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RESEARCH ARTICLE

KI 67 ANTIGEN IMMUNOHISTOCHEMISTRY IN INTRACRANIAL MENINGIOMA AMONG SUDANESE PATIENTS

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ABSTRACT

Introduction: Meningiomas are predominantly benign tumors, which arise from the arachnoids' cap cells. The development mechanism is unknown but they may result from an adverse effect of cranial irradiation and trauma. Antigen KI-67 also known as Ki-67 or MKI67 is a protein that in humans is encoded by the MKI67 gene (antigen identified by monoclonal antibody Ki-67). The antigen KI-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. Inactivation of antigen KI-67 leads to inhibition of ribosomal RNA synthesis. Ki-67 is an excellent marker to determine the growth fraction of a given cell population, the fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer.

Materials 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 ki 67 antigen.

Results: Positive immmuno-staining for Ki67 was identified in 88% of the patients. Labelling indexes of 11-20% were reported in 40% of the Ki 67 group. Mostly in fibrous and atypical subtypes of meningioma.

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 (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. Despite more recent data on meningiomas, traditional nomenclature consisting of histogenetically and clinically irrelevant terms continues to be used because of the extremely broad morphologic spectrum exhibited by meningiomas. The old term "angioblastic meningioma" that included hemangiopericytomas is a misnomer because hemangiopericytomas are derived not from meningothelial cells, but more likely from pericytes, hence, they are considered sarcomas. Today, the term "meningioma" refers strictly to tumors originating from meningothelial, or arachnoidal cells, regardless of histology. 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 (Nakasu *et al.*, 1985). 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 and Abdul-Rahman (1988) reported similar results in a material of 127 cerebral tumors during 10 years time (Abu Salih and Abdul-Rahman 1988). In Sudan, cancer registry has faded

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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. However, during childhood female preponderance is less pronounced and boys are more affected than girls. Meningiomas are tumors of adults with main age of incidence ranging between 20-40 years and peak of incidence around 40 years. In Children meningiomas account for less than 2% and less than 2% of intracranial tumors of childhood. In this regard, long-term follow-up data from the Danish Cancer Registry found that meningiomas are rare tumors in children, but these children had an overall survival of 35% and a mean survival of only 10 years. Young patients should be watched more carefully, as their meningiomas may grow more rapidly than adult tumors. Most benign meningiomas, regardless of patient gender, acquire a variety of hormone receptors during tumor genesis, the best established of which is the progesterone receptor. The association between hormone receptor expression and meningiomas has been used to explain the discordant prevalence of meningioma in females, where the overall ratio is 2:1 in the brain and up to 10:1 in the spinal cord (Staneczek and Janisch 1992). However, the hormonal stimulation hypothesis in women is complex, since meningiomas in men and children may also express the same receptors (Korhonen *et al.,* 2006). The frequency of meningiomas has been the topic of relatively few reports, and those provide information from either hospital- or population-based studies. Hospital-based brain tumor series indicate that the incidence is approximately 20% of all intracranial tumors, a figure derived from the combined results of several large series (Nayar *et al*., 2010).

Ki 67 antigen

Antigen KI-67 also known as Ki-67 or MKI67 is a protein that in humans is encoded by the MKI67 gene (antigen identified by monoclonal antibody Ki-67) (Verheijen *et al.,* 1989). The antigen KI-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation (Verheijen *et al.,* 1989). Inactivation of antigen KI-67 leads to inhibition of ribosomal RNA synthesis (Cole *et al.,* 1992). Ki-67 is an excellent marker to determine the growth fraction of a given cell population, the fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer (Cole *et al.,* 1992; Kill *et al.,* 1994; Fonatsch *et al.,* 1991; Maier *et al.,* 1997). The Ki-67 antigen (pKi-67) was first identified by virtue of its reactivity with Ki-67 antibodies (Gerdes 1983). pKi-67 is detected in the nucleus of proliferating cells in all active phases of the cell division cycle, but is absent in non-proliferating cells. During interphase, pKi-67 is localised mainly in the nucleolus (Gerdes 1983).

MATERIALS 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 ki 67 antigen. Immunostaining Accordin to Dako standard protocol for immunostaining (Karamitopoulou *et al.,* 1998) 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 by professional pathologist and histotechnologist, Labeling index for ki67 antigen 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 processing and statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) 13 software with reference P.value of 0.05 was considered statistically significant.

RESULT

The immunostaining results of Ki67 antigen was displayed in Table (I, II, III, IV). Positive immmuno-staining for Ki67 was identified in 88% of the patients. Labelling indexes of 11-20% were reported in 40% of the Ki 67 group. Mostly in fibrous and atypical subtypes of meningioma.

Table I. showed the frequency of positive and negative immunostain of Ki67 antigen in meningioma tumors

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	81	88.0	88.0	88.0
	negative	11	12.0	12.0	100.0
	Total	92	100.0	100.0	

Table 2. showed the frequency of Ki67 antigen labeling index in meningioma tumors

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-5%	37	45.7	45.7	45.7
	6-10%	11	13.6	13.6	59.3
	11-15%	30	37.0	37.0	96.3
	16-20%	3	3.7	3.7	100.0
	Total	81	100.0	100.0	

Table 3. showed cross tabulation between Ki67 antigen labeling index and histological subtypes of meningioma. P>0.002

Crosstab

			labelingki67				Total
			1-5%	6-10%	11-15%	16-20%	
histopathology	fibrous	Count	11	1	12	0	24
		% within histopathology	45.8%	4.2%	50.0%	.0%	100.0%
	atypical	Count	10	0	15	2	27
		% within histopathology	37.0%	.0%	55.6%	7.4%	100.0%
	meningiothelial	Count	4	5	2	0	11
		% within histopathology	36.4%	45.5%	18.2%	.0%	100.0%
	mixed	Count	8	3	0	0	11
		% within histopathology	72.7%	27.3%	.0%	.0%	100.0%
	angiomatous	Count	1	1	0	0	2
		% within histopathology	50.0%	50.0%	.0%	.0%	100.0%
	psammomatous	Count	1	0	0	0	1
		% within histopathology	100.0%	.0%	.0%	.0%	100.0%
	anaplastic	Count	0	1	0	1	2
		% within histopathology	.0%	50.0%	.0%	50.0%	100.0%
	clear cell	Count	1	0	1	0	2
		% within histopathology	50.0%	.0%	50.0%	.0%	100.0%
	secretory	Count	1	0	0	0	1
		% within histopathology	100.0%	.0%	.0%	.0%	100.0%
Total		Count	37	11	30	3	81
		% within histopathology	45.7%	13.6%	37.0%	3.7%	100.0%

Table 4. showed cross tabulation between Ki67 antigen labeling index and WHO grading of meningioma. P >0.000

Crosstab

			labelingki67				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 the recurrence cases within the labeling index of Ki67 antigen

		recuryes				Total
		1-5%	5-10%	11-15%	16-20%	
histopathology	fibrous	3	1	3	0	7
	atypical	2	2	5	1	10
	anaplastic	0	0	0	1	1
	secretory	1	0	0	0	1
Total		6	3	8	2	19

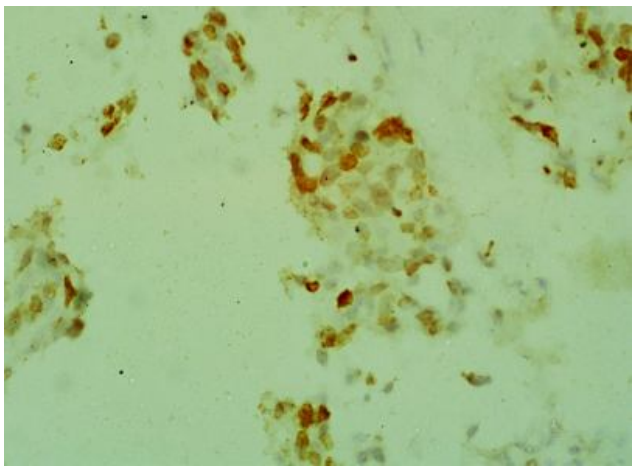

Fig.1. showed ki 67 labeling index 11-15%, in atypical meningioma

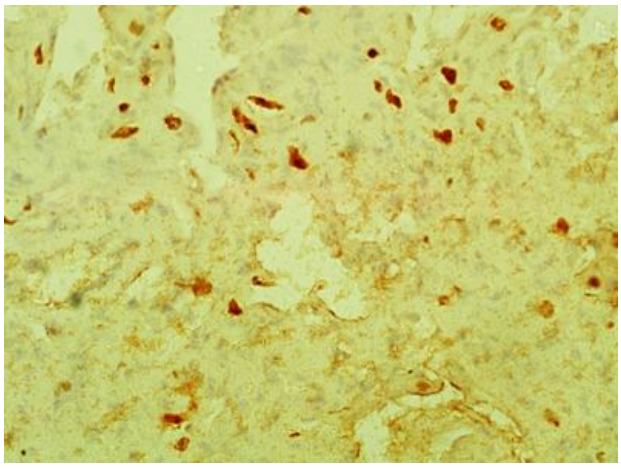

Fig.2. showed ki 67 labeling index 6-10%, in fibrous meningioma

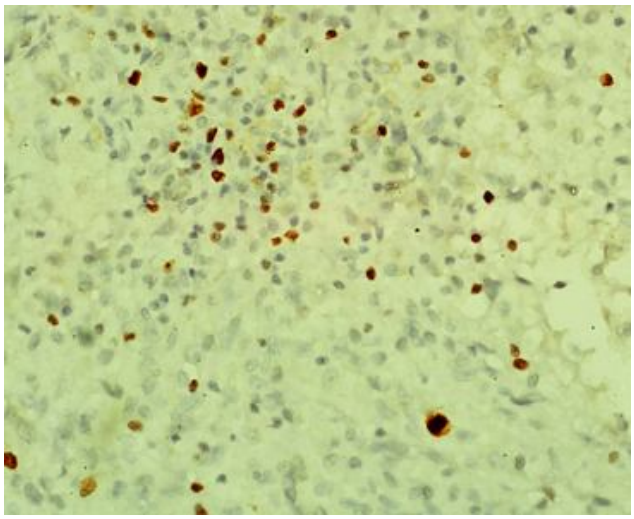

Fig.3. showed ki 67 labeling index 11-15%, in atypical meningioma

DISCUSSION

In the present study immunoreactivity of, Ki-67 antigen was identified in 81 of the cases (88%). In these cases, high labeling index of Ki67 (11-15%) was identified in 50% of the fibrous and 55% of the atypical subtypes. This finding revealed that fibrous histolological subtype of meningioma behave like grade 11 atypical meningioma. Recurrence among the Ki67 immuno-reactive group was reported in 19 cases, 12 of whom were grade II and III meningioma and the remaining 7 cases (38%) were fibrous subtype. Different studies have reported on association of Ki67 Li and recurrence, (Abramovich and Prayson 1999; Torp *et al.*, 2005; Schiffer *et al.*, 2005), however, the results of the present study do not support Ki 67 Li as a sole predictor of recurrence since more than 60% of meningioma patients with Ki67Li of more than 11% did not report with recurrence.

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