma, paraganglioma, 2 cases each of hemangiopericytoma, glioma, meningioma, 3 cases of glioblastoma and remaining two were spinal tumors that is plasmacytoma and schwannoma. All the cases were sporadic and had no familial history for CNS tumor. The age of patients ranged from 7 years to 63 years. The histopathological features of central nervous system tumors are described (see Table 1).

DNA extraction from blood and tissue specimen

Tumor tissue biopsy of histologically confirmed tumors and peripheral blood collected in EDTA which would serve as control of the same patient was taken. DNA was isolated from tissue as well as blood using phenol-chloroform extraction method (10).

MSI Analysis

Six mono - and four dinucleotide microsatellite markers were examined for MSI in CNS tumors (Table 2). The primer sequences for each microsatellite marker were searched from human genomic data base (10). Polymerase Chain Reaction (PCR) amplification of DNA was done with marker specific primers of concentration of 6 pmol, 200 μ M dNTPs, 10 mM Tris - Cl (pH 8.3), 50 mM

KCl, 1.5 mM MgCl₂, 0.25 units of Taq polymerase (MSI, Fermentas), 100 ng DNA and 2 μ Ci [α -³²P] dCTP (specific activity: 4000 Ci/mM) (BRIT, India) in a total volume of 20 - 26 μ l. PCR conditions involved an initial denaturation at 95°C for 3 min followed by 30 cycles (95°C for 1 min, 50°C to 60°C for 2 min and 72°C for 3 min) and a final extension at 72°C for 8 min. All the PCR products were mixed with equal volume of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% Xylene cyanol), denatured for 3 min at 95°C and loaded onto an 8% polyacrylamide gel containing 7 M urea. Gels were run at 55 W for 2 hours, transferred onto a Whatman sheet, dried under vacuum and autoradiography was done.

The tumor was called unstable if its PCR product had altered band pattern when compared to alleles in corresponding matched blood DNA (11).

RESULTS

Microsatellite Alterations

Ten microsatellite markers were examined by PCR in tumor tissue and blood separately of the corresponding matched individuals in 16 brain and 2 spinal tumors. Out of the total central nervous system tumors/ masses analyzed, 39% (7/18) of them were found to have genetic alterations. Two of the six mononucleotide and three of the four-

Table 2. Characteristic features of microsatellite markers examined in central nervous system tumors

Name of marker	Repeat pattern	Chromosomal location	PCR product size
BAT 26	(A) ₂₆	5 th intron of hMSH2, 2p	117-130 bp
BAT 40	(A) ₄₀	2 nd intron of β hydroxy steroid dehydrogenase	94 - 112 bp
BAX (38 - 41)	(G) ₈	19q13.3 - q13.4	94 bp
TGF β RII (665 - 737)	(A) ₁₀	3p22	73 bp
IGFIIR (4030 - 4140)	(G) ₈	6q26 - 27	110 bp
hMSH3 (381 - 383)	(A) ₈	5q	150 bp
D2S123	(CA) ₁₃ TA (CA) ₁₅ (T/GA) ₇	2p16	197-227 bp
D9S283	(CA) _n	9q13 - q22	178-203 bp
D9S1851	(CA) _n	9q22.3	143-159 bp
D18S58	(GC) ₅ GA(CA) ₁₇	18q22.3	144-160 bp

dinucleotide markers showed instability in one or more tumors (Table 3).

Twenty eight percent (5/18) of the cases showed either contraction or expansion of alleles at BAT - 26 locus whereas only expansion at BAT - 40 locus in tumor tissue as compared to blood of the corresponding matched patient. However, none of the other mononucleotide markers - BAX, TGF β RII, IGFIIR and hMSH3 exhibited altered pattern. Out of the total cases examined, only 17% (3/18) had microsatellite instability at dinucleotide microsatellite loci. D2S123 did not show any change in tumoral DNA when compared to reference DNA of the same patient. D18S58 situated on chromosome 18 and D9S283 on chromosome 9 showed loss of heterozygosity (LOH) in 11.1% (2/18) and 5.5% of the cases respectively. D9S1851 present on

chromosome 9 exhibited biallelic alteration (BA) in only one case. Changes were observed at BAT - 40, BAT - 26, D9S1851, D9S283 and D18S58 loci (Fig. 1) while no abnormally migrating bands at BAX, TGF β RII, IGFIIR, hMSH3 and D2S123 were observed in brain and spinal tumors/ masses as (Fig 2).

On classifying the gross microsatellite instability into MSI - L and MSI - H observed at five microsatellite loci (two mono and three dinucleotide repetitive motifs), 71.4% (5/7) and 28.6% (2/7) of the total unstable cases demonstrated MSI - L and MSI - H respectively.

On correlating total MSI observed in 16 brain and 2 spinal tumors/ masses with the age of patients, we found 33.3% (4/ 12) of the patients with ages ranging from 11 years to 40 years had microsatellite

Table 3. Genetic alterations at ten different microsatellite markers examined in central nervous system tumors

Case nos.	Alterations at microsatellite loci									
	Mononucleotide markers						Dinucleotide markers			
	BAT	-26 BAT	-40 TGFII β RII	GFIIR	BAX	hMSH3	D2S123	D9S1851	D9S283	D18S58
BT 1	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 2	↓	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 3	MSS	MSS	MSS	MSS	MSS	MSS	MSS	BA	MSS	LOH
BT 4	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	LOH	MSS
BT 5	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 6	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 7	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 8	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 9	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 10	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 11	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 12	↑	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 13	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 14	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 15	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 16	MSS	↑	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 17	MSS	↑	MSS	MSS	MSS	MSS	MSS	MSS	MSS	MSS
BT 18	MSS	↑	MSS	MSS	MSS	MSS	MSS	MSS	MSS	LDH
Microsatellite stable (MSS); Contraction of allele size (↓); Expansion of allele size (↑); Biallelic alteration (B A); Loss of Heterozygosity (LOH)										

alterations as compared to 50% (3/ 6) of the patients with ages above 40 years.

DISCUSSION

The pathogenesis of cancer in human central nervous system is very complex. It involves chromosomal alterations that occur as a result of genetic instability as an inherent property of cancer cells. Genotypic alterations like somatic mutations causing loss of function in p53, p16, PTEN tumor suppressor genes in gliomas (12), activation of proto - oncogenes N - MYC in neuroblastomas (13),

c - MYB in glioblastomas, EGFR in gliomas, c - MYC in medulloblastomas (14) and rare HRAS1 allele in glial neoplasms (15). Recently another type of genetic abnormality resulting from defect in mismatch repair genes has been reported in many tumors of brain. Various studies in different types of brain tumors came up in the last ten years but they showed conflicting results (16). The abnormal function of mismatch repair genes revealed by presence of widespread MSI acts as a novel mechanism in tumor development.

Fig 1. Altered allelic pattern observed in DNA of brain and spinal tumors (T) as compared to control DNA (N) indicated by an arrow at (A) BAT - 26 locus represents insertion; (B) BAT - 40 shows borderline increase in allele size; (C) D9S1851 undergoes biallelic alteration; (D) and (E) D9S283 and D18S58 respectively show loss of heterozygosity (LOH)

Fig 2. Stable pattern of alleles observed in control (N) and tumor tissue (T) at (A) BAX (lanes 1 - 6); (B) TGFβRII (lanes 1 - 6); (C) IGFIR (lanes 1 - 6); (D) hMSH3 (lanes 1 - 4) and (E) D2S123 (lanes 1 - 6) in tumors of central nervous system

We undertook the present study to find out the role of MSI in Indian patients with CNS tumors. We observed 39% of total MSI in 16 brain and 2 spinal tumors of varied histological types. Cases presenting with epidermoid, plasmacytoma, hemangiopericytoma, grade III glioma and pituitary macroadenoma showed 5.5% of MSI but no change was observed in glioblastoma. However, one of the previous studies showed low MSI in glioblastomas but not in astrocytomas with different panel of markers (17). One of our cases was of multiple tuberculomas of brain while another child with acute lymphoblastic leukemia who once developed leukemic infiltration of brain along with fungal granuloma formation also exhibited MSI. We did not observe any MSI in meningiomas, Schwannoma and paraganglioma which is not in concordance with an earlier study demonstrating 2.4% MSI in meningiomas, 3% in astrocytic tumor, 17.4% in oligodendro-gliomas and 3.2% in pituitary adenoma using different microsatellite markers (18).

In present study, contraction at BAT - 26 in multiple intracranial tuberculoma; expansion at BAT - 26 and BAT - 40 in plasmacytoma, hemangiopericytoma, grade III glioma and pituitary macroadenoma; biallelic alteration at D9S1851 in epidermoid and LOH at D9S283 and D18S58 in aspergilloma with leukemic infiltration, epidermoid and pituitary macroadenoma were observed. No change was noticed in glioblastomas in our study with present set of markers and it differs from the previous finding which demonstrated genetic defect as LOH in 10% of glioblastomas (19).

Out of the ten loci studied, the loci present on β hydroxy steroid dehydrogenase gene, hMSH2 (2p) and 9q13-q22 showed allelic shifts and imbalance, which may be indicative of defective, mismatch repair system. LOH at D9S283 and D18S58 may hypothesize the presence of putative tumor suppressor gene at chromosomes 9 and 18 near to these loci. Other four mononucleotide repetitive motifs TGF β RII, IGFIR, BAX and hMSH3 present in exonic regions did not exhibit any change in CNS tumors while an earlier study demonstrated the presence of MSI at these loci in gliomas (20). D2S123 situated in intronic region of 2p16 was found to be microsatellite stable (MSS) for all CNS tumors. Therefore, absence of instability rules out the possibility of any genetic defect at TGF β RII, IGFIR, BAX, hMSH3 and D2S123 loci.

Following the criterion of MSI classification into MSI - L and MSI - H we observed MSI - H in only 28.6% of unstable CNS tumors as compared to MSI - L which was found in 71.4% of unstable cases. Low incidence of MSI - H suggests that CNS tumors differ from HNPCC where MSI - H is

intimately associated with tumor initiation as well as progression. Therefore, it can be hypothesized that MSI only helps in the evolution of tumor in central nervous system but not in cancer initiation and progression that is later on overtaken by other genetic pathways.

We observed higher incidence of MSI (50%) in patients above 50 years of age as compared to tumors in younger age group but more studies would be required to substantiate this observation because our group is too small to make a conclusion.

In conclusion, our study shows that 39% of histologically varied CNS tumors harbor microsatellite alterations at low level but instability at multiple loci is infrequent. These results extend the observation that pathway for tumorigenesis in human central nervous system is different from HNPCC and thus low incidence of MSI - H hypothesizes that MSI in CNS tumors may result in tumor evolution but not in its initiation and progression. The presence of tumor suppressor gene at chromosomes 9 and 18 involving in tumorigenesis may be hypothesized. These studies open new avenues to focus MSI analysis in large number of central nervous system tumors with expanded panel of microsatellite markers for better understanding the mechanism of their pathogenesis.

ACKNOWLEDGEMENT

One of the authors Minal Vaish is thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for awarding research fellowship.

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