THE RELATIONSHIP OF GLIOMAS WITH *TP53* RS1042522 C > G AND GLIOMAS IN DUHOK

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ABSTRACT

A tumor suppressor gene TP53 has a central role in controlling the cell cycle, apoptosis, as well as DNA damage repair. A common polymorphism in TP53 is the Arg72Pro exon 4 polymorphism. Polymorphism has been proposed to be associated with genetically determined susceptibility in different types of cancers, including glioma. This study was conducted to estimate the distribution of glioma within age groups, gender, smokers, and residence of individual also to investigate the distribution of TP53 Arg72Pro SNPs genotype in glioma, and determine whether TP53 Arg72Pro polymorphism is a possible relevance in susceptibility to glioma using RFLP-PCR analysis. Enrolled were 65 patients (glioma tissues matched age and gender) and 70 healthy individuals as a control. The findings in glioma samples 40(61.54%) were homozygous for arginine (Arg / Arg), 19 (29.23%) heterozygous for (Arg / Pro), and 6 (9.23%) homozygous for proline (Pro / Pro). Three separate frequencies of genotypes of Arg72Pro; 33 (47.14%), 28 (40.0), and 9 (12.86%) were identified in healthy individuals, respectively. The allele Frequencies for the Pro 72 and Arg 72 gliomas were 16 (24.62%) and 49 (75.38%), respectively. In the Pro 72 and Arg 72 controls, the allele frequencies were 23 (32.86%) and 47 (67.14%), respectively. Finally, there was no significant relationship between age group, gender, dwellers, non-smokers and smokers in different genotypes of codon 72 of TP53 gene (P < 0.05).

KEYWORDS: PCR- RFLP; TP53; Codon72; SNPs; Brain Cancer (Gliomas).

INTRODUCTION

In TP53 gene, Arg72Pro polymorphism has been suggested to be associated with genetically determined susceptibility in various types of cancers including brain tumors.

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be associated with genetically determined susceptibility in various types of cancers including brain tumors. Our objective was to investigate the possible association between TP53 Arg72Pro polymorphism with brain tumor susceptibility and to examine its correlation with the clinico-pathologic variables of tumor cases for oncologic prognosis of patient arg72Pro polymorphism has been suggested in the *TP53* gene to be associated genetically with determining susceptibility in different types of cancers including brain tumors (Sarkar *et al.,* 2009). Variable glioma reports have been reported worldwide with inconsistent results on this polymorphism. Other polymorphic loci consequences of glioma are not much discussed (Prerana *et al.,* 2010). In adults, gliomas account for about 2 percent of the malignant tumors. In Asia and the Pacific region, the incidence of glioma was lower than in Europe, America and Australia (Bondy *et al.,* 2008). The etiology of brain tumors is mostly anonymous, and risk factors for brain tumors are just exposure to ionized radiation (Baldwin *et al.,* 2004).

Studies by Sadetzki *et al.,* (2005) and Umansky *et al.,* (2008) have confirmed that only

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a few environmental risk factors, such as long-term ionizing radiation sequelae. An additional possible risk from low-frequency electromagnetic fields (e.g. the use of cell phones) has been proposed and lately being debated (Inskip *et al*., 2010; Cardis *et al*., 2011). Most malignancies have a higher prevalence in males than in females (Chien *et al.,* 2016; Scarbrough *et al.,* 2014). Whereas in Kashmir, study of Khan *et al.,* (2015) showed no specific differences in age or gender among Brain Tumour clusters. In Sweden (1984-2005), Europe (1988-1997) and the United States (2005-2009), the average incidence rates of pediatric brain tumours were 4.2 (Lannering *et al.,* 2009), 2.9 (Peris-Bonet *et al.,* 2006), and 5.0 per 100,000 children up to 15 years of age (Dolecek *et al.,* 2012). In boys, pediatric brain tumors are significantly more common than in girls. In adults, brain tumors are comparatively more common than in children; in the United States (2005-2009), for instance, there was an occurrence of 26.8/100,000 in adults (20 + years) (Dolecek *et al.,* 2012).

The gene *TP53* located at 17p13, is generally known as a gene that suppresses tumors. P53 has been shown to play an important role in controlling the cell cycle, cell formation, differentiation, proliferation and apoptosis as a 53 kDa protein encoded by the *TP53* gene (Kruiswijk *et al.,* 2015). In addition to mutations that inactivate TP53 functions, many SNPs have been specified in the *TP53* that also influence the molecular function of p53 protein as the guardian of the genome (Yang Z *et al*., 2013). Arg72Pro is a polymorphism of TP53 that affects *TP53's* protein expression (Dastjerdi, 2011). The different outcome of G to C in codon 72, which means alteration of arginine with proline (Hu *et al.,* 2010 and Khan *et al.,* 2015). More efficiently than the Pro72 form, the *TP53* Arg72 form induces apoptosis (Dumont et al., 2003; Pim and Banks, 2004). It has been shown

that more than one form of cancer is significantly associated with polymorphisms of the *TP53* codon 72 gene, such as nasopharyngeal cancer (Zhuo *et al.,* 2009), prostate cancer (Rogler *et al.,* 2011), bladder cancer (Lin *et al.,* 2012), lung cancer (Qiao and Hu, 2013), epatocellular carcinoma, breast cancer, thyroid cancer, cervical cancer, osteosarcoma, and Wilms tumor (Rebbani *et al.,* 2015) (Hossain *et al.,* 2017 and Liu P *et al.,* 2019).

To our Knowledge, no previous study has been reported yet in Kurdistan region of Iraq investigating the possible association between *TP53* polymorphism with brain tumor susceptibility. Therefore, the present study was conducted to evaluate the distribution of glioma within age groups, gender, and individual residence in this region. The current study also seeks to determine whether SNP in *TP53* may play a role in this tumor as a risk factor.

MATERIALS AND METHOD

Sample collection:

The Staff Neurosurgeons at Duhok Accident and Emergency Hospital (Teaching) collected a total of 65 samples of fresh glioma tissue from patients with brain tumors. 38 (58.5 percent) males and 27 (41.5 percent) females were included in the current investigation (Table 1). The samples were immediately put in a sterilized cup containing absolute ethanol. Tumor tissue samples were sent for histopathological diagnosis and all were diagnosed as glioma. In both groups (glioma and control), part of the infected tissue was taken and another seventy healthy individuals were enrolled as control as a source of mutant DNA. Another groups included 44 (62.9 %) males and 26 (37.1 %) females used as a category of controls (Table 1). All samples in the Duhok Governorate were taken from both rural and urban areas.

Table (1): Distribution of patients with glioma and healthy (controls) according to age groups, gender, and the inhabitants (Rural and Urban).

	Characteristics	Study groups No. (%)			
		Patients (n=65)		Control (n=70)	
Age groups	20-30	5	7.7	6	8.6
	31-40	8	12.3	10	14.3
	41-50	13	20.0	15	21.4
	51-60	17	26.2	18	25.7
	>60	22	33.8	21	30.0
Gender	Male	38	58.5	44	62.9
	Female	27	41.5	26	37.1

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Smoker	Yes	20	30.8	18	25.7
	No	45	69.2	52	74.3
Residence (Dwelling)	Rural	42	64.6	36	51.4
	Urban	23	35.4	34	48.6

The study was conducted at the Scientific Research Center, College of Science of Duhok University. Both tumour tissues and normal blood samples were transported to the Scientific Research Centre's medical biotechnology laboratory and Store condition (at -8°C in the Freezer for example) for study. DNA was extracted (from both tumor and blood samples) using the protocol described by Maniatis *et al.*, (1982) updated by Bass *et al*., (1984). The concentration and purity of the extracted DNA were estimated by using the NanoDrop 1000 spectrophotometer.

All samples were subjected to PCR amplification of the Arg72Pro polymorphism region found in the TP53 gene using primers shown in Table(2) previously used by Pinto *et al.*, (2008).

Table (2):

Primer of gene TP53	Sequence (5'- 3')	Length bp	Amplicon bp
Arg72Pro-Forword	5'- GAA GAC CCA GGT CCA GAT GA-3'	20	152
Arg72Pro-Reverse	5'- CTG CCC TGG TAG GTT TTC TG-3'	20	

Primers that used for amplification of TP53 gene codon72 Arg72Pro SNPs

Polymerase chain reaction (PCR):

TP53 Arg72Pro polymorphism detection was performed by PCR-RFLP. For amplification in a 25 μL reaction mixture, the genomic DNA (100 ng) template was used, For amplification, the Cinagen PCR ready master mix kit was used as follows: 12.5 μL Master mixture, 8.5 μL D.D.W, 1uL Forward primer, 1uL Reverse primer 1uL. The amplification conditions were as follows; first step calling pre-PCR at 95 ° C for 5 min denaturation, then applying 35 cycles at 94 ° C for 30 s. denaturation, 54 ° C for 30 s. annealing, 72 ° C for 30 s. extension, and 5 min. extension as a final step within 72 ° C. After running the product bands visualized by UV light, PCR products were run on 2 percent agarose electrophoresis (Pinto *et al*., 2008).

Analyzing RFLP:

The *TP*53 Arg 72Pro SNP amplicons were digested overnight with BstU I restriction enzymes at 37 ° C (Fermentas, Germany). After ethidium bromide staining, the effects of enzymatic digestion were visualized by UV light in a 3 percent agarose gel by electrophoresis.

DATA ANALYSES

The characteristics of the patients and control groups were presented by percentage and number in a Pearson Chi-squared test, then compared. The comparison of allele and genotype prevalence between controls and patients. The relevant difference amount was estimated with a P-value of less than 0.05. Statistical studies were carried out using the version 25 social science statistics kit (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

RESULTS

The samples in the current study were composed of 65 cases of new brain tumor tissues, all of which were histopathologically diagnosed as glioma, and 70 healthy cases as a

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control. The distribution of samples according to the gender for patient cases were 38 (58.5 percent) males and 27 (41.5 percent) females (M / F ratio = 1.41). The mean age was 43 years (range 22-64. Whereas, the healthy control blood samples consisted of 44 (62.9%) males and 26 (37.1%) females (M / F = 1.7), the mean age was 46 years (range = 25-67 years). There were no variations in age and gender differences (p>0.05) observed among were glioma patients. The patients 20 smokers and 45 non-smokers, while the control groups were 18 smokers and 52 non-smokers, respectively. The findings of the statistical in the present study showed that there was no substantial difference (p>0.05) in the smoking status of patients with glioma and of individuals in the control group, as well as in the residence status (rural or urban) of individuals (Table 3).

Analyzing PCR:

Both sample groups, 65 glioma and 70 controls, were successfully amplified and polymorphic alleles were analysed for *TP*53 codon 72. The results of 152bp amplicon is shown in Figure 1.

Table (3): Comparison of general characteristics of patients with glioma and healthy controls

Characteristics		Study groups No. (%)				P-value
		Patients (n=65)		Controls (n=70)		
Age groups	20-30	5	7.7	6	8.6	
	31-40	8	12.3	10	14.3	
	41-50	13	20.0	15	21.4	0.988
	51-60	17	26.2	18	25.7	
	>60	22	33.8	21	30.0	
Gender	Male	38	58.5	44	62.9	
	Female	27	41.5	26	37.1	0.601
Smoker	Yes	20	30.8	18	25.7	
	No	45	69.2	52	74.3	0.514
Residence (Dwelling)	Rural	42	64.6	36	51.4	
	Urban	23	35.4	34	48.6	0.121

Fig. (1): Represent the amplicon bands of *TP53* Arg72Pro SNPs by 2% agarose gel electrophoresis.

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Analyzing RFLP:

With the BstU I restriction enzyme, the amplified fragments of *TP*53 Arg 72Pro SNP were digested (Figure 2). The findings were that a single band (152 bp) indigested PCR product identified as the Pro/Pro variant (CC) representing homozygotes proline, two fragments of 50 and 102bp representing homozygotes arginine (Arg/Arg) wild (GG), and heterozygotes for codon 72 Arg/Pro variant (GC) identified by three fragments representing 50, 102 and 152 bp (Khan et al., 2015).

The current research showed 6 (9.23%) glioma patients with 152 bp undigested PCR product fragment representing homozygotes proline (Pro / Pro), 40 (61.54%) with two 50 and 102 bp fragments representing homozygotes arginine (Arg / Arg), and 19 (29.23%) with three 50, 102, and 152 bp fragments representing heterozygotes for codon 72 (Arg / Pro).

Results of control genotypes showed 9 (12.86%) individuals with a fragment of 152 bp representing homozygotes proline (Pro/Pro), 33 (47.14%) with two fragments representing homozygotes arginine (Arg / Arg), and 28 (40.0%) with three bands representing heterozygotes (Arg / Pro) for codon 72 (Table 4) (Figure 3).
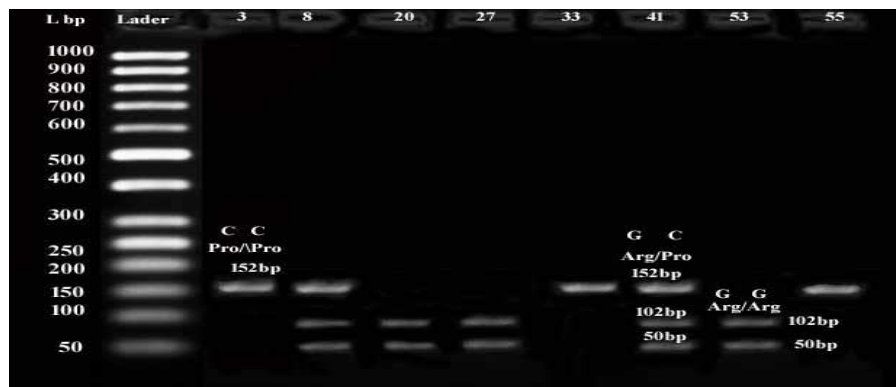

Fig. (2): PCR- RFLP study illustrates *TP*53 codon 72 SNPs by electrophoresis of agarose gel (3%). After digestion, PCR products are   shown: samples 3, 33 and 55, Pro / Pro (CC); samples 20, 27 and 53, Arg / Arg (GG); and samples 8 and 41, Arg / Pro (GC), markers of 50 bp DNA scale.

Table (4): Comparison of prevalence of allele and genotype between controls and patients.

Genotypes	Study groups No. (%)				P-value
	Patients (n=65)		Controls (n=70)		
TP53 Arg72Pro					
Arg/Arg	40	61.54	33	47.14	
Arg/Pro	19	29.23	28	40.0	0.245
Pro/Pro	6	9.23	9	12.86	
Allele frequency					
Arg72 allele	49	0.75	47	0.67	0.291
Pro 72 allele	16	0.25	23	0.33	

Characteristics	Genotype/Allele	Study groups No. (%)				P-value
		Patients (n=65)		Controls (n=70)		
Male (p.n=38, c.n=44)	Arg/Arg	28	0.73	33	0.75	
	Arg/Pro	8	0.21	8	0.18	0.791
	Pro/Pro	2	0.05	3	0.07	
	Allele frequency					
	Arg72allele	32	0.8	37	0.84	0.597
	Pro72allele	6	0.2	7	0.16	
Female (p.n=27, c.n=26)	Arg/Arg	12	0.44	9	0.35	
	Arg/Pro	11	0.41	15	0.57	0.293
	Pro/Pro	4	0.15	2	0.08	

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Allele frequency					
Arg72allele	19	0.7	17	0.6	0.340
Pro72allele	9	0.3	9	0.4	

The findings of the current study indicate that there are no substantial variations (P>0.05) in the frequency of Pro72allele in patients as compared to control groups. Similarly, with regard to the frequency of the Arg72allele, there are no substantial variations (P>0.05) between the frequency of the Arg72allele in control group patients (Table 4).

The current research indicates no substantial differences (P>0.05) between the frequency of Arg72allele

and Pro72allele in glioma-affected females as opposed to the frequency of Arg72allele and Pro72allele in the control groups. In relation to the frequency of Arg72allele and Pro72allele in males, the same result is shown (Table 4).
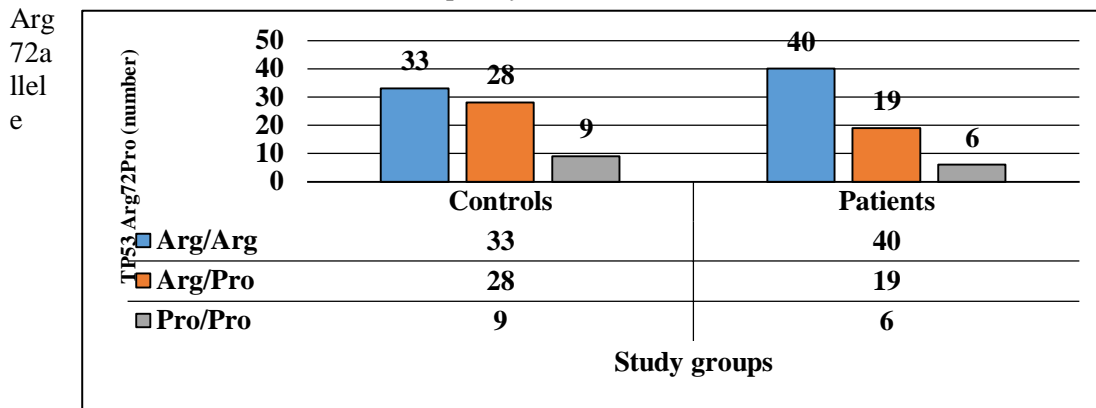

TP53 Arg72Pro (number)	Controls	Patients
Arg/Arg	33	40
Arg/Pro	28	19
Pro/Pro	9	6

Study groups

Fig. (3): Frequencies of allele and genotype between controls and patients.

DISCISSION

Single Nucleotide polymorphisms are considered very important common modifications in human DNA and have been frequently studied in the search for genetic predisposition of various diseases, because they are not time consuming with low cost and reliable outcomes of supply. The detection of these nucleotides in human DNA may be considered risk factors and in patients with malignancies may help diagnosis and follow-up. The role of these SNPs as risk factors that could influence prevention, diagnosis and also as a prognostic factor has been stated by Kirk *et al.,* (2002).

The current study shows that no clear gender, age, smoking status and residential (rural or urban) variations (P<0.05) between the glioma groups (Table 3). Khan *et al.,* (2015) Kashmir study showed the same outcome as that found in the current study, with no major variations between age groups or gender in Brain Tumor patients. Likewise, no major difference in

dwelling and smoking status was seen. Previous studies have shown that brain tumours vary according to gender, age, ethnicity, and geography. In Sweden (1984-2005), Europe (1988-1997) and the United States (2005-2009), the average incidence rates of pediatric brain tumours were 4.2 (Lannering et al., 2009), 2.9 (Peris-Bonet et al, 2006), and 5.0 per 100,000 children up to 15 years of age (Dolecek *et al,* 2012).

In boys, pediatric brain tumors are significantly more common than in girls. Brain tumors are relatively more common in adults than in children; the incidence in adults (20 + years) was 26.8/100,000 in the United States (2005-2009), for example (Dolecek *et al.,* 2012). No association between cigar smokers and glioma disease was shown in the Baldwin *et al* (2004) study. The etiology of brain tumors remains largely unknown, with exposure to ionizing radiation being the only reported risk factors for brain tumors (Baldwin *et al.,* 2004). Studies by Sadetzki *et al.,* (2005) and Umansky *et al.,* (2008) have confirmed that only a few

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environmental risk factors, such as long-term ionizing radiation sequelae, have been identified. An additional possible risk (e.g. cell phone use) from low-frequency electromagnetic fields has been proposed and is currently under consideration (Inskip *et al.,* 2010; Aydin *et al.,* 2011; Cardis et al., 2011).

TP53 codon 72 genotypes 9.23 % glioma patients as a proline homozygote (Pro / Pro), while the same genotype showed in 12.86% healthy individuals in control groups. Arginine homozygotes (Arg / Arg) was 61.54%, while 47.14% individuals in control groups. Heterozygotes (Pro / Arg) for the codon 72 gene, was 29.23%, while 40.0% individuals in controls (P=0.245). No substantial differences (P<0.05) have been reported in all genotypes, as well as in the allele frequencies between glioma patients and control groups. This results In agreement with the study Hamajima *et al.,* (2002), they found that the *TP*53 codon 72 gene polymorphism in patients with colorectal cancer was not significantly associated with the same codon polymorphism in a normal person in Japan. The stomach polymorphism analysis by Khadand *et al.,* (2007) also showed non-significant variations in genotype frequencies and allele frequencies of *TP*53 Arg72Pro, colorectal adenocarcinoma and breast cancer patients when compared with healthy people in the Southern Iran population. The same findings suggest no connection between *TP*53 Arg 72 Pro polymorphism and glioma cancer when examined in the Southeast Brazilian population (Pinto *et al.,* 2008). The study conducted by Onrat *et al.,* (2009) in Turkey and Japan did not find a correlation between the prevalence of *TP*53 gene polymorphism with colorectal cancer, and their study showed that the frequency of alleles was consistent between the control group and colorectal cancer cases.

The study by Khan *et al.,* (2015) of Proline allele in the *TP*53 gene, Arg72Pro polymorphism in a Kashmir population, showed that no statistical difference was observed between patients and the control group in the *TP*53 gene, Arg72Pro polymorphism. Moradinasab *et al.,* (2017) showed no major connection between breast cancer and codon 72 polymorphism in *TP*53. Fang *et al.,* (2020) found that there was no important link between *TP*53 gene polymorphism and neuroblastoma susceptibility.

Another report disagrees with the current study's outcome. A research conducted by Zhou

et al ., (2009) concluded that *TP*53 Arg 72 Pro SNP is a risk factor for the development of many cancers, including cervical head and neck, colorectal, breast cancers and many others. Similarly, the molecular genetic analysis by Mammano *et al.,* (2009) found that the genetic risk factor for colorectal cancer was codon 72 polymorphism, the genotype frequencies of *TP*53 codon 72 in patients with sporadic colorectal adenocarcinomas and normal individuals from the Isfahan province of Iran in 2008 showed a substantial difference between the controls and the Arg / Arg genotype case group compared to Arg / Pro genotype.

Study of Doosti *et al,* (2011) in the colorectal cancer population of South West Iran, which reported that the *TP*53 codon 72 genotype frequencies among the cases and control groups were not closely consistent with the Hardy-Weinberg equilibrium rule and their outcome showed substantial differences in allele and genotypic frequencies between control groups and patients. Their findings also showed a decline in the homozygous genotype of Proline and a rise in patients' homozygous genotypes of Arginine and an increase in the frequency of Arg allele. There is no research conducted with distinct genetic analysis in general that has shown a clear correlation and it is still elusive (Khadang *et al.,* 2007).

CONCLUSION

Based on our observations, we may conclude that there is no association between *TP*53 Arg72Pro and increased glioma susceptibility in Duhok / Iraq.

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