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RESEARCH ARTICLE

P53 IMMUNOHISTOCHEMISTRY AND DIRECT SEQUENCING OF CODON 72 EXON 4, IN INTRACRANIAL MENINGIOMA AMONG SUDANESE PATIENTS

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ABSTRACT

Introduction: Meningiomas are predominantly benign tumors, which arise from the arachnoids' cap cells. They account for 20% of all primary intracranial neoplasms. The p53 tumor suppressor is a key regulator of cell cycle progression, such that its inactivation promotes increased cell growth and tumorigenesis. Inactivation of p53 as a consequence of *p14* ARF loss and *MDM2*-mediated degradation may contribute to meningioma progression.

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. All meningioma tumors at Elhassan Medical Laboratory for histopathology and cytology during the above mentioned period were processed for P53 protein immunohistochemistry. Data were analyzed using SPSS 13 software with reference P.value of 0.05 was considered statistically significant

Results: The sequencing results showed heterozygosity of T>G in exon 4 at protein position (Ala 70Ala) in 16 samples (10 fibrous, 5 atypical and one anaplastic). Within the afore-mentioned 16 samples, 6 samples showed heterozygosity of C>T in exon 4 at position Ala 69 Val, (this polymorphism had 5 features). Positive immmuno-staining for p53 was identified in 76% of the patients . Labelling indexes of 11-20% were reported in 32.8% of the p 53 group, mostly in fibrous and atypical subtypes of meningioma.

INTRODUCTION

Meningiomas are predominantly benign tumors, which arise from the arachnoids' cap cells (Louis *et al.,* 2007). The development mechanism is unknown but they may result from an adverse effect of cranial irradiation and trauma (Harrison *et al.,* 1991). Meningiomas may occur intracranially or within the spinal canal in some cases. As a tumor suppressor, p53 is essential for preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress. Following various intracellular and extracellular stimuli, such as DNA damage (by means including ionizing radiation, UV radiation, application of cytotoxic drugs or chemotherapeutic agents, and infectious virus), heat shock, hypoxia, and oncogene over expression, wt p53 is activated and emerges as a pivotal regulatory protein which triggers diverse biological responses, both at the level of a single cell as well as in the whole organism. (Jallepalli *et al.,* 2003) p53 activation involves an increase in overall p53 protein level as well as qualitative changes in the protein through extensive posttranslational modification, thus resulting in activation of p53-targetedgenes (Hess *et al.*,1994). The polymorphic variant at codon 72 has been shown to be an intragenic modifier of mutant p53 behavior, (Schneider-Stock *et al.,* 2004). 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 (Bellini *et al.,* 2010), have aslo found that in Arg/Pro germ line heterozygote, the Pro allele is preferentially lost and the Arg allele is preferentially mutated (Bellini *et al.,* 2010). Several studies in the past have demonstrated a proportionate increase in proliferative index with increasing tumor grade (Amatya *et al.,* 2004). Though assessment of proliferative activity is a good indicator of tumor aggressiveness, p53 expression was studied to evaluate its role in tumor biology. p53 is responsible for tumor progression in many malignant tumors and its over

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expression has been detected in these tumors. Earlier studies have demonstrated variable results with regards to alteration in p53 pathway in meningiomas (Amatya *et al.,* 2004). The diversity in expression always correlated with the tumour grade and aggressive biology (Karamitopoulou *et al.,* 1998). A high proliferative index and p53 expression in grade 11 and 111 meningiomas confirms their aggressive nature. A uniformly high expression of p53 in grade 11 and 111 meningiomas identifies its role in causing genomic instability in this subgroup of aggressive meningioma.

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. For immunohistochemistry, all meningioma tumors at Elhassan Medical Laboratory for histopathology and cytology during the above mentioned period were processed for P53 protein immunohistochemistry. 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, ImmunostainingAccordin to Dako standard protocol for immunostaining (Ruttledge *et al.,*1996) the all intracranial meningioma tissue from archival paraffin embedded blocks from Elhassan Medical Laboratory for Histopathology and cytology at Khartoum Sudan, were sectioned, special immunostaining slides were used, 4 µl sections were obtained, and then incubated in the oven at 65 oC for overnight, then all sections were treated with xyline, absolute ethanol, 90% ethanol, 70% ethanol, respectively and washed in water, then in retrieval solution at 95 oC in water path for 30 minutes, Dako pen was used for drawing a circle around the reaction area on the slides, all slides were placed in washing buffer for 10 minutes, then hydrogen peroxide was performed to all sections for peroxidase blocking, then washed in washing buffer for 15 minutes, the primary antibody was added to all sections for 30 minutes, then washed in buffer for 15 minutes, followed by link solution (HRP) for 25 minutes, and washed in buffer for 15 minutes, and then diluted DAB solution was added for 10 minutes, then washed twice in water and washing buffer respectively, then Mayers heamatoxyline was used as a counter stain, then after that cover slips and DPX mounting medium were used for mounting and preserving sections, all sections were examined under the light microscope, Labeling index for p53 protein, was calculated as the percentage of tumor cell nuclei that stain positive out of the total number of tumor cell nuclei counted, as (1-5%, 6-10%, 11-15%, 16-20%). Data were analyzed using the Statistical Package for Social Sciences (SPSS) 13 software with reference P.value of 0.05 was considered statistically significant.

RESULTS

The immunostaining results p53 protein were displayed in Table (1, 2, 3, 4) Positive immmuno-staining p53 was identified in 76% of the patients respectively. Labelling indexes of 11-20% were reported in 32.8% of the p 53 group, mostly in fibrous and atypical subtypes of meningioma. The sequencing results showed heterozygosity of T>G in exon 4 at protein position (Ala 70Ala) in 16 samples (10 fibrous, 5 atypical and one anaplastic). Within the afore-mentioned 16 samples, 6 samples showed heterozygosity of C>T in exon 4 at position Ala 69 Val, (this polymorphism had 5 features). (Table 5)

Table 1. showed the frequency of positive and negative immunostain of p53 protein in meningioma tumors

P =0.002

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	70	76.1	76.1	76.1
	nagative	22	23.9	23.9	100.0
	Total	92	100.0	100.0	

Table 2. showed the frequency of p53 protein labeling index in meningioma tumors

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-5%	35	50.0	50.0	50.0
	6-10%	12	17.1	17.1	67.1
	11-15%	13	18.6	18.6	85.7
	16-20%	10	14.3	14.3	100.0
	Total	70	100.0	100.0	

DISCUSSION

Loss of heterozygosity in p53 tumor suppresser gene had been studied in different types of brain tumors (Whibley *et al.,* 2009), 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 (Ruttledge *et al.,* 1996). The most common genetic abnormalities were the loss of heterozygosity in chromosome 17p where p53 is located (Phelan *et al.,* 1995). The fact that mutated p53 protein has an extended half life due to protein accumulation, made it possible to be detected by immunohistochimistry techniques. Direct sequencing of p53 gene showed, heterozygosity of T>G in exon 4 at protein position (Ala 70Ala) in 16 samples (10 fibrous, 5 atypical and one anaplastic). Within the afore-mentioned 16 samples, 6 samples of recurrent tumors, (4 fibrous and 2 atypical), showed heterozygosity of C>T in exon 4 at position Ala 69 Val. A study done in Korea, found that the atypical and malignant meningiomas showed higher rate of p53 gene mutation than benign meningioma. (Cho *et al.,* 1999). Another study from China has investigated the frequency of TP53 gene mutations in human tumors derived from meningeal tissues including 2 malignant and 4 atypical meningiomas, as well as 2 hemangioblastomas and 3 hemangiopericytomas, and were screened by immunohistochemistry. (PCR-SSCP) and direct sequencing, has reported p53 mutation only in the malignant

Table 3. showed cross tabulation between p53 protein labeling index in meningioma and histopathology. P>0.057

Crosstab							
			labelingp53				Total
			1-5%	6-10%	11-15%	16-20%	
histopathology	fibrous	Count	9	2	4	3	18
		% within histopathology	50.0%	11.1%	22.2%	16.7%	100.0%
	atypical	Count	11	1	7	6	25
		% within histopathology	44.0%	4.0%	28.0%	24.0%	100.0%
	meningiothelial	Count	2	6	2	0	10
		% within histopathology	20.0%	60.0%	20.0%	.0%	100.0%
	mixed	Count	6	2	0	0	8
		% within histopathology	75.0%	25.0%	.0%	.0%	100.0%
	angiomatous	Count	1	0	0	0	1
		% within histopathology	100.0%	.0%	.0%	.0%	100.0%
	psammomatous	Count	3	0	0	0	3
		% within histopathology	100.0%	.0%	.0%	.0%	100.0%
	anaplastic	Count	1	0	0	1	2
		% within histopathology	50.0%	.0%	.0%	50.0%	100.0%
	clear cell	Count	2	1	0	0	3
		% within histopathology	66.7%	33.3%	.0%	.0%	100.0%
Total		Count	35	12	13	10	70
		% within histopathology	50.0%	17.1%	18.6%	14.3%	100.0%

Table 4. showed cross tabulation between p53 protein labeling index in meningioma and WHO grading P> 0.18

Crosstab							
			Labelingp53				Total
			1-5%	6-10%	11-15%	16-20%	
WHOgrade	grade 1	Count	26	10	14	0	50
		% within WHOgrade	52.0%	20.0%	28.0%	.0%	100.0%
	grade 11	Count	11	0	16	2	29
		% within WHOgrade	37.9%	.0%	55.2%	6.9%	100.0%
	grade 111	Count	0	1	0	1	2
		% within WHOgrade	.0%	50.0%	.0%	50.0%	100.0%
Total		Count	37	11	30	3	81
		% within WHOgrade	45.7%	13.6%	37.0%	3.7%	100.0%

Table 5. showed sequencing results of p53 gene codon 72 in exon 4

Frequency of wt. allele	Frequency of mutant allele	wt.Cdon	Mutant codon	rs. Number/variation	wt. aa	Mutant aa	Type of mutation
CC=0.83	TT=0.17	GCT	GTT	Variation:TP53 g-11437C>T	Alanine	Valine	Missense variant
TT=0.52	GG=0.48	GCT	GCG	novel	Alanine	Alanine	silent

types of meningioma and concluded that p53 gene mutation may be considered as a marker for malignant transformation in meningioma (Wang *et al.*, 1995). The findings of the present study have indicated that fibrous meningioma, atypical and malignant meningioma showed similar pattern of p53 mutation. Furthermore 22.2% of the fibrous meningiomas in this study expressed p53 Li of 11-15%, and 28% of the atypical meningiomas had the same Li of p53 protein., Several studies in the past have demonstrated a proportionate increase in p53 proliferative index with increasing tumor grade (Amatya *et al.*, 2004; Karamitopoulou *et al.*, 1998). The diversity in expression always correlated with the tumour grade and aggressive biology (Karamitopoulou *et al.*, 1998). A high proliferate index and p53 expression in grade 11 and 111 meningiomas was reported and attributed its role in causing genomic instability in aggressive meningioma (Amatya *et al.*, 2004). The results of this study have shown p53 mutation and high prolifrative index in fibrous as well as atypical menigioma. This finding might indicate more aggressive behavior of fibrous meningioma which was considered as benign type. This is further supported by alteration of alanine to valine at codon 72 in position Ala69Val, in the fibrous meningioma as well as in atypical and malignant

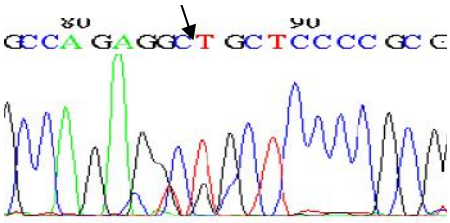

Fig. 1. showed heterozygosity of T>G at position (Ala70Ala)

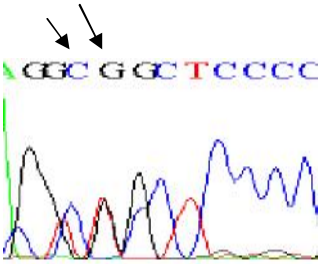

Fig. 2. showed typical heterozygosity of T>G at position (Ala70Ala) and C>T at position (Ala69Val)

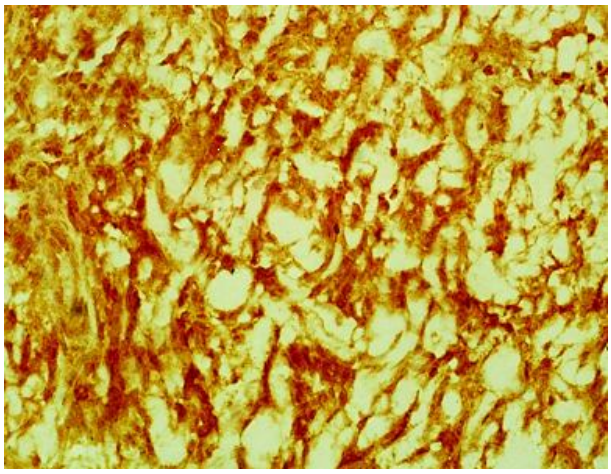

Fig. 3. showed p53 labeling index more than 16-20%, in the fibrous subtype of meningioma

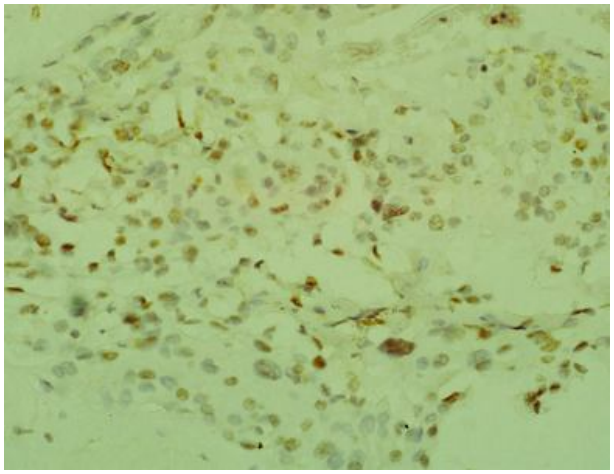

Fig. 4. showed p53 labeling index 16-20%, in atypical subtype of meningioma

meningiomas. Wolf *et al.* (2004) found that, Psoriasis patients exposed to high cumulative doses of psoralen + ultraviolet A frequently exhibit so-called "psoralen + ultraviolet A keratoses" (i.e., hyperkeratotic lesions with varying degrees of histologic atypia). In these patient there was significant mutations of both p53 and Ha-ras, with ultraviolet fingerprint type (C>T or CC>TT transitions at dipyrimidine sites). In the present study, C>T mutation was found in six recurrent cases, 4 were fibrous and two were atypical meningioma. C>T mutation may indicate possible association with tumor recurrence, a finding that necessitate further investigation in bigger number of recurrent sample.

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