Ki-67 cell proliferation in familial and in sporadic unilateral retinoblastoma: case report

Avaliação da proliferação celular com marcador Ki-67 em pacientes com retinoblastoma unilateral esporádico e familiar: relato de caso

ABSTRACT

Purpose: Ki-67 is a nuclear protein that is expressed at all phases of the cell cycle except the resting phase. This study is a clinicopathologic observational case report that aims to report on the cell proliferation rates, as measured by the Ki-67 antigen, in two enucleated retinoblastoma eyes.

Methods: One unilateral familial (mother with unilateral disease - patient 1) and one unilateral sporadic retinoblastoma (patient 2) patients were submitted to enucleation without previous treatment. The tumor cell proliferation rate was assessed by the Ki-67 antigen labeling index (stained cells / 100 cells) in five different fields of the tumor.

Results: Patient 1 was 23 months old and the tumor was exophytic with associated neovascularization of the iris; patient 2 was 6 years old and the tumor was endophytic with coarse vitreous seeds. Both enucleated eyes presented optic nerve with free surgical margins. Positive Ki-67 cell index in patient 1 varied from 75 to 90 (MD ± SD: 79.5 ± 6.61) and in patient 2 from 38 to 60 (MD ± SD: 46.6 ± 8.2).

Conclusions: The familial retinoblastoma, besides the earlier age presentation, showed 45.8% more Ki-67 positive cells than the same stage sporadic one. This proliferation rate may explain the earlier presentation age of the tumor in the inherited disease.

INTRODUCTION

Retinoblastoma is the most common intraocular malignancy in childhood\(^1\). This highly malignant neoplasm is known to emerge from the nuclear layers of the retina following two genetic bouts that suppress both alleles of the RB1 gene\(^2\). The diagnosis of the site of mutation, only in the tumor or, in the tumor and elsewhere in the body is the straight way to separate sporadic from familial disease. Since in the detection of the mutation through genetic mapping or through the indirect method of the esterase D activity\(^3\) cell culture is involved and is not generally available or cost effective, indirect clinical clues are used to assume the double germ mutation in a retinoblastoma case. The clinical indicators for inherited disease are early appearance age, bilateral disease and positive family history. Quantitative immunohistochemical analysis of cell proliferation, apoptosis and angiogenesis, have been reported recently in oncology\(^4\)\(^-\)\(^10\), and in other areas in ophthalmology\(^11\). For retinoblastoma it was shown that the proliferation index, instead of the apoptotic rate\(^4\)\(^-\)\(^9\), and the vascular pattern\(^4\)\(^-\)\(^9\) correlated better with the prognosis. The cell proliferation rates as measured by the Ki-67 antigen in two enucleated unilateral retinoblastoma eyes are reported.
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One unilateral familial (mother with unilateral disease - patient 1) and one unilateral sporadic (patient 2) retinoblastoma patients were submitted to enucleation only. For immunohistochemical evaluation of Ki-67 the tissue sections were deparaffinized and hydrated using xylene and graded alcohol. Endogenous peroxidase was quenched with 3.0% hydrogen peroxide in methanol for 10 minutes. Following phosphate-buffered saline (PBS) rinses, sections were immersed in citrate buffer (10 mM citric monohydrate, adjusted to pH 6.0) and heated in a microwave over at boiling point for 20 minutes. Then, the microwave-irradiated sections were cooled to room temperature and washed with PBS. They were then incubated in protein blockage solution (Zymed Laboratories, San Francisco, CA, USA) for 10 minutes. Sections were incubated with primary antibodies; mouse anti-human monoclonal antibody (Neomarkers, Fremont, CA, USA) diluted in 1:100 PBS for Ki-67 immunostaining. After PBS rinse, sections were treated with biotinylated second antibody (Zymed) for 10 minutes and enzyme conjugate (HRP-streptavidin, Zymed) for 10 minutes at room temperature(12). Once Ki-67 positive cell density may not be uniform inside the same tumor(4), the tumor cell proliferation rate was assessed by the Ki-67 stained cells, in 100 cells, of five different fields of the tumor. The mean, called proliferation index (P) and pattern deviation (Mean ± SD), as well as the median of positive nuclear stained cells in the 5 fields, was calculated and the differences between the two patients are shown in percentage.

RESULTS

Patient 1 was a 23-month-old boy with the right eye affected. The enucleation was chosen as the only treatment based on the presence of extensive iris neovascularization. The anatomicopathologic analysis showed a large, mostly exophytic tumor (17 mm at the base), 60% necrotic with sparse calcification and Hommer-Wright rosettes. The tumor invaded the choriocapillaris but spared the choroids themselves; the optic nerve was invaded up to the lamina cribosa with free surgical margins. The P value in this patient varied from 75 to 90 (79.5 ± 6.61), median: 84 (Figure 1 A, B). Patient 2 was a 6-year-old girl with bilateral disease, however, P was not significantly higher for advanced clinical stage than for less advanced disease. It was suggested that unregulated cell growth contributes to a more aggressive disease phenotype for tumors without a germinal loss of pRb(7). We demonstrated a higher proliferation index in the inherited case (mean: 79.5, median 84) than in the sporadic one (mean: 46.6, median 45) in the same tumor stage. These two high P indices are in accordance with the literature for advanced stage disease but our patients are free of systemic disease in the follow-up period of 2 years and therefore considered cured. Though it was shown that high prolifera-

DISCUSSION

The balance between proliferation and cell death is the major determinant of tumor growth. For retinoblastoma it was shown that the proliferation rate, instead of the apoptotic rate(4-9), and the vascular pattern(8-9) correlated better with the prognosis. Antigen Ki-67 reacts with a DNA associated antigen in the nuclei at all phases of the cell cycle except the resting phase and it is considered a gold standard for cell proliferation in many tissues(8,10-11) with the advantage related to its availability and technical easiness. Recent reports showed a positive correlation between P and clinical stage(5,6). In a series of 33 retinoblastomas (5 bilateral) the reported mean P value was 21 ± 2.1 and, if the proliferative index was in excess of 40, it was clearly associated with unfavorable diagnosis(5). In another report of 62 unilateral and 24 bilateral cases of nonfamilial retinoblastoma, a highly significant correlation between stage and P value for unilateral disease was shown; for the advanced stages (3 and up), P was 81.25 ± 6.78 and for stages 1 and 2 P was 69.50 ± 9.45 (p=0.001). Among children with bilateral disease, however, P was not significantly higher for advanced clinical stage than for less advanced disease. It was suggested that unregulated cell growth contributes to a more aggressive disease phenotype for tumors without a germinal loss of pRb(7). We demonstrated a higher proliferation index in the inherited case (mean: 79.5, median 84) than in the sporadic one (mean: 46.6, median 45) in the same tumor stage.

Arq Bras Oftalmol. 2007;70(2):347-9
tion rate is correlated with a poor prognosis\textsuperscript{5,8}, a well-performed enucleation with free surgical margins seems to play an important role in the prevention of a disseminated disease. The inherited retinoblastoma, besides its earlier age presentation, showed 45.8% more Ki-67 positive cells than the sporadic one, in the same stage. The difference in the P index in our patients could be explained by the presence of a different amount of Rb protein (pRb) in the tumor cell nuclei of the familial and the sporadic retinoblastoma. Although it was unexpected, different amount of this suppression factor (pRb) was found in retinoblastoma and, such as in other malignancies\textsuperscript{11}, its expression was shown to relate inversely with the P index \textsuperscript{(7)}. The high P in patient 1, associated with other factors, may have been responsible for the earlier appearance of the tumor in this inherited disease. If future prospective investigations with antigen Ki-67 labeling confirm the present study, and an index for familial and sporadic cases is settled, Ki-67 labeling could be an additional tool in retinoblastoma genetic counseling.

CONCLUSION

The inherited retinoblastoma, besides its earlier age presentation, showed 45.8% more Ki-67 positive cells than the sporadic one, in the same stage.

REFERENCES