Differentiation in Retinoblastoma and Histopathological Risk Factors in Mexico

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ABSTRACT

**Purpose:** To describe the relationship between retinoblastoma differentiation, histopathological risk factors, age at enucleation, laterality, and genetic type (hereditary or sporadic) in a series of enucleated eyes in a referral hospital. The criteria used in other studies to classify retinoblastoma differentiation are discussed.

**Methods:** The authors retrospectively studied histopathological preparations of eyes with retinoblastoma treated with primary enucleation. Tumors were classified as well differentiated, undifferentiated, and moderately differentiated. Patient age at enucleation, laterality of disease, genetic form (hereditary or sporadic), and presence of histopathological risk factors (massive choroidal infiltration, postlaminar optic nerve invasion, tumor in optic nerve cut, scleral invasion, and involvement of orbital soft tissues) were analyzed. The chi-square test was used for categorical variables and analysis of variance for test mean differences.

**Results:** Histopathological risk factors were present in 23 (36%) of 63 eyes. Moderately differentiated tumors occurred at advanced ages and were more frequently associated with histopathological risk factors.

**Conclusions:** A consensus is needed to establish the histopathological criteria of retinoblastoma differentiation. The value of rosettes as a marker of cell differentiation should be reviewed.


INTRODUCTION

Retinoblastoma is the most common intraocular malignancy of childhood. Degrees of differentiation range from poorly differentiated neuroblastic cells to the formation of Homer-Wright and Flexner-Wintersteiner rosettes. Several studies support the hypothesis that retinoblastoma becomes progressively less differentiated with time.\(^1,2\) But different criteria are used to classify tumor differentiation. In Mexico, a high frequency of this tumor has been documented with a usually late diagnosis and with enucleation almost always being the initial treatment.\(^3\) The objective of this study was to describe the relationship between retinoblastoma differentiation, histopathological risk factors, age at enucleation, laterality, and genetic form (hereditary or sporadic) in a series of eyes enucleated as primary treatment in a referral hospital in Mexico.

PATIENTS AND METHODS

Histopathological preparations of enucleated eyes with retinoblastoma from 2000 to 2011 were reassessed by light microscopy. The specimens were previ-
ously fixed in 10% formalin and processed. All specimens were evaluated by one of the authors (MER-G).

We excluded cases treated prior to enucleation (chemotherapy and/or radiotherapy). Tumor differentiation was classified according to the estimated percentage of Flexner-Wintersteiner rosettes. Tumors were considered well differentiated when rosettes were present in more than 80% of their area and undifferentiated when there were no rosettes; the remaining tumors were considered moderately differentiated.

Histopathological risk factors analyzed were massive choroidal infiltration, postlaminar optic nerve invasion, tumor in optic nerve cut, scleral invasion, and involvement of orbital soft tissues. The definition of massive choroidal infiltration was in accordance with the international consensus.4

Variables such as patient age at enucleation and laterality were obtained from medical records. Eyes with bilateral disease and unilateral cases with multiple tumors were considered as hereditary retinoblastoma. Data were analyzed using SPSS software (version 20; SPSS, Inc., Chicago, IL). The chi-square test was used to determine the statistical significance of categorical variables and analysis of variance was used to test mean differences; a \( P \) value less than .05 was considered statistically significant. The study was approved by the ethics and research committee of the hospital.

**RESULTS**

There were 73 eyes with retinoblastoma enucleated in the ophthalmology department of the hospital during the study period, with 65 cases (89%) being primary enucleation. Of these 65 eyes enucleated, two cases were excluded because histopathological preparations were not available for evaluation. In total, 63 enucleated eyes of 62 patients were evaluated. General characteristics of the tumors studied are presented in Table 1. Nineteen of 63 eyes (30%) had hereditary retinoblastoma (14 bilateral and 5 unilateral tumors with multiple tumor foci).

Undifferentiated retinoblastoma was identified in 50% of cases and well-differentiated in 27%. Moderately differentiated tumors were less common, occurring in 23% of eyes examined. Well-differentiated tumors were more frequent in eyes enucleated prior to 1 year of age (10 of 17 cases, 59%), whereas most undifferentiated (26 of 32, 81%) and moderately differentiated (13 of 14, 93%) tumors were identified after that age. Histopathological risk factors were found in 23 (36%) eyes: 14 had only one risk factor (11 massive choroidal infiltration, 3 postlaminar optic nerve invasion) and the rest had two or more risk factors. Hereditary tumors were enucleated earlier (mean: 14.9 months, standard deviation [SD]: 11.4) than sporadic tumors (mean: 28.8 months, SD = 18.9) (\( P = .003 \)) with no observed differences in the distribution of the tumoral degrees of differentiation. Sporadic retinoblastoma was more frequently associated with histopathological risk factors (20 of 44 cases) than hereditary retinoblastoma (3 of 19 cases) (\( P = .044 \)).

Patients with bilateral retinoblastoma had earlier enucleation (mean: 10.2 months, SD: 9.1, range: 0.5 to 32) than those with unilateral retinoblastoma (mean: 28.3 months, SD: 18.4, range: 2 to 90) (\( P = .001 \)). Well-differentiated tumors were predominant in bilateral retinoblastoma, undifferentiated tumors were present in 35% of cases, and there were no cases of moderately differentiated tumors. Median age at enucleation of patients with undifferentiated bilateral retinoblastoma was 11 months.

Conversely, undifferentiated tumors were predominant among unilateral retinoblastoma, with almost one-third of the cases being moderately differentiated. Well-differentiated tumors accounted for only 16% of unilateral retinoblastoma. Distribution

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
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<tbody>
<tr>
<td>Type of retinoblastoma</td>
<td></td>
</tr>
<tr>
<td>Hereditary</td>
<td>19 (30%)</td>
</tr>
<tr>
<td>Sporadic</td>
<td>44 (70%)</td>
</tr>
<tr>
<td>Mean age at enucleation (months) (range)</td>
<td>24.3 (0.5–90)</td>
</tr>
<tr>
<td>Histopathological types</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>32 (50%)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>14 (23%)</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>17 (27%)</td>
</tr>
<tr>
<td>Histopathological risk factors</td>
<td></td>
</tr>
<tr>
<td>Massive choroidal infiltration</td>
<td>17</td>
</tr>
<tr>
<td>Postlaminar optic nerve invasion</td>
<td>9</td>
</tr>
<tr>
<td>Scleral invasion</td>
<td>6</td>
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<tr>
<td>Tumor in optic nerve cut</td>
<td>3</td>
</tr>
<tr>
<td>Involvement of orbital soft tissues</td>
<td>1</td>
</tr>
</tbody>
</table>

**TABLE 1**

General Characteristics of Enucleated Eyes (n = 63)
of clinical characteristics of the tumors according to their differentiation is shown in Table 2.

Overall, well-differentiated tumors were enucleated at a younger age and were less frequently associated with histopathological risk factors. However, with the criteria used to define differentiation, patients with moderately differentiated tumors were older at the time of enucleation and tumors were more frequently associated with histopathological risk factors.

**DISCUSSION**

Retinoblastoma is a primitive neuroectodermal tumor that demonstrates varying degrees of differentiation. Several studies have established a relationship between phenotypic appearance and biological behavior of the tumor: well-differentiated tumors are more frequent in eyes enucleated at younger ages and are less often associated with histopathological risk factors. Conversely, undifferentiated retinoblastoma tends to occur in eyes enucleated at later ages and is more frequently associated with histopathological risk factors. In this study, age at enucleation and the prevalence of the undifferentiated type were similar to those reported previously by other authors.

The lack of moderately differentiated tumors in hereditary retinoblastoma and the presence of undifferentiated tumors in eyes with bilateral retinoblastoma enucleated prior to 1 year of age support the possibility mentioned by Madhavan et al. that well-differentiated and undifferentiated tumors represent events at different levels of retinal development and, therefore, not all tumors experience a process of time-dependent de-differentiation.

What is the best criterion to classify differentiation in retinoblastoma? Traditionally, most published studies base differentiation on the percentage of rosettes shown by the tumor, but this percentage was established arbitrarily by each investigator. In a study of 170 cases in India, tumors with rosettes in more than 80% of their area were classified as well-differentiated retinoblastoma. Gupta et al. established only the presence of rosettes as a criterion for well-differentiated retinoblastoma, without determining the percentage. Recently, Kashyap et al. defined well-differentiated tumors as those with more than 50% rosettes. None of these authors established an intermediate degree of differentiation, and few authors described the clinicopathological characteristics of retinoblastoma.

Arce et al. classified the degree of differentiation according to the type of rosette in the tumor, defining undifferentiated tumors as those not forming rosettes, poorly differentiated as those only presenting Homer-Wright rosettes, and well-differentiated as those simultaneously presenting Flexner-Wintersteiner and Homer-Wright rosettes. Interestingly, in Arce et al.’s study the intermediate form of differentiation presented itself at a constant rate at all ages.

Orellana et al. used the same classification system that we did and reported frequencies similar to ours for undifferentiated (60%) and moderately differentiated (23%) tumors and lower frequencies of well-differentiated (8%) tumors. Possible explanations for this difference may be the percentage of unclassified tumors and older ages at the time of enucleation, especially bilateral retinoblastoma (median: 21.3 months). Unfortunately, these authors did not
analyze the relationship between tumor differentiation and histopathological risk factors.\(^{11}\)

Assuming that the reported histological types represent various phases of progression of the tumor, moderately differentiated retinoblastoma should represent an intermediate stage in the transformation from differentiated to undifferentiated tumors. We found no correlation between clinical and biological behavior and the undifferentiated and moderately differentiated types of retinoblastoma. However, the relationship between phenotypic appearance and biological behavior of tumors is controversial. The chemoresistance of well-differentiated retinoblastoma has been known for years.\(^{12}\) Schouten-van Meeteren et al. demonstrated a distinct sensitivity to cytotoxic drugs according to tumor differentiation,\(^ {13}\) and Filho et al. reported the expression of P-glycoprotein to be directly proportional to the degree of retinoblastoma differentiation.\(^ {14}\) Contrary to that, Krishnakumar et al. found no relationship between differentiation, expression of P-glycoprotein, and pulmonary protein resistance, with invasiveness and treatment response.\(^ {15}\)

The number of eyes analyzed in this study is small and prevents making definitive conclusions; however, it is clear that unlike other tumors such as neuroblastoma and Wilms’ tumor where the histology is related to tumor behavior and plays a prognostic role, in retinoblastoma there are no uniform criteria to classify differentiation. With the exception of massive necrosis\(^ {16}\) and the presence of rosettes, other phenotypic characteristics related to the clinical and biological behavior of retinoblastoma have not been identified. Despite the traditional approach for classifying differentiation of retinoblastoma based on the presence of rosettes, probably because of their association with low metastatic potential, there is evidence that rosette formation is unrelated to differentiation of retinoblastoma cells.\(^ {17}\) Unification of criteria to define the degree of retinoblastoma differentiation will allow for clarification of the relationship between phenotypic characteristics and clinical and biological behavior of the tumor, allowing new perspectives in the knowledge of this fascinating tumor.

This study showed that despite the fact that undifferentiated retinoblastoma was most frequent, moderately differentiated tumors were more frequently associated with increasing age at enucleation and histopathological risk factors. Due to the different approaches reported in the published literature and current evidence, it is essential to unify the criteria for classifying retinoblastoma differentiation and to review the role of rosettes as a marker of cell differentiation.

**REFERENCES**