Original Article

Evaluation of rs1957106 Polymorphism of NF-κBI in Glioblastoma Multiforme in Isfahan, Iran

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Abstract

Background: The kB family of nuclear factor (NF-κB) is a series of transcription factors that plays a key role in regulation of immunity, cell growth, and apoptosis and is considered as the main downstream component of epidermal growth factor receptor for which there are evidence of excessive activity in most cases of glioblastoma multiform (GBM). Thus, the current information has gained evidence on NF-κBIA tumor suppressor role in GMB. *SNP rs1957106* was diagnosed as a new polymorphism which affected the expression of NF-κBI and causes activation of NF-κB in GBM patients. **Materials and Methods:** This study was conducted on 100 cases of GBM including 47 paraffin-embedded brain tissue samples and 53 blood samples from another 53 GBM patients and 150 controls. The NF-κBI *rs1957106 SNP* was identified by the NCBI, and genotyping was performed by high-resolution melt (HRM) assay. Melt curves from HRM which suspected to single-nucleotide polymorphism (SNP) were selected and subjected to direct sequencing. **Results:** The distribution of allele A of NF-κβ gene in patients with GBM with 31% was not significantly different from healthy participants (27.3%) ($P = 0.375$). Furthermore, the distribution of AG and GG genotypes in comparison with AA genotypes did not show a significant correlation with GBM incidence ($P > 0.05$). **Conclusion:** Findings of the present study provide evidence that the *rs1957106 SNP* in NF-κBIA is found more in GBM patients, but it was not statistically significant. As there are conflicting studies showing significant higher rate of this SNP in GBM, further study is suggested.

Keywords: *Glioblastoma,* NF-κBI, *polymorphism*

Introduction

Glioblastoma (GBM) is the most common and invasive form of primary malignant brain tumors with a survival rate of about 1 year.[1-3] Low survival rates have led to provide a treatment method in order to tumor removal or increase survival in GBM, and unlike the current treatment regimens, GBM survival of 5 years has still been reported to be <10%.[4-6]

This cancer has a poor prognostic as well as its molecular mechanisms are less known.[7,8]

Many studies have shown that a number of signaling pathways would be unregulated during the multistage GMB carcinogenesis,[9-14] and there are evidence of excessive activity of epidermal growth factor receptor (EGFR) in the most cases of GMB.[15] The κB family of nuclear factor (NF-κB) is a series of transcription factors that plays a key role in regulation of immunity, inflammatory responses, cell growth, and apoptosis and is considered as the main downstream component of EGFR[14,16] which consists of a group of homodimer and heterodimer protein complexes;[16,17] among them, the heterodimer complex of p50/p65/p53 is the most common complex in many types of cells.[18,19]

In nonstimulated cells, the inactive NF-κB complex is present in the cytoplasm and has been bind to a variety of NF-κB inhibitor proteins called NF-κBI, including NF-κBIA, NF-κBIB, IKBγ, IKBε, bc1-3, p100, and p150.[20] In many cases, NF-κB activation is performed with NF-κBIA signal induced destruction, and as a result, the transcription factor is released and transferred to the nucleus.[21-23] The family of NF-κBI proteins is characterized by the presence of multiple ankyrin repeats and their ability to physical connection to NF-κB proteins. Among this family,

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NF-κBIA has three sections: the N-terminal section, which has phosphorylation sites and is involved in degradation regulation, the ankyrin repeat section, and the C-terminal PEST section that controls the basic degradation.[24-28]

Mutations, polymorphisms, and NF-κBIA haplotype have been reported in Hodgkin's lymphoma, colorectal cancer, melanoma, hepatocellular carcinoma, breast cancer, and multiple melanomas,[25-27,29-38] as well as an inappropriate structural activation of NF-κB has been reported in GMB.[39-41,28] In addition, the current information has gained evidence on NF-κBIA tumor suppressor role in GMB.[42]

In addition, GMB cells that did not respond to chemotherapy were found to have lower mRNA expression, and increased expression of NF-κBIA inhibited tumor malignancy.[42] These results suggest that this single-nucleotide polymorphism (SNP) can be used to predict the survival rate of the affected patients. From the limited impact of EGFR target treatment in GMB, it can be understood that the effect of EGFR inhibitor treatment may be reduced by interfering transmission pathways, mutation, or augmentation of growth factor receptors such as PDGR, PDGFRA, ERBB2, or MET.[14] Because NF-κBIA is the main downstream factor in these transmission pathways, NF-κBIA-stabilizing treatments can be much more effective in controlling oncogenic pathways.[42]

Given the evidence presented in our report on *SNP rs1957106*, this study was aimed to find a way to better prognosis and treatment for patients with malignant tumor GBM by examining this polymorphism in cancerous specimens that still have unspecified aspects.

Materials and Methods

This case–control study was approved by the Research and Ethics Committee of Isfahan University of Medical Sciences. After providing sufficient information, a written consent was obtained from all participants or their legal guardians before involvement in the project. This study was conducted on 100 cases of GBM including 47 paraffin-embedded brain tissue samples that were taken from the Department of Pathology of Alzahra University Hospital and 53 blood samples from another 53 GBM patients, who were under therapy for this disease from Milad Hospital, and 150 controls from population of Isfahan, Iran, from 2013 to 2015.

DNA was extracted from brain tissue samples using PFET-DNA extraction kit (Yektatajhiz Inc., Tehran, Iran) and from blood samples using blood-DNA extraction kit (Yektatajhiz Inc., Tehran, Iran) according to the manufacturer's protocol.

The *NF-κBI rs1957106 SNP* was identified by the NCBI, and ensemble databases and primers were designed by Beacon Designer 8.1 ((Premier Biosoft International, USA) and synthesized by Bioneer (Bioneer, Korea). The forward primer was 5′-sequence-3′ and reverse primer was 5′-sequence-3′. Genotyping was performed by high-resolution melt (HRM) assay using a Rotor-Gene 6000 instrument (Corbett Life Science, Australia) [Figures 1 and 2].

Polymerase chain reaction (PCR) reactions were carried out in triplicate in 10 μL of final volume using the type-it HRM kit (Qiagen, Germany) according to the manufacturer's protocol. The PCR program consisted of an initial denaturation-activation step at 95°C for 10 min, followed by a 40-cycle program (denaturation at 95°C for 15 s, annealing conditions 60°C for 20 s, 72°C for 20 s; an HRM step from 75°C to 95°C rising at 0.1°C/s). Curves for each triplicate were checked on the shape, melting pattern, and Tm to meet reproducibility.

Melt curves from HRM which suspected to SNP were selected and subjected to direct sequencing.

The Hardy–Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies with the expected frequencies among samples, so that the genotype distribution of *NF-κβ rs1957106* was compatible with the HWE in our patients.

Finally, the collected data were entered into the SPSS software (version 20; SPSS Inc., Chicago, Ill., USA), and mean ± standard deviation or *n* (%) was used to show the data. Moreover, Fisher's exact test and independent *t*-test were applied for statistical analysis of frequency distribution of gender and mean age. To show the relationship between the genotype, *NF-κβI rs1957106* allele, and GBM disease, logistic regression was used and odds ratio was reported. Furthermore, to increase the precision of the study, variables such as age and gender were taken under control as the confounding variables. In all the analyses, $P < 0.05$ was considered statistically significant.

Results

In the present study, in the control group, 150 healthy individuals consisting of 80 (53.3%) males and 70 (46.7%) females were enrolled. The mean age of the control group was 49.67 ± 15.12 years. Meanwhile, in the case group, 100 patients with GBM including 55 (55%) males and

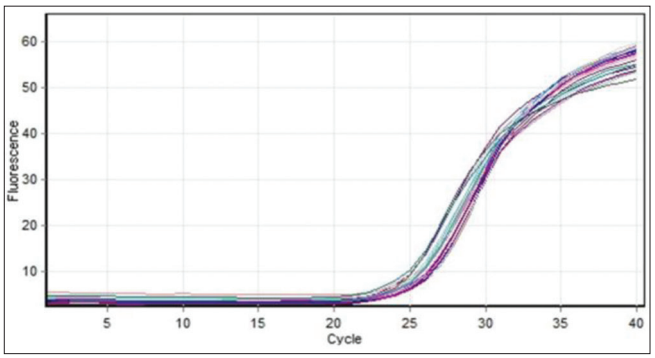

Figure 1: Amplification plots

45 (45%) females were assigned. The mean age in this group was 51.63 ± 13.27 years. Accordingly, the two groups were matched in terms of age and gender ($P > 0.05$) [Table 1].

On the other hand, the distribution of allele A of NF-κβ gene in patients with GBM with 31% was not significantly different from healthy participants (27.3%) ($P = 0.375$).

Furthermore, the distribution of AG and GG genotypes in comparison with AA genotypes did not show a significant correlation with GBM incidence ($P > 0.05$). In addition, by controlling the age and sex, there was still no association between the allele and genotype of *NF-κβ rs1957106* with GBM [Table 2].

Discussion

Genetic polymorphisms in NF-κBI gene are noticed due to their role in Hodgkin's lymphoma, colorectal cancer, melanoma, hepatocellular carcinoma, breast cancer, and multiple myelomas.[25-27,29-38] In this study, we analyzed the alterations of this gene in GBM. We analyzed the DNA sequence of the NF-κBIA gene from blood samples taken from 100 GBM patients and 100 noncancerous controls who were adjusted for sex and age.

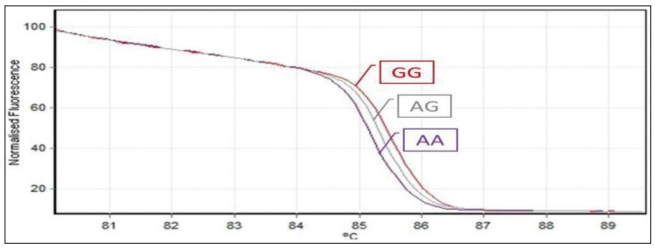

Figure 2: Melt curves and genotypes

Table 1: Demographic characteristics of patients in two groups

Characteristics	Controls (*n*=150), *n* (%)	Cases (*n*=100), *n* (%)	*P*
Sex (%)			
Male	80 (53.3)	55 (55.0)	0.897
Female	70 (46.7)	45 (45.0)	
Age (year)	49.67±15.12	51.63±13.27	0.281

It is to be noted that the prevalence of GBM was higher in men and its mean age was 51.63 ± 13.27 years. Thus, it can be concluded by adding other epidemiologic results that generally the GBM is more prevalent in men and it mostly occurs in elderlies rather than the youth.

On the other hand, the analysis of association of allele and genotype of NF-κBI gene with the occurrence of GBM showed that despite the frequency of A allele or AG and AA genotype was higher in GBM patients, this relation was not statistically significant. Furthermore, the presence of A allele was more frequent than G allele in these patients, and also, AG and AA genotypes had higher frequency rates that the difference was not significant. These results remained unchanged by adding sex and age as confounding variables, so we may conclude that sex or age did not have a role in the presence of this allele of genotype in these patients.

Accordingly, in the study of Zhao *et al*., they studied on the association between different genotypes of the *NF-κBIA rs1957106 SNP* and the gene copy number, mRNA level, and protein expression of NF-κBIA. The *SNP rs1957106* AG and AA genotypes were associated with lower NF-κBIA protein levels and a poor prognosis of GBM patients. They revealed that the SNP rs1957106 AG and AA genotypes in GBM were associated with a comparatively shorter survival rate.[28] Thus, their results suggest a role for NF-κBIA in the suppression of GBM tumors. Their results were in agreement with Bredel *et al*.,[42] who showed that a higher NF-κBIA expression was associated with a longer survival by studying bigger sample size of patients.

The study of Zhao *et al.* on 24 GBM patients and 8 controls showed SNP rs1957106 as a newly diagnosed polymorphism of NFKBI gene. Thus the conflict of our results may be due to fewer samples studied in the research of Zhao *et al*. comparing to 100 samples of GBM and 150 noncancerous patients in our study.

Table 2: Genotype and allele frequencies of nuclear factor-Kβ rs1957106 in two groups

Genotype/allele	Control (*n*=150), *n* (%)	Case (*n*=100), *n* (%)	OR (95% CI)	*P*
Genotype				
GG	81 (54.0)	48 (48.0)	Reference	
AG	56 (37.3)	42 (42.0)	1.266 (0.740-2.164)	0.389
			1.268 (0.741-2.171)[a]	0.387[a]
AA	13 (8.7)	10 (10.0)	1.298 (0.529-3.188)	0.569
			1.234 (0.498-3.057)[a]	0.649[a]
AG + GG	48 (48.0)	52 (52.0)	1.272 (0.766-2.111)	0.353
			1.262 (0.759-2.098)[a]	0.370[a]
Allele				
G	218 (72.7)	138 (69)	Reference	
A	82 (27.3)	62 (31)	1.194 (0.806-1.769)	0.375

[a]Adjusted for age, sex. OR: Odds ratio, CI: Confidence interval

Finally, it might be necessary to say that one of the limitations of this study was difficulties in gathering GBM samples, because larger samples may lead to more reliable results. Furthermore, another weakness of our study was the lack of other clinical features and comorbid disease of these patients. Thus, the duration of disease, family history of GBM, and other comorbid diseases might affect the distribution of mentioned allele or genotypes in these patients. Hence, further study noticing these factors in a bigger sample size is recommended.

Conclusion

To the best of our knowledge, the results of the present study support that the rs1957106 SNP in NF-κBIA is found more in GBM patients, but it was not statistically significant. As there are conflicting studies showing significant higher rate of this SNP in GBM, further study is suggested.

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Conflicts of interest

There are no conflicts of interest.

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