P53 Gene Allele Frequency in Linguistic Affiliation of Different Sudanese Tribes

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Abstract

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 (2) and Abdul-Rahman (1988) reported similar results in a material of 127 cerebral tumors during 10 years' time (2).

Tumor suppressor genes such as the p53 gene play a role in normal cell division and DNA repair and are critical for detecting inappropriate growth signals in cells (3). If these genes, as a result of inherited or acquired mutations, become unable to function, genetic mutations in other genes can proceed unchecked, leading to neoplastic transformation.
A total of 180 intracranial meningioma patients were included in the present study, males were 60 constituting 33.3% and females were 120 constituting 66.7%, with male to female ratio 1:2,
However, frequencies of Pro72 superior to 0.40 have been observed in African-Americans (7), and in Chinese's (8). In the present study , significant correlation was observed between P53 gene codon 72 alleles and Linguistic affiliation of the different Sudanese tribes, a finding that necessitate further investigation in bigger number of sample.

Keywords:

P53 Gene, Allele Frequency, Linguistic Affiliation, Different Sudanese Tribes, neoplastic transformation

Background

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 (1) 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 (2) and Abdul-Rahman (1988) reported similar results in a material of 127 cerebral tumors during 10 years time (2). 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 occur in both sexes but afflict women more often than men; male/female ratio ranges from 1:1.4 to 1:2.8. The female preponderance is more pronounced in the black population.

Tumor suppressor genes such as the p53 gene play a role in normal cell division and DNA repair and are critical for detecting inappropriate growth signals in cells (3). If these genes, as a result of inherited or acquired mutations, become unable to function, genetic mutations in other genes can proceed unchecked, leading to neoplastic transformation. As with most genes, 2 alleles are present that encode for each tumor suppressor gene. A defective copy of one gene may be inherited, leaving only one functional allele for the individual tumor suppressor gene. If a mutation is acquired in the other allele, the normal protective mechanisms of the tumor suppressor gene are lost, and dysfunction of other protein products or DNA damage may escape unregulated, leading to cancer.

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 histologically 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. Clinical and demographic data were collected using predesigned structural interview questionnaire. The personal data of all patients were obtained from the registry

data base in the National Center of Neurological Sciences, and the laboratory data were collected from meningioma tissue PCR results according to the band size of p53 gene codon 72Pro/Arg variants,

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 proteinase 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.

PCR amplification of p53 gene

Primers for p53 gene codon 72 arg/pro was obtained from the published data (9), 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. In a 0.5 ml PCR tube the following solutions were placed in a total volume of 25 µl, for 1X reaction, 2.5µl PCR buffer with concentration of 200mM, Magnesium chloride 1.5 µl with final concentration of 50mM, dNTPs 1µl final concentration of 10mM, Taq polymerase 0.3µl with final concentration of 500 unite, extracted meningioma DNA 2µl (Approximately 200 ng) repeated cycles 35 times using a PCR thermal cycler (TC-3000) at 95 C◦ for 3minutes, 94 for 20-20 seconds, 65 for 45 seconds, 72 for one minute, 57 for 45 seconds, and at 72 for 10 minutes.

Using 2% Agarose gel electrophoresis amplified PCR product were visualized

Results

A total of 180 intracranial meningioma patients were included in the present study, males were 60 constituting 33.3% and females were 120 constituting 66.7%, with male to female ratio 1:2, (Table.1). Linguistic affiliation of the study group showed that meningiomas were more common in Afro Asiatic tribes, 126 patients (70%),followed by Nilo Saharan in 48 patients (26.7%) and only 5 patients (2.8%) in Niger Congo tribes., (Table.2). Genotype of p53 gene showed that Arg/arg variant was more common in Afro-Asiatic group in 110 of the cases, followed by Nilo-Saharan in 44 cases, Table (3).

sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	60	33.3	33.3	33.3
	female	120	66.7	66.7	100.0
	Total	180	100.0	100.0	

Table 1 showed the frequency of male and female in meningioma patients

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		1	.6	.6	.6
	Afro Asiatic	126	70.0	70.0	70.6
	Nilo Saharan	48	26.7	26.7	97.2
	Niger Congo	5	2.8	2.8	100.0
	Total	180	100.0	100.0	

Table 2 showed frequency of linguistic affiliation in intracranial meningioma

Count

		p53T			
		arg/arg	arg/pro	pro/pro	Total
ftribe	Afro Asiatic	110	2	14	126
	Nilo Saharan	44	0	4	48
	Niger Congo	3	1	1	5
		0	1	0	1
Total		157	4	19	180

Table 3 showed cross tabulation of p53 gene alleles and Linguistic affiliation in intracranial meningioma

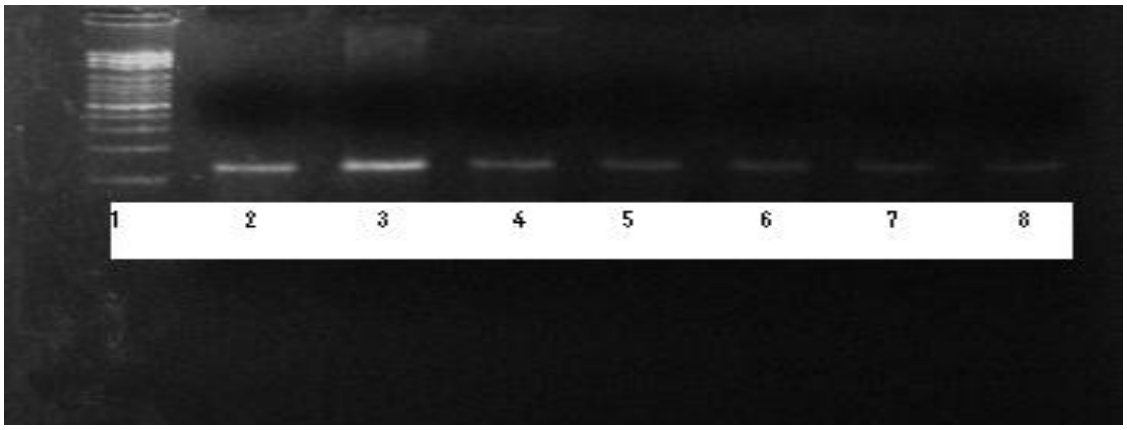

Fig 2 showed gel electrophoresis of P53 gene in meningioma.
Lane 1: DNA ladder (100bp) , Lane 2,3,4,5,6,7,8: Arg/arg variant (140 bp).

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 (4), 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, (5). In this study there was statistically significant correlation between meningioma incidence and the linguistic affiliation of the different Sudanese tribes. The Afro-Asiatic tribes were the most vulnerable group to have cranial meningioma. This finding could not be attributed to the geographical location of these tribes since members of the same tribe living in distant geographical areas have the tendency to develop meningioma. This is further supported by the finding that members of tribes affiliated to the other linguistic groups and sharing the same geographical locations with Afro-Asiatic tribes, did not report to have the same incidence of the disease. Of particular interest are the Niger- Congo affiliate tribes who are least prone to develop meningioma. These findings point towards possible genetic role that needs further search. Different studies were observed sharp ethnic differences in codon 72 allele frequencies. In the Northern hemisphere, the Pro72 allele shows a North-South gradient, from 0.17 in Swedish Saamis to 0.63 in African Blacks (Nigerian), (6). In Western Europe (France, Sweden, and Norway), North America (USA), Central and South America (Mexico, Costa-Rica, Peru) and Japan, the most common allele is Arg72, with frequencies ranging from 0.60 to 0.83. However, frequencies of Pro72 superior to 0.40 have been observed in African-Americans (7), and in Chinese's (8). In the present study , significant correlation was observed between P53 gene codon 72 alleles and Linguistic affiliation of the different

Sudanese tribes, a finding that necessitate further investigation in bigger number of sample.

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