Allele Frequency Of P53 Gene Arg72Pro In Sudanese Meningioma Patients And Controls

Alsadig Gassoum, Mohamed A.Arbab, Sawsan A. H. Aldeaf, Lamya A. Elhassan, Elshibli Elshibli, Ahmed M. Elhassan,

Abstract: The Meningiomas are one of the commonest intracranial tumors and account for 20% of all primary intracranial neoplasms. However, the true incidence is likely to be much higher, since many benign meningiomas do not produce symptoms. In autopsy studies, 2.3% of individuals harbored undiagnosed asymptomatic meningiomas, suggesting that such tumors are up to 1000 times more common than their clinically detected counterparts. Material and Method: This is a cross-sectional study that had been performed at the National Center for Neurological Sciences during February 2011 to December 2013. Result: Molecular screening of the 180 meningioma specimens, showed that the most common allele of p53 gene codon 72, in meningioma , was the arginine variant (Arg/arg), in 157 (87.2%) of the cases.

Key words: meningioma, P53 gene,

Introduction

Coined by Harvey Cushing, the term meningioma refers to a set of tumors that arise contiguously to the meninges. Meningiomas are predominantly benign tumors, which arise from the arachnoids' cap cells (1). The development mechanism is unknown but they may result from an adverse effect of cranial irradiation and trauma (2).

Incidence

The Meningiomas are one of the commonest intracranial tumors and account for 20% of all primary intracranial neoplasms. However, the true incidence is likely to be much higher, since many benign meningiomas do not produce symptoms. In autopsy studies, 2.3% of individuals harbored undiagnosed asymptomatic meningiomas, suggesting that such tumors are up to 1000 times more common than their clinically detected counterparts (3). In Africa, the frequency of meningiomas is even higher and reaching 30% of all brain tumors. This race differences extend to Africans Americans as reports indicate more meningiomas incidence among Africans Americans compared with white Americans. In Sudan, Abu salih(4)and Abdul-Rahman (1988) reported similar results in a material of 127 cerebral tumors during 10 years time (4).

- Alsadig Gassoum, Mohamed A.Arbab, Sawsan A. H. Aldeaf, Lamya A. Elhassan, Elshibli Elshibli, Ahmed M. Elhassan,
- Department of surgery faculty of medicine university of Khartoum
- National center for neurological sciences
- Institute of endemic diseases university of Khartoum
- school of medicine Ahfaad university for women
- Faculty of medicine Moghtarbeen university
- Neelain University, Statistic, section

In Sudan, cancer registry has faded away since the early seventies and thus the incidence of cancer including meningiomas is poorly documented, however, based on the data from the National Center for Neurological Sciences, meningioma is the most common intracranial tumor in Sudan. Meningiomas typically exhibit variable growth rates and recurrence independent of the histopathologic features (histology type, grade and stage). Because of the heterogeneous natural history of the meningiomas, numerous studies have attempted to correlate morphologic and biological characteristics of the tumor with the natural history (aggressive behavior and rapid recurrence). Unfortunately, and till now no reliable predictive factors that can help in management of patients has been identified, although some and limited subjective morphological features (5). Determining the proliferation index, however, provides adjunct objective information about a neoplasm's behavior, and a direct correlation has been shown to exist between the proliferation rate and the biologic aggressiveness. In one study that has been done demonstrated a statistically significant correlation between the MIB-1 (Ki-67antigen) and bromodeoxyuridine (BUdR) proliferation indices in meningiomas and concluded that MIB-1 proliferation indices could be substituted for BUdR proliferation indices to determine the proliferative potential of meningiomas (6).

P53 gene

Human p53 is a nuclear phosphoprotein of MW 53 kDa, encoded by a 20 –kb gene containing 11 exons and 10 introns, which is located on the small arm of chromosome 17. It belongs to a highly conserved gene family containing two other members, p63 and p73.Wild – type p53 protein contains 393 amino acids and is composed of several structural and functional domains. The polymorphic variant at codon 72 has been shown to be an intragenic modifier of mutant p53 behavior, (7). Arg72-containing allele was preferentially mutated and retained in squamous cell tumours arising in Arg/Pro germline heterozygotes and was more potent in neutralizing p73-induced apoptosis and cooperating with EJ-Ras to transfrom cells. Other studies in colorectal, lung, and head and neck cancers (8) ,have aslo found that in Arg/Pro germ line heterozygote, the Pro allele is preferentially lost and the Arg allele is preferentially mutated(8). In intron 4, a SNP was recently shown, to affect the activity of the internal promoter of TP53 controlling the production of dN133p53 isoform(8). Many

studies have investigated the associations of TP53 polymorphisms with increased risk for cancers (9). Codon 72 (Arg/Pro), intron 6 (G>A) and intron 3 duplication are the more extensively studied. However, in several cases, putative associations have been challenged by subsequent studies. In lung cancer for example, the codon 72 Pro/Pro genotype has been associated with an elevated risk (9) . Another study showed that the Arg/Pro genotype contributes to heritable susceptibility for smoke-induced lung carcinoma (10). Several studies in the past have demonstrated a proportionate increase in proliferative index with increasing tumor grade(11) .Though assessment of proliferative activity is a good indicator of tumor aggressiveness, p53 expression was studied to evaluate its role in tumor biology. Earlier studies have demonstrated variable results with regards to alteration in p53 pathway in meningiomas (11).

Material and methods

This is a cross-sectional study that had been performed at the National Center for Neurological Sciences during February 2011 to December 2013. The study included samples from intracranial meningioma patients histologicaly diagnosed at the National Center of Neurological Sciences, during the above mentioned period. The study was conducted in accordance with the guidelines of the local ethical committee. Native tumors specimens were obtained from 180 intracranial meningiomas' treated at the National Center for Neurological Sciences, all tumor tissue were classified according to the WHO guidelines(2007), tissue samples were taken in sterile containers, and kept in -80 ºC till used, the all samples were processed for DNA extraction. The corresponding blood samples were taken in sterile containers that contain Ethylin Diamin Tetra Acitic acid (EDTA) anti coagulant and processed for DNA extraction, DNA was successfully obtained from 94 cases . Clinical and demographic data were collected using predesigned structural interview questionnaire,. Furthermore 20 tumors which were positive immunoreactivity of p53 protein were sent to (Macrogen Korea) for sequencing . Eighteen reactions were successfully obtained for the p53 gene codon 72 arg/pro , with especial emphasis to arginine variant at codon 72. The personal data of all patients were obtained from the registry data base in the National Center of Neurological Sciences. The following variables concerning each case were recorded (age, gender, occupation, residence, tribe, histopathology of tumor, WHO criteria, tumor genotyping of p53 gene at codon 72 variants .More over 150 blood samples were collected from healthy individuals as control.

DNA extraction from meningioma tissue samples

The DNA extraction was done, using **AccuPrep** Genomic DNA Extraction Kit **Cat. No.: K-3032** method. A total of 200 tissue lyses buffer was added to the samples for homogenization, then 20µl proteinas K was added to digest all types of proteins, then samples were incubated at 60 ºC for 1 hour ,samples were vortexed 3 times during incubation period, after the incubation, the samples were shacked and then treated with 200 µl from Binding buffer (GC) and immediately mixed by vortex mixer, then samples were incubated at 60 ºC for 10 minutes. Following the last incubation 100 µl of Isopropanol was added, after that the samples were mixed gently and transferred into the upper reservoir of the binding column tube (fit in a 2 ml tube) then centrifuged in micro centrifuge at 8000 RPM for 1 minute. The Binding column tubes were transferred into a new 2 ml tubes for filtration 500 µl of Washing buffer solution 1(W1) was added without wetting the rim , and then tubes were closed and centrifuged at 8000 RPM for 1 minute , after this step the solution was poured from the 2 ml tube into a disposal bottle , then 500 µl of Washing buffer solution 2(W2) was added without wetting the rim , and then tubes were closed and centrifuged at 8000 RPM for 1 minute, and then tubes were centrifuged at 12000 RPM for 1 minute again to completely remove ethanol ,after that the Binding Column tubes were transferred to a new 1.5 ml tubes, and then 200 µl of Elution buffer(EL) was added to each tube, and then after incubation at room temperature (25 ºC) for 5 minutes , and after that the tubes were centrifuged at 8000 RPM for 1 minute, about 180 to 200 µl of eluent can be obtained,the eluted genomic DNA can be used immediately or store at 4 ºC.

DNA extraction from blood samples

The DNA extraction was done by using guanidine chloride method , 2 ml from each meningioma blood was placed into Falcon tube (15 ml), 10 ml from red cell lysis buffer was added, then tubes gently were mixed by using vortex mixer, after that the tubes were centrifuged at 6000 RPM for 10 minutes , this step was repeated until clear pallet was obtained, then 2 ml from White cell lysis buffer, 1 ml from guanidine chloride , 350 µl of ammonium acitate and 20 µl of proteinase K were added , tubes were vortexed and then incubated at 37 C◦ for over night , after incubation the tubes were vortexed and 2 ml from pre chilled chloroform was added, the tubes were mixed by using vortex mixer , after that the tubes were centrifuged at 6000 RPM for 10 minutes , then the supernatant was transferred into a new Falcon tube (15 ml) , 8 ml of pre chilled ethanol was added to each tube with gentle mixing to precipitate the DNA, for completion of DNA precipitation the tubes were incubated at -20 C◦ for 2 hours , after incubation the tubes were centrifuged at 6000 RPM for 10 minutes , then the ethanol was poured into disposal bottle , after that 4 ml of 70% alchol was added and after that the tubes were centrifuged at 6000 RPM for 10 minutes , the 70% alchol was poured into disposal bottle , and after that the tubes were blotted on filter paper , and then left to air dry, after completion of drying 100 µl of Elution buffer was added, then after that the tubes were incubated at 4 C◦ for completion of DNA elution. Deoxynucleotides were prepared by adding 10µl of each nucleotides (total volume 40), in 60 µl of sterile deionized water to a final concentration of 10 mM. The mixture was vortexed to collect any dNTPs from the tube surface in the button of the tube.Master mix (MM) was prepared using P53- F and P53- R, according to the number of samples to be processed with an extra one more sample to compensate pipetting errors. 24µl of MM was added into each PCR tube, all reagents were placed in a frozen-cryo-rack. To avoid contamination, separate area for PCR was prepared, and all work material needed for PCR were carefully handled. According to the international PCR protocol all the steps were been followed. Primers for p53 gene codon 72 arg/pro was obtained from the published data(114), 5 tcc ccc ttg ccg tcc caa 3 forward primer and 5

ctg gtg cag ggg cca cgc 3 , reverse primer were used for amplification of arginine allele at codon 72, and ,5 gcc aga ggc tgc tcc ccc 3 forward primer and ,5 cgt gca agt cac aga ctt 3 primer were used for amplification of prolin allel at codon 72. Each of the forward and reverse primers were prepared by adding10µl of each stock primer (100 µM) to 90µl deionized water, the solution was mixed carefully using vortex mixer. Using 2% Agarose gel electrophoresis amplified PCR product were visualized

Molecular results

Molecular screening of the 180 meningioma specimens, showed that the most common allele of p53 gene codon 72, in meningioma , was the arginine variant (Arg/arg), in 157 (87.2%) of the cases. This was followed Proline variant (pro/pro) and Arg/pro variants in 10.6% and 2.2% respectively, P.value >0.000. (Table 1). Genotyping of p53 gene of the collected blood samples was performed in 94 cases. In 68 samples (72.3%) Arginine\arginine was identified, followed by , Arginine/proline (arg/pro) variant in 15 samples and Proline/proline (Pro/pro) variant in 11 samples, P.> 0.000 (Table.2). The three genotypes i.e. Arg/arg, Pro/pro and Arg/pro were identified and found to be matching in both tumor specimens and blood samples of the 68 patients. The remaining 26 patient's samples disclosed mismatching between the genotypes identified in the blood versus those identified in tumor tissue, P> 0.000. Cross tabulation of the histopathological subtypes and p53 gene genotyping showed dominance of Arg/arg variant in all subtypes (Table 3, 4). The most common variant in control samples was Arg/arg in 123 cases (82%), (table 5)

Table.1 showed the frequency of p53 gene variants in meningioma tumors P =0.000

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	arg/arg	157	87.2	87.2	87.2
	arg/pro	4	2.2	2.2	89.4
	pro/pro	19	10.6	10.6	100.0
	Total	180	100.0	100.0	

Table.2 showed the frequency of p53 gene variant in the blood of meningioma patients P=0.000

	Observed N	Expected N	Residual
arg/arg	68	31.3	36.7
arg/pro	15	31.3	-16.3
pro/pro	11	31.3	-20.3
Total	94		

Table.3 showed cross tabulation between p53 gene genotyping of meningioma tumors and histopathology P >0.41

		p53T arg/arg	arg/pro	pro/pro	Total
histopathology	fibrous	64	1	11	76
	atypical	34	0	2	36
	meningiothelial	24	1	3	28
	mixed	17	1	3	21
	angiomatous	4	0	0	4
	psammomatous	3	1	0	4
	anaplastic	2	0	0	2
	clear cell	5	0	0	5
	secretory	4	0	0	4
Total		157	4	19	180

Table.4 showed cross tabulation between p53 gene genotyping of meningioma patients blood samples and histopathology P >0.9

		p53B arg/arg	arg/pro	pro/pro	Total
histopathology	fibrous	29	7	5	41
	atypical	10	1	3	14
	meningiothelial	13	4	1	18
	mixed	6	2	2	10
	angiomatous	2	1	0	3
	anaplastic	2	0	0	2
	clear cell	3	0	0	3
	secretory	3	0	0	3
Total		68	15	11	94

Table 5 showed frequency of P53 variants in control samples from healthy individuals

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	arg/arg	123	82.0	82.0	82.0
	arg/pro	6	4.0	4.0	86.0
	pro/pro	21	14.0	14.0	100.0
	Total	150	100.0	100.0	

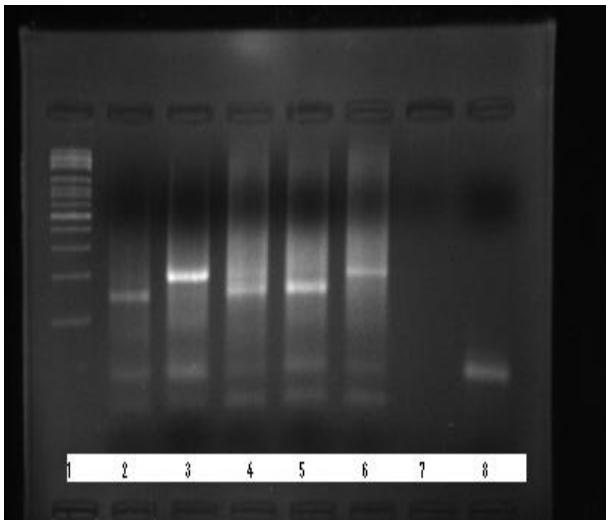

Fig.1 showed gel electrophoresis of P53 gene in meningioma .
Lane 1: DNA ladder (100bp) , Lane 2: Arg/arg variant (140 bp), Lane 3 Pro/pro variant (180 bp)

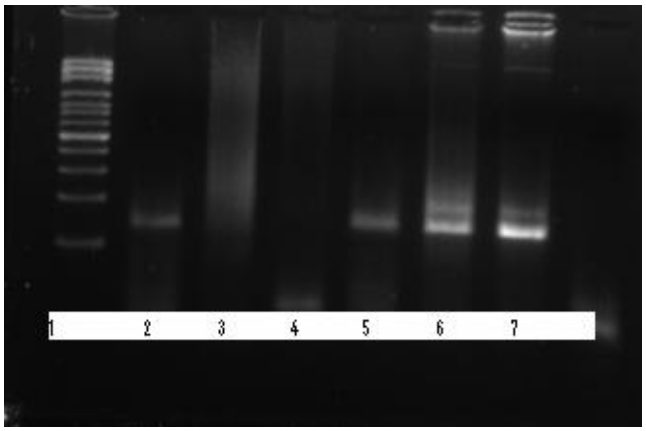

Fig. 2 showed gel electrophoresis of P53 gene in meningioma .
Lane 1: DNA ladder (100bp) , Lane 2: Arg/arg variant (140 bp), Lane 3,4 negative , Lane 6,7 Arg/pro variant (140/180 bp)

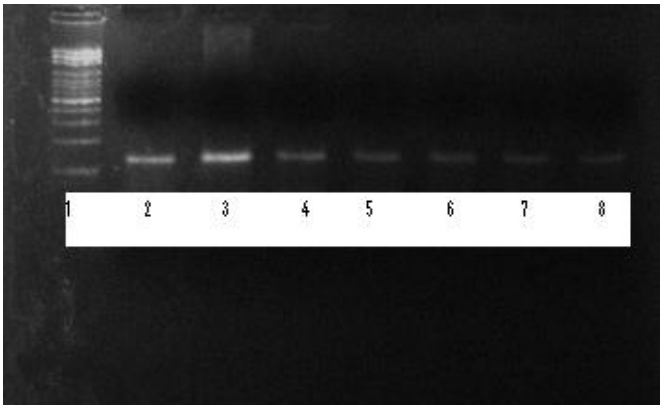

Fig 3 showed gel electrophoresis of P53 gene in meningioma .
Lane 1: DNA ladder (100bp) , Lane 2,3,4,5,6,7,8: Arg/arg variant (140 bp).

Discussion

The results of the present study showed that among the 180 patients been studied, 120 patients were female with female::male ratio of 2:1. P>0.000, The female predominance has been suggested to be hormonal dependent (12), however, in a Turkish study it was postulated that the high incidence of meningioma in women can not be explained only by difference of sex hormone receptors and thus other hidden causes should be looked for, (13). The distribution of the ages of the studied material revealed that 68.3% of the patients were above the age of 40 years, this finding did not differ from the international incidence of intracranial meningioma among age group in male and females (12, 13, 14). In this study, out of the fifteen histolopathogicaly subtypes, only nine variants were identified, of these the fibrous was the most common. More over, clear discrepancy was noted between the fibrous and atypical sub types. Though the fibrous meningioma is considered to be benign, however, its biological behavior seemed to be more aggressive. This has been manifested either by the relatively large tumor volumes or the aggressive behavior as revealed by the images Loss of heterozygosity in p53 tumor suppresser gene had been studied in different types of brain tumors (15), but little is known about p53 gene in meningioma. NF-1, NF-2 and p53 were reported to be involved in development of brain tumors. Alteration of the Nf-1 gene was associated with development of astrocytomas and neurofibromas, NF2 associated with the meningioma and V111 nerve neuronomas (16). The most common genetic abnormalities were the loss of heterozygosity in chromosome 17p where p53 is located (17) The fact that mutated p53 protein has an extended half life due to protein accumulation, made it possible to be detected by immunohistochimistry techniques. In the present study, our findings revealed that, the most common genotype of p53 gene in tumor specimens as well as blood was Arg/Arg. Different studies in Sudan that investigated P53 Codon 72 Polymorphism and cancer in Sudan , showed significant differences in frequency and genotype association between different types of cancer. Breast and cervical carcinoma showed excess of homozygous Arg genotype as compared to controls, while in Burkit lymphoma and oral cancer the most dominant genotype was Arg/pro (18, 19, 20, 21, 22).

Association of breast carcinoma and P53 genotype was reported world-wide (23, 24, 25, 26). In China significant correlation between gastric cancer and p53 genotypes was reported (27).An other study from Brazil studied polymorphism and methylation of p53 in extra axial brain tumors, reported that patients who had TP53 pro allele at codon 72 had greater risk to develop extra-axial brain tumors including meningioma (28). A study from Iran that investigated P53 polymorphism and basal cell carcinoma suggested that Arg allele at codon 72 might affect the risk to ultraviolet light in basal cell carcinoma (29). A report from Sweden showed increased risk of meningioma associated with specific P53 genotype in patients with family history of cancer (30). Another study done in India found significant association of codon 72 TP53 Pro/Pro genotype with the risk of prostate cancer, (31). A study from Turkey did not find association between Polymorphism p53 Arg72Pro and gastric or colorectal carcinoma, (32). These differences of P53 genotypes and cancers might suggest different pathways of these cancers.

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