RESEARCH ARTICLE

4G/5G and A-844G Polymorphisms of Plasminogen Activator Inhibitor-1 Associated with Glioblastoma in Iran - a Case-Control Study

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Abstract

Background: Glioblastoma is a highly aggressive and malignant brain tumor. Risk factors are largely unknown however, although several biomarkers have been identified which may support development, angiogenesis and invasion of tumor cells. One of these biomarkers is PAI-1. 4G/5G and A-844G are two common polymorphisms in the gene promotor of PAI 1 that may be related to high transcription and expression of this gene. Studies have shown that the prevalence of the 4G and 844G allele is significantly higher in patients with some cancers and genetic disorders. Materials and Methods: We here assessed the association of 4G/5G and A-844G polymorphisms with glioblastoma cancer risk in Iranians in a case-control study. All 71 patients with clinically confirmed and 140 volunteers with no history and symptoms of glioblastoma as control group were screened for 4G/5G and A-844G polymorphisms of PAI-1, using ARMS-PCR. Genotype and allele frequencies of case and control groups were analyzed using the DeFinetti program. Results: Our results showed significant associations between 4G/5G (p=0.01824) and A-844G (p = 0.02012) polymorphisms of the PAI-1 gene with glioblastoma cancer risk in our Iranian population. Conclusions: The results of this study supporting an association of the PAI-1 4G/5G (p=0.01824) and A-844G (p = 0.02012) polymorphisms with increasing glioblastoma cancer risk in Iranian patients.

Keywords: Biological markers - glioblastoma - plasminogen activator inhibitor 1 - polymorphisms - risk factors

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Introduction

Glioblastoma multiform which grade IV of glioma (according to The World Health Organization classification) is most aggressive primary brain tumor and most deadliest forms of cancer with very poor prognosis, so survival rate of patients is about 1-2 years (Bleeker et al., 2012; Jiang et al., 2012). New studies have found few biomarkers (including MGMT, IDH, TP53, and EGFR and so on) that participated in pathogenesis and prognosis of glioblastoma (Das et al., 2013; McNamara et al., 2013; Serao et al., 2011). Evidences have shown that a high plasma level of plasminogen activator inhibitor-1 (PAI-1) is one of the most biomarkers of a poor prognosis in several cancer types (Dano et al., 2005; Andreasen et al., 2007). In normal Plasma Level, PAI 1 plays some important biological functions specially in cell adhesion and Migration, also PAI 1 can control cell adhesion by regulating of Urokinase plasminogen activator (uPA) and Cell Migration by regulating of Attachment-detachment cycle of Integrins (Binder et al., 2002; Czekay et al., 2011; Yasar Yildiz et al., 2014). PAI-1 physiological function is not only regulation of uPA, but also plays a crucial role in other biological activities which include: wound healing, atherosclerosis, bone remodeling, rheumatoid arthritis, sepsis, and others (Binder et al., 2002; Gomes-Giacoia et al., 2013). In the other hand studies have shown that any change in gene expression of PAI 1 can have positive effect on tumor cells invasion and development, for example high elevated plasma level of PAI 1 can plays a crucial role in tumor invasion in some type of cancers like colorectal cancer, Breast cancer, liver carcinogenesis and skin carcinogenesis by controlling of degradation of the extracellular matrix by tumor cell-associated proteases (Berger et al., 2002; Dano et al., 2005; Brandal et al., 2011). Moreover Studies have shown increased level of PAI 1 associated with supporting angiogenesis in neuroblastoma tumors and increasing of the risk of developing coronary artery disease as well as the extent of coronary sclerosis, restenosis, myocardial infarction, and it has a protective effect against apoptosis of tumor cells (Bajou et al., 2001; Isogai et al., 2001; Fang et al., 2012). 4G/5G and A-844G polymorphisms are two

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common SNPs in gene promotor of PAI 1 that can cause of increasing in PAI 1 gene transcription and expression, in 4G/5G insertion/deletion, one guanine nucleotide deletion at position 675 in this gene promotor can block on of transcriptional repressor, thus the transcription of PAI 1 gene will be higher than wild-type genotype. Besides in A-844G polymorphism, a guanine nucleotide will exchange with adenine nucleotide at position 844 in gene promotor that cause high expression of this gene (Eriksson et al., 1995; Grubic et al., 1996).

The goal of this study was investigating association between 4G/5G and A-844G polymorphisms of PAI 1 with patients affected with GBM in Iranian population.

Materials and Methods

Our study population was included of 71 patients with GBM, who were hospitalized in the Shohada-e-Tajrish Specialist Hospital in Tehran (Tehran, Iran). Control group consisted of 140 volunteers who had no symptoms or history of glioblastoma that approved by department of pathology of Shohada-e-Tajrish hospital. Brain Tissue samples were taken by surgery from patients and venous blood samples were taken from all healthy group members. DNA extracted and isolated from Tissue samples by using GeNet Bio PrimePrep Genomic DNA Extraction Kit (GeNet Bio, Korea). DNA obtained and extracted from Blood samples by using Zymo Research Quick-gDNA Blood MiniPrep (Zymo Research, USA). Then Nucleic Acid Quality control, were performed by Nano Drop spectrophotometer.

We have used ARMS-PCR for amplification of polymorphic segments. Three primers (Normal, Mutant and Common) have been designed by using Primer designing tool (NCBI primer designing tools online program). PCR mixture were containing as follow: 6 μL of Taq DNA Polymerase 2x Master Mix RED (Ampliqon), 1.5 μL of DNA samples, 1.5 μL of primer mix (Primer A/B + primer C) + 3 μL of H_2O added to a final volume of 12 μL.

The ARMS-PCR were performed as following cycles: 95°C for 5 min as primary Denaturation, followed by 30 cycles of 95°C for 35 second as secondary denaturation, 60°C for 35 second for primers annealing, 72°C for 50 second for DNA extension and 72°C for 5 min for final extension (Table 2).then DNA samples (were amplificated by ARMS-PCR) have loaded and run on Agarose Gel (3%) Electrophoresis, we have used GEL red for DNA staining. To confirm the results collected by using ARMS-PCR, chosen samples (every seventh) were subjected to DNA sequence analysis. Analysis of our results and genotypes and alleles distribution in case and control groups has calculated by means of DeFinetti program software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) with considering the chi2 square test and the 95% confidence intervals (CI). The level of significance association was set at $p<0.05$.

Results

According to our results, Genotype frequencies of 4G/5G polymorphism showed significant differences in case and control groups (listed in table 1) .in case group genotype frequency were obtained as following: 4G/4G=29.6%, 4G/5G=42.3%, 5G/5G=28.1%.genotype frequency in control group were obtained as following: 4G/4G=9.3%, 4G/5G=60%, 5G/5G=30.7% .This results were showed significantly differences in genotypes and alleles frequency in case and control groups (Table 1, Table 3). According to this results when 5G/5G wild-type genotype exchange to 4G/4G Genotype there is a significant association between mutant genotype with glioblastoma cancer risk (P=0.02523), therefore, it could be suggested that the 4G/4G genotype probably have positive and supportive effect for the glioblastoma cancer risk or the other hand 5G/5G genotype probably have protective/negative effect for the risk of Glioblastoma. Moreover, when if 5G/5G wild-type genotype change to 4G/5G genotype (heterozygous genotype) there is no significant association (P=0.44230), accordingly it seems that 5G allele type have more protective effect than 4G allele's positive and supportive effect for glioblastoma cancer risk in heterozygous genotype.

Otherwise Genotype frequencies of the A-844G polymorphism were generated within the control group as following: AA = 3.6 % AG = 57.9 %, GG = 38.5 %, while the frequencies in the case group were as following: AA=19.7, AG=46.5 and GG= 33.8 % (Table 2). According to this part of our results, if GG wild-type genotype exchange to AA Genotype there is a significant association between mutant genotype with glioblastoma cancer risk (P=0.00059), Thus, it could be suggested that the AA genotype probably have positive and supportive effect for the risk of Glioblastoma development or the other way GG wild-type genotype probably have protective/negative effect for the risk of Glioblastoma (Table 3). Moreover, when if GG wild-type genotype exchange to AG genotype (in heterozygous genotype) there is no significant association (P=0.78611). Therefore, it seems that G allele type have more protective effect than A allele positive and supportive effect for glioblastoma cancer risk in heterozygous genotype.

Table 1. Genotype Frequency of 4G/5G Polymorphism of PAI-1 in Case and Control Group

Genotype	Case	Control
4G/4G	21(29.6%)	13(9.3%)
4G/5G	30(42.3%)	84(60%)
5G/5G	20(28.1%)	43(30.7%)
Total	71	140

Table 2. Genotype Frequency of A-844G Polymorphism of PAI-1 in Case and Control Group

Genotype	Case	Control
AA	14(19.7%)	5(3.6%)
AG	33(46.5%)	81(57.9%)
GG	24(33.8%)	54(38.5%)
Total	71	140

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Table 3. Final Analysis of the Study Results Including Alleles and Genotype Frequency Differences (by means Definetti Program Software)

SNP	Tests for deviation from Hardy-Weinberg equilibrium		Tests for association (C.I.: 95% convidence interval)				
	Controls	Cases	allele freq. difference	heterozygous	homozygous	allele positivity	Armitage's trend test
4G5G	4G5G n11=43 (51.61)	n11=20 (17.25)			**Risk allele 2**		
	n12=84 (66.79)	n12=30 (35.49)	[5G]<->[4G]	[5G5G]<->[5G4G]	[5G5G+]<->[4G4G]	[5G5G]<->[5G4G+4G4G]	common odds ratio
	n22=13 (21.61)	n22=21 (18.25)	Odds_ratio=1.590	Odds_ratio=0.768	Odds_ratio=3.473	Odds_ratio=1.130	Odds_ratio=1.758
	f_a1=0.61 +/-0.025	f_a1=0.49 +/-0.045	C.I.=[1.058-2.388]	C.I.=[0.391-1.508]	C.I.=[1.453-8.304]	C.I.=[0.602-2.122]	
	F=-0.25775	F=0.15476	chi2=5.01	chi2=0.59	chi2=8.15	chi2=0.15	chi2=5.57
	p=0.002290 (Pearson)	p=0.192218 (Pearson)	p=0.02523 (P)	p=0.44230	p=0.00430	p=0.70265	p=0.01824
	p=0.001919 (Llr)	p=0.191332 (Llr)			**Risk allele 1**		
	p=0.002712 (Exact)	p=0.234231 (Exact)	[4G]<->[5G]	[4G4G]<->[5G4G]	[4G4G]<->[5G5G]	[5G5G+5G4G]<->[4G4G]	common odds ratio
			Odds_ratio=0.629	Odds_ratio=0.221	Odds_ratio=0.288	Odds_ratio=0.244	Odds_ratio=0.583
			C.I.=[0.419-0.945]	C.I.=[0.099-0.496]	C.I.=[0.120-0.688]	C.I.=[0.113-0.524]	
			chi2=5.01	chi2=14.57	chi2=8.15	chi2=14.35	chi2=5.57
			p=0.02523 (P)	p=0.00013	p=0.00430	p=0.00015	p=0.01824
A844G	n11=54 (63.79)	n11=24 (23.10)			**Risk allele 2**		
	n12=81 (61.42)	n12=33 (34.80)	[G]<->[A]	[GG]<->[GA]	[GG+]<->[AA]	[GG]<->[GA+AA]	common odds ratio
	n22=5 (14.79)	n22=14 (13.10)	Odds_ratio=1.564	Odds_ratio=0.917	Odds_ratio=6.300	Odds_ratio=1.230	Odds_ratio=2.059
	f_a1=0.68 +/-0.023	f_a1=0.57 +/-0.043	C.I.=[1.032-2.371]	C.I.=[0.489-1.719]	C.I.=[2.038-19.477]	C.I.=[0.676-2.236]	
	F=-0.31868	F=0.05161	chi2=4.47	chi2=0.07	chi2=11.81	chi2=0.46	chi2=5.40
	p=0.000163 (Pearson)	p=0.663660 (Pearson)	p=0.03447 (P)	p=0.78611	p=0.00059	p=0.49775	p=0.02012
	p=0.000064 (Llr)	p=0.663799 (Llr)			**Risk allele 1**		
	p=0.000187 (Exact)	p=0.636019 (Exact)	[A]<->[G]	[AA]<->[GA]	[AA]<->[GG]	[GG+GA]<->[AA]	common odds ratio
			Odds_ratio=0.639	Odds_ratio=0.146	Odds_ratio=0.159	Odds_ratio=0.151	Odds_ratio=0.540
			C.I.=[0.422-0.969]	C.I.=[0.049-0.436]	C.I.=[0.051-0.491]	C.I.=[0.052-0.438]	
			chi2=4.47	chi2=14.26	chi2=11.81	chi2=14.99	chi2=5.40
			p=0.03447 (P)	p=0.00016	p=0.00059	p=0.00011	p=0.02012

Legend: The tests for association are adapted from Sasieni PD (1997); n11(e): Genotype 11,Wild Genotype (expected); n12(e): Genotype 12 (expected); n22(e): Genotype 22,Mutant Genotype(expected); f a1: Frequency of allele 1 +/- standard deviation; F: Inbreeding coefficient; p (Pearson): Pearson's goodness-of-fit chi-square (degree of freedom = 1); p (Llr): Log likelihood ratio chi-square (degree of freedom = 1); p (Exact): Exact test; The following equations correspond to risk allele 2; Odds ratio (allele freq. difference): (Case_a2 * Control_a1) / (Case_a1 * Control_a2); Chi2 (allele freq. difference): (P) = Pearson's goodness-of-fit chi-square (df=1), (F) = Fisher's exact test; Odds ratio (heterozygous): (Case_12 * Control_11) / (Case_11 * Control_12); Odds ratio (homozygous): (Case_22 * Control_11) / (Case_11 * Control_22); Odds ratio (allele positivity): ((Case_12+Case_22) * Control_11) / (Case_11 * (Control_12+Control_22)); Common odds ratio: (Case_12*Control_11/N01 + Case_22*Control_12/N12 + 4*(Case_22*Control_11/N02))/; (Case_11*Control_12/N01 + Case_12*Control_22/N12 + 4*(Case_22* Control_11*Case_11*Control_22)**0.5/N02)

Discussion

A number of potential biomarkers of glioblastoma were identified and classified in last studies, SERPINE1 (PAI 1 gene) is one of these biomarkers (Sreekanthreddy et al., 2010). Many recent studies focused on researching the association polymorphisms of PAI 1 (Especially 4G/5G) with several cancer risks (Wang et al., 2013). For example studied have investigated that 4G/4G genotype will increase breast cancer, Ovarian cancer and colorectal cancer susceptibility, besides this polymorphism will be one cause of poor prognosis of patients in these cancers (Halamkova et al., 2013; Ren et al., 2013; Serce et al., 2013). 4G/5G insertion/deletion in gene promotor of PAI 1 will increase transcription of this gene and an exchange of guanine to adenine nucleotide in position 844 of PAI 1 gene will cause of high expression of it. Nonetheless there is a lack of studies about the association of 4G/5G and A-844G polymorphisms of PAI 1 with glioblastoma. For the first time, we have studied the association between 4G/5G and A-844G polymorphisms of plasminogen activator inhibitor 1 with the risk of glioblastoma. Our results have showed the presence of the 4G and A alleles in case group were higher than control group, and there was significantly difference between 4G/4G and AA genotypes frequency in case and control group. Nevertheless it seemed that protective effect of 5G allele is higher than 4G allele effect in heterozygous genotype (4G/5G) thus probably 5G allele has neutralized 5G allele in this genotype, also in A-844G polymorphism presumably G allele has more protective effect against A allele in heterozygous genotype, thus G allele has neutralized A allele effect in this genotype.

In conclusion, the results of this Study supporting an association of the PAI-1 4G/5G (p=0.01824) and A-844G (p=0.02012) polymorphisms with increasing Glioblastoma cancer risk in Iranian patients.

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