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Research Article

A Prospective Analysis on Association of Polymorphism of XRCC7 Gene and Risk of Glioma: A Case-Control Study

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Introduction:

The incidence of central nervous system (CNS) tumors in India ranges from 5 to 10 per 100,000 population , with an increasing trend and accounts for 2% of all malignancies.[1,2] The age-standardized rate of incidence of gliomas was 4.7/100,000 person-years. They represent a substantial burden in terms of morbidity and mortality.

In 2016 WHO updated its primary brain tumor classification schema to directly incorporate genetic information for the first time, in which IDH mutation has become definitional for infiltrating gliomas in adults, with 1p/19q codeletion further characterizing the type. [3] Astrocytomas (38.7%) were the most common primary tumors, with the majority being high-grade gliomas i.e., glioblastomas (59.5%).[4] More interestingly, the median age of gliomas was seen to be at least a decade earlier in indian population than reported in the Western population, which can be explained by a larger proportion of younger population and lower life expectancy.[5] The etiology of GBM is still unclear. However, various environmental factors and genetic factors have been found to be associated. It is known that exposure to ionizing radiation can increase the risk of glioblastoma development.[6]

Human beings are exposed to a large number of exogenous and endogenous agents that damage genes. These agent directly or indirectly affects DNA structure by inducing DNA breaks, particularly, double-stranded DNA

breaks (DSBs). Secondary effects are the generation of ROS that oxidize proteins and lipids, and also induce several damages to DNA, like the generation of basic sites and single-strand breaks (SSB). Collectively, all these changes induce apoptosis and mitotic failure.[7]

Such DNA damage is usually repaired by DNA repair-pathway genes via homologous recombination (HR) and non-homologous end joining (NH+EJ) pathways that restore genomic integrity.[8,9] If this mechanism of repair fails, it leads to development of carcinomas.

The XRCC7 is a NHEJ repair gene, which encodes the catalytic subunit of DNA-activated protein kinase (DNA-PKcs).[10] DNA-PKcs belongs to the phosphatidylinositol 3-kinase-related kinase protein family. The DNA-Pkcs protein is a serine/threonine protein kinase comprising a single polypeptide chain of 4,128 amino acids. DNA-PKcs is recruited to the site of DSBs by the Ku70/Ku80 heterodimer to form an active DNA-PK complex that is essential for the progression of the pathway.[11] Defects in the XRCC7 gene make the DNA-activated protein kinase activity undetectable and these cells sensitive to ionizing radiation.[12]

So we hypothesized that DNA repair gene polymorphism can be associated with glioma risk. Few studies have investigated the role of DNA repair gene polymorphism in glioma risk. The current study was conducted to confirm the association between the gene XRCC7 polymorphism and glioma in the Indian population.

Material and methods:

Study population:

A Prospective observational study done in the Department of Neurosurgery, in collaboration with the Department of Biochemistry, ESIC Medical College, faridabad And Research, New Delhi, included 30 newly diagnosed and histologically confirmed glioma patients as cases and 30 patients unrelated to cases and having non-cancerous pathologies as controls. Each eligible participant was interviewed to obtain data regarding age, sex, and ethnicity. After giving informed consent, each participant donated 2 ml of blood collected in EDTA tubes. The research protocol was approved by the ethical committee.

Genotyping

We used the commercially available Qiagen kit (Qiagen Inc., Valencia, CA) to extract DNA from peripheral blood leukocytes. The purified DNA was used to determine the genotypes for the

XRCC7 G6721T polymorphism, the primers used were obtained from the study published by Wang et al.[13] The sense primer was 5'-CGGCTGCCAACGTTCTTTCC-3' (nucleotides 6626– 6645), and the antisense primer was 5'-TGCCCTTAGTGGTTCCCTGG-3' (complementary to nucleotides 6974–6993; GenBank accession no. L27425; Ref. 10). The primers amplified 368-bp fragment containing the G/T variant in intron 8, which was then subjected to fast restriction digestion with PvuII (New England BioLabs, Inc., Beverly, MA) at 37°C for 15 min. The homologous GG allele had only one band of 368 bp; the heterozygous allele (GT) had three bands of 368, 274, and 94 bp; and the homozygous TT allele had two bands of 274 and 94 bp because of the gain of the restriction site. The 20µl PCR mixture contained 50 ng of genomic DNA, 5.0 pmol of each primer, 0.1 mM each deoxynucleoside triphosphate, 1 x PCR buffer [50 mM KCl, 10 mM Tris HCl (pH 9.0 at 25°C), and 0.1% Triton X-100], 1.5 mM MgCl2, and 1.0 units of Taq polymerase (Sigma- Aldrich Biotechnology, Saint Louis, MO). The PCRs were performed with a PTC-200 DNA Engine (MJ Research, Inc., Watertown, MA). The PCR profile consisted of an initial melting step of 95°C for 5 min; 30 cycles of 95°C for 30 s, 61°C for 30 s, and 72°C for 45 s; and a final elongation step of 72°C for 10 min, with a minor modification for the XRCC7 gene.

Statistical Analysis.

The data was entered in a Microsoft Excel spreadsheet, and analysis was done using Epi-Info, JASP, and Statistical Package for Social Sciences (SPSS) version 23.0.

Continuous variables are represented as mean ± SD or medians with interquartile range. Categorical variables are represented as numbers and percentages (%).

The quantitative variables were tested for normality with the Shapiro-Wilk test for normality, Q-Q plots,+ and visual inspection of the histograms.

All tests of significance were two-tailed, and statistical significance was defined as $P < 0.05$.

Results

In our study, there was a male preponderance in the cases in the ratio of 1.5: 1 and a female preponderance in the control group in the ratio of 1.5:1. However, both the groups were well matched with respect to gender distribution (p value 0.196).

The mean age of cases and controls was, respectively, 42.43 ± 15.08 and 40.67 ± 15.71 years. The vast majority of our cases (n = 24, 80%) as well as

controls (n=21, 70%) belonged to the age group 20 to 60 years. Both groups were well matched with respect to the mean age (p value 0.658) as well as the distribution (p value 0.614) (Table 1). Our study included a dominant number (n=16, 53.33%) of GBM patients (Grade 4).

The control group consisted of 12 (40%) patients of GG Genotype and 9 (30%) subjects each of GT and TT genotypes. Thus, 60% (n=18) of the control subjects had at least 1 T allele. On the other hand, there were only 2 (6.67%) patients of the GG Genotype in the Cases. The remaining 28 (93.33%) cases had at least 1 T allele (13 of GT and 15 of TT genotype). Thus, the prevalence of the GT or TT genotypes was significantly (p value 0.009) higher in the Cases (93.33%) as compared to the Controls (40%). (Table 2)

There was a significant difference (p value 0.003) in the distribution of the alleles between the 2 groups with a significantly higher prevalence of T allele in the cases as compared to the control group. (table 3). On comparing the cases and controls in different age groups and of different gender, we found that cases in the age group 40-60 years and male gender had a significantly higher proportions (p value <0.001) of GT and TT genotypes versus the GG genotype. (table 4).

In our study, we did not find any difference with respect to the histological subgroups. (Table 5)

Discussion:

In our study, we selected nonhomologous end joining (XRCC7) repair pathways to investigate the associations of their common polymorphisms with risk of glioma. We conducted a age and sex matched case control study to assess the correlation betwwen XRCC7 gene polymorphism and risk of glioma. GT and TT genotype of XRCC7 are more common among cases as compared to controls. The T allele frequency is higher in cases as compared to controls. The increased risk of glioma associated with the XRCC7 variant genotypes is more pronounced in older (46–60 years) and male cases. In our study, there is no correlation with histological subtypes which may be due to small sample size. Our study is one of the first studies to compare the XRCC7 polymorphisms in Glioma patients in the Indian setting and our results are resonant with those of international studies such as Wang et al[13] done previously. This data could thus help in planning further studies on these lines. Limitations of our study was controls were patients admitted to

the hospital with another indication and were not healthy community-based controls. This could play a role if the XRCC7 polymorphism also plays a role in non-neoplastic conditions such as AV malformations and CVA.

Table 1: Table comparing the Gender distribution and Age (in years) of cases and controls

	CASE	CONTROL	TOTAL	P Value	Test Performed
GENDER					
Female	12 (20%)	18 (30%)	30 (50%)	0.196	Chi square test
Male	18 (30%)	12 (20%)	30 (50%)		
TOTAL	30 (50%)	30 (50%)	60 (100%)		
AGE (YEARS)					
Number	30	30	60	0.658	Student's T Test
Mean ± SD	42.43 ± 15.08	40.67 ± 15.71	41.55 ± 15.29		
Median	41.5	40.5	40.5		
IQR	22.5	25.5	23.25		
AGE GROUP				0.614	Chi square Test

<20	1 (1.67%)	3 (5%)	4 (6.67%)		
20-40	14 (23.33%)	10 (16.67%)	24 (40%)		
40-60	10 (16.67%)	11 (18.33%)	21 (35%)		
>60	5 (8.33%)	6 (10%)	11 (18.33%)		
TOTAL	30 (50%)	30 (50%)	60 (100%)		

Table 2:- Table showing distribution of XRCC7 genotypes in cases and controls

XRCC7 (G6721T) GENOTYPES	CASES	CONTROLS	TOTAL	P Value	Test Performed
GG	2	12	14 (23.33%)	0.009	Chi Square Test
GT	13	9	22 (36.67%)		
TT	15	9	24 (40%)		
TOTAL	30 (50%)	30 (50%)	60 (100%)		

Table 3 :- Table showing the allele frequency in cases and controls

	CASES	CONTROLS		

ALLELE	COUNT	PREVALENCE	COUNT	PREVALENCE	P VALUE	TEST APPLIED
T ALLELE	43	0.71	27	0.45	0.003	Chi Square
G ALLELE	17	0.28	33	0.55		

Table 4:- Table showing subgroup analysis of XRCC7 genotypes in cases and controls versus age groups and gender

XRCC7 (G6721T) GENOTYPES	CASES			CONTROL			P VALUE	TEST PERFORMED
	GG	GT	TT	GG	GT	TT		
GENDER								
Males	0	7	11	8	2	2	<0.001	Fisher's Exact Test
Females	2	6	4	4	7	7	0.828	Fisher's Exact Test
AGE GROUP								
Age <40 Years	1	5	9	6	3	4	0.063	Fisher's Exact Test
Age 40-60 Years	0	6	4	6	3	2	0.029	Fisher's Exact Test
Age >60 Years	1	2	2	0	3	3	0.98	Fisher's Exact Test

Table 5:- Table showing subgroup analysis of XRCC7 genotypes in cases versus histological grade and subtype

XRCC7 (G6721T) GENOTYPES	CASES			P Value	Test Performed
	GG	GT	TT		
HISTOLOGICAL GRADE					
GRADE 2	1	2	2	0.63	Fisher's Exact Test
GRADE 3	0	3	6		
GRADE 4	1	8	7		
HISTOLOGICAL SUBTYPE					
Astrocytoma	0	1	4	1	Fisher's Exact Test
Glioblastoma	1	8	7		
Oligodendroglioma	1	4	4		

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