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Original Article

An Immunohistochemical Study of P63 Protein Expression in Meningioma

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ABSTRACT

Background and Objective: **Histological grading has prognostic and therapeutic implications in meningioma. However, histological criteria are sometimes not fulfilled to predict the biological behavior of meningiomas. P63 gene is a novel P53 family member, known as tumor suppressor factor, with multiple isoforms and shows high expression in many normal and neoplastic human tissues. In this study, we investigated expression of P63 in different histological grades of meningiomas.**

Material and Methods: **For this purpose, we studied and analyzed the immunohistochemical expression of P63 by a monoclonal antibody (clone 4A4) that recognizes all P63 isoforms in 52 cases of meningioma including WHO grade I (42 cases), grade II (7 cases) and grade III (3 cases). Correlation between histological grade and parameters like cell nuclear immunoreactivity to P63 antibody, age, gender and site of meningioma was analyzed by parametric and non-parametric statistical tests.**

Results: **Among 52 patients analyzed, there were 35 females (67.3 %) and 17 males (32.7%) between 13 and 75 years old. Histological grading revealed 42 cases (80.8%) as WHO grade I, 7 (13.5%) as grade II and 3 (5.8%) cases were grade III. Expression of P63 protein was found in 83.2% of cases with grade I but in grade II and III, all of the cases were immunoreactive, so as in grade III, 100% of cases were with higher percent of nuclear reactivity in our study. Correlation between histological grade and nuclear immunoreactivity was highly significant (p=0.001).**

Conclusion**: We conclude that the immunohistochemical staining of P63 will be valuable in discrimination of different grades of meningiomas.**

Key words: Meningioma, p63 protein, Immunohistochemistry

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Introduction

Meningioma as only neoplasm exhibiting morpho-logic and immunophenotyping evidences of an origin from meningothelial cells are mostly encountered in middle and later adult life. Females are more afflicted and most meningiomas arise within the cranial cavity. They are mostly benign tumors that can be cured by surgical resection. Because meningiomas tend to recur, long term management in patients remains controversial (1;2). Histological grading of meningioma has high prognostic and clinical therapeutic value but histological analysis has limited value to predict the biological behavior of meningiomas. Previous studies have shown many indicators for prediction of behavior of meningiomas such as proliferative markers Ki67, ER, PR, AgNOR and expression of P53 and P73 in this tumor (3-6).

P63, a novel P53 family member, has remarkable structural similarity with P53 and analogous biological functions as tumor suppressor and apoptotic factor. These factors regulate key cellular processes and over-expression of p63 may act via a negative effect on the endogenous p53 for regulation of cell cycle and apoptosis (7;8). In this study, we analyzed expression of P63 protein in various types of meningioma for finding possible correlation between P63 expression and histological grading of this tumor.

Materials and Methods

In this retrospective study, 52 patients who had meningioma in extra- and intra-cranial cavity and referred to Ghaem hospital of Mashhad medical center were selected. All of surgical specimens of these cases underwent additional sectioning and reviewed by two pathologists for grading based on WHO grading system for meningioma. Then, additional sections at 4- micrometer thickness were prepared for immunohistochemical staining. Imm unoperoxidase,streptoavidin-biotin procedure was performed. After deparraffinization and hydration, slides incubated with 3% hydrogen peroxide for 20 minutes. Antigenic retrieval was done by incubation with 1% citrate buffer (pH=6) in microwave oven for 20 minutes. Then, slides incubated with anti-human p63 protein (Clone 4A4, Dako Company, Denmark) for 60 minutes at room temperature. In the next step, biotinylated link, anti-mouse and anti-rabbit immunoglobulin and streptoavidin-peroxidase (DAKO LSAB 25 system, Peroxidase kit, Denmark) was used. Peroxidase was shown by diaminobenzidin hydrochloride (DAB). Counterstaining was done with Hematoxylin-Mayer and mounted in Canadabalsam.

For negative control, antibody was omitted from this process and for positive control, normal skin and prostate tissue was used. Microscopic slides after immunohistochemistry evaluated and interpreted by two pathologists. In this study, Spearman's and Chi-square T statistical tests were performed using SPSS version 14.

Results

We studied the immunohistochemical expression of p63 in 52 cases of meningiomas including 17 males (32.7%) and 35 females (67.3%). Mean age of studied patients were 49 ± 14.4 years (the youngest was 13 years and the oldest was 75 years old), for female patients it was 47.6 ± 13.7 years and for males it was 51.9 ± 15.8 years. No significant correlation between these two groups was found out using statistical T test (p=0.32).

Out of 52 cases with different histological type of meningioma, 80.8% (42 cases) were benign, 13.5% (7 cases) were atypical and 5.8% (3 cases) were anaplastic. In addition, 7 cases (13.5%) did not label for P63 marker and 23 cases (44.2%) had a nuclear immunoreactivity of 1+ (1-25% of cells) and12 cases (23.1%) had a reactivity of 2+ (26-50% of cells), 5 cases (9.6%) had a reactivity of 3+ (51-75% of cells) and in 5 cases (9.6%), more than 75% of cells were immunoreactive for p63 (Figure1). Anatomic site of meningioma in our patients was intra-cranial for 48 cases (92%) and it was extra cranial for 4 cases (8%).

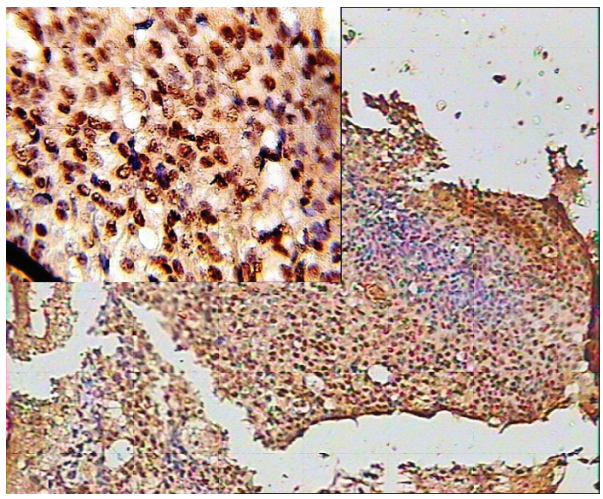

Figure 1: Nuclear Immunoreactivity to p63 antibody in WHO grade III meningioma cells [more than 75% of cells are rective] – (p63 Immunohistochemical staining, × 100-400)

In this study, Spearman's statistical test showed significant correlation between histological grade of meningioma and expression of p63 in meningioma cells (p=0.001) (Table 1). Also, Chi-square test showed a significant statistical difference between histological grade and gender of patients but no such reactivity was found out between reactivity of meningioma cells and gender. (p=0.03).

Table 1: p63 immunoreactivity in different histologic subtypes of studied cases

Count				
P63 Immunoreactivity	Histologic Grade			Total
	Benign	Atypical	Anaplastic	
Negative	7			7
1-25% +	20	2		22
26-50% +	9	3		12
51-75% +	4	1		5
76-100% +	2	1	3	6
Total	42	7	3	52

Discussion

Meningiomas are tumors derived from arachnoidal cells within the arachnoidal villi and the stroma of perivascular spaces and choroidal plexus. They account for about 13-19% of intracranial tumors. Meningioma usually became clinically evident at middle age and there is a marked female predominance (3/1). Loss of heterozigocity for loci on chromosome 22 has been demonstrated in 40-80% of sporadic tumors and this correlates with mutations in the NF2 gene in most cases. Its incidence appears to be related to histological type. Numerous variations of meningiomas have been described reflecting the mesenchymal and epithelial histogenic potential of arachnoidal cell. Although the majority of these variations exhibit similar biological behavior, some are associated with systemic disease. For example, papillary, clear cell and chordoid variants behave in a more aggressive fashion which includes a very high recurrence rate and spinal seeding. Histological grading of meningiomas has prognostic and clinical therapeutic implications that based on WHO grading system. According to this system, meningiomas were histologically classified into 3 different grades: well differentiated (grade I), atypical (grade II), and anaplastic (grade III). The grade I meningioma is a lobular, well differentiated lesion, generally meningotheliomatous, psammomatous, fibrous or microcystic type without evidences of brain invasion, necrosis and rare mitoses. The atypical meningioma has intermediate cytological and histological grade. Lesion has 4 or more mitoses per 10 HPF or demonstrates 3 or more of the following variables: loss of lobular architecture, prominent nucleoli, increased cellularity, high nucleocytoplasmic ratio, and foci of necrosis and brain invasion. Anaplastic type of meningioma exhibits atypical feature in addition to mitotic activity 20 or more per 10 HPF. According to WHO system, both rhabdoid and papillary lesions are assigned grade III.

However, these histological criteria are sometimes not fulfilled and other criteria are necessary for prediction of biological behavior of meningioma. Many surveys have been done for solving this problem such as evaluation of cell proliferation index (MIB-1, AgNOR), cytological studies, and immunohistochemical assessment. Cytogenetic analysis revealed loss of heterozygosity of chromosomes 1, 6, 10, 14 in meningioma that are associated with more aggressive lesion. The most common cytogenetic abnormality in meningioma is monosomy 22. Also, atypical and malignant meningiomas have usually loss or losses on chromosome 1p. Immunohistochemical studies have shown that meningioma as a whole is immunoreactive for EMA and vimentin and focally positive for S-100 protein and over half of meningiomas are positive for progesterone and staining for estrogen receptors is usually negative. Immunoreactivity for progesterone has also been correlated with good outcome. In one study MIB-1 labeling index was significantly higher in the progesterone receptor negative lesion. In another study atypical and malignant meningiomas were more frequently negative for progesterone receptor (6;9).

Mutation of p53 tumor suppressor gene has been confirmed in many primary tumors of central nervous system. P63 as a novel member of p53 family has structural and biological similarity with p53 as a tumor suppressor protein. P63 like p53 is powerful in transactivation and apoptosis assays. Alteration in p63 expression has been proved in many human cancers when a cell incurs DNA damages. In this respect p63 down-regulates and allowing p53 to step into action, protecting the cell from damaged DNA. Deregulating and over-expression of p63 would interfere with the protective role of p53. Osada et al reported mutations in p63 and its over-expression in many carcinoma and amplification of chromosomal location of p63 gene has been observed in cervical carcinomas supporting

the role of deregulated p63 expression in epithelial tumorogenesis and over-expressed p63 was found out in large series of urothelial cancers (10-13). These studies confirm the role of p63 over-expression and offer clinical usefulness of this factor in predicting behavior of neoplasms. The predominant isoform of p63, i.e. Np63 is limited to proliferating basal and suprabasal cells but is gradually lost when these cells withdraw from the stem cell compartment. Although the discovery of this homologous p53 like p63 and p73 has been an instant excitement and quick, but their precise role in tumor biology is still a challenge and there are evidences that these genes (p53, p63, and p73) play an important role in human cancers (14-21). For these reasons, we decided to investigate p63 expression in most common tumors of meningothelial cells and to evaluate correlation between p63 expression, histological grade and biological behavior of meningioma.

Conclusion

It was found out that there exists a significant statistical correlation between histologic grade of meningioma and p63 expression. Considering the sensitivity of immunohistochemistry method and probably its technical failures, further studies are needed for confirmation of the results of this study.

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