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MENINGIOMAS AND HORMONAL RECEPTORS

IMMUNOHISTOCHEMICAL STUDY IN TYPICAL AND NON-TYPICAL TUMORS

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ABSTRACT - The authors assessed 116 cases of meningiomas classified as typical, atypical and anaplastic and they used an immunohistochemical technique for estrogen and progesterone receptors attempting to determine if there is any difference between typical and non-typical tumors in relation to hormone receptors. The immunohistochemical technique to estrogen receptors was negative in all meningiomas studied. Progesterone receptors were detected in 58.3% of typical, and in 48.2% of non-typical meningiomas. This difference was not statistically significant. However, individually considering the criteria used for selection of non-typical tumours, those that concurrently displayed brain invasion and increased mitotic activity or necrosis, as well as the summation of those three features, were predominantly negative for progesterone receptors (respectively p=0.038; p=0.001; and p=0.044). The authors conclude that estrogen receptors were not present in meningiomas; that progesterone receptors in isolation are not enough to predict a higher tumoral malignancy but can be useful associated with other histological features.

KEY WORDS: meningioma, progesterone receptor, estrogen receptor, immunohistochemistry.

Meningiomas e receptores hormonais: estudo imuno-histoquímico em tumores típicos e não típicos

RESUMO - Os autores avaliam 116 meningiomas classificados em típicos, atípicos ou anaplásicos usando técnica imuno-histoquímica para receptores de estrógeno (ER) e progesterona (PR) com o objetivo de determinar se existe diferença entre tumores típicos e não típicos em relação aos receptores hormonais. Todos os tumores estudados foram negativos para ER. Os receptores de progesterona foram detectados em 58,3% dos meningiomas típicos e em 48,2% dos tumores não-típicos. Essa diferença não foi estatisticamente significativa. Entretanto, considerando os critérios utilizados para seleção dos não-típicos, os tumores que apresentavam, de forma concomitante, invasão do sistema nervoso central e aumento da taxa mitótica ou necrose, bem como a soma das três características, foram predominantemente negativos para PR (p=0,038; 0,01 e 0,044, respectivamente). Os autores concluem que: ERs não estão presentes em meningiomas; PRs estão presentes na maioria dos meningiomas; a negatividade para PR isoladamente não é suficiente para predizer maior malignidade mas pode ser útil se associada a outras características histológicas.

PALAVRAS-CHAVE: meningioma, receptores de progesterona, receptores de estrógeno, imuno-histoquímica.

Meningiomas are the most common meningeal tumors of the central nervous system. These tumors arise from the arachnoidial layer of meninges and, although they are generally benign, there are many cases showing atypical and anaplastic features[1]. Epidemiological data suggest that female sex hormones may play a role in the growth of meningiomas. They show a higher incidence in

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female than in male (2/3 of patients are women). Also, it has been observed that these tumors often grow during pregnancy and luteal phase of the menstrual cycle[2] and there is an association between meningiomas and breast cancer and other tumors wich have hormonal dependency[3,4]. Based in these data, there have been many reports which have dealt with the presence of estrogen receptor (ER) and progesterone receptors (PR) in meningiomas[5-15]. In recent years, the development of monoclonal antibodies specific for steroid hormone receptors has permitted the detection of PR and ER direct in tissue. This approach has been proved to be better than earlier methods[9,16,17].

There is a general agreement that the majority of meningiomas contain the PR and are devoid of ER, but the significance of receptor status in the progression of tumour remain unclear. There are few papers[9,10,13,17] about the correlation between PR and ER and tumor grade in meningiomas while in other tumors, like breast and uterine cancer, there is a correlation between hormonal immunoreactivity and overall outcome[18].

In our study, we compared different grade of meningiomas (benign, atypical and anaplastic) with the PR and ER status by immunohistochemical method.

MATERIAL AND METHODS

Our sample include 116 cases of meningiomas selected from 246 cases which undergo to histopathological diagnosis in Departament of Pathology of Fundação Faculdade Federal de Ciências Médicas de Porto Alegre from March, 1968 to March, 1993. The cases were selected from the Department of Pathology files and included patients operated in different hospitals. Slides from all cases were reviewed to confirm histopathological diagnostic and evaluated for tumour grade (benign, atypical and anaplastic) and histological subtype[17,19]. After that, we selected 116 cases including 56 non-typical tumours (all but 5 without enough material) and 60 typical tumours randomly selected. Immunohistochemical detection for ER and PR was done in these cases (n:116).

The tumor was considered atypical when exhibited at least two of the following features: hypercelullarity, high nuclear cytoplastic ratio, prominent nucleoli, brisk mitotic activity (at least 5 mitotic figures in 10 high power field), presence of zonal necrosis and brain invasion without anaplasic features. Anaplastic meningiomas already display cytological anaplasia, large number of mitosis with atypical ones and brain invasion.

The clinical data were reviewed for evidence of recurrence.

Immunohistochemical detection for ER and PR was performed by streptoavidin-biotin method using anti-PR monoclonal antibodies (Novocasta) in 1:40 dilution, and anti-ER mouse monoclonal antibodies (DAKO-ER M 7047) in 1:100 dilution.

Briefly, sections of formalin fixed, paraffin-embebed surgical specimens were deparaffinized in xylene and processed through ethanol. Tissue sections were microwaved, to reverse the effect of formalin fixation, in citrate buffer solution (pH 6.0) at high power until the solution came to a boil, then reset to a moderate temperatures and heating was continued for 15 minutes to maintain a gentle boil with stops to replace lost liquid when necessary. The endogen peroxidase activity was blocked with methanol containing 0.3% H_2O_2 for 30 minutes. Slices were rinsed with PBS and nonespecific reactions were blocked by incubation with normal horse serum. Tissue sections were then incubated with primary antibody in wet box overnight in refrigerator. After washing with PBS, the sections were incubated with anti-mouse secondary antibody during 20 minutes and the avidin-biotin-peroxidase complex for 40 minutes, with washing PBS between steps.The antigen-antibody binding was visualized with 3-3' diaminobenzidine/H_2O_2 solution and coverstained with hematoxylin.

Negative control sections were prepared by omitting the primary antibody during overnight. Positive controls were breast tumours immunoreactive for PR and ER.

The immunoreactivity was independently confirmed by two examiners under light microscopy. The specimens were considered positive when more than 1% of immunoreactive nuclei were shown. If only cytoplasm was stained, the tumor was considered negative.

The statistical analysis was done by qui-square test and Fisher's exact test with differences considered significant when $p<0.05$.

RESULTS

Among all meningiomas (246), 185 (75.22%) were considered typical meningiomas, 47 (19.1%) were atypical and 14 (5.68%), anaplastic, in 3 cases with papillary features. Among typical tumors, the meningothelial subtype was more frequent (Fig 1).

The age ranged between 3 and 80 years old, with mean age of 48 years old among typical cases and 44 years old among non-typical, without statistical difference. In both groups, the female gender predominated (37:24 in non-typical meningiomas and 130:55 in typical ones).

Thirteen percent of tumors recurred, including 3.78% of the typical, 42.5% of the atypical and 45.5% of anaplastic (Fig 2).

Sixty-two (53.4%) of 116 cases of meningiomas selected for immunohistochemical detection of PR and ER contained cell nuclei exhibiting a positive reaction for the PR, ranging from a few cells to over almost all of the tumor cell population. Immunoreactivity was nuclear, not found in cytoplasm, connective tissue or blood vessel. The specific PR immunoperoxidase staining was heterogeneous in distribution with one or several focal nuclear positivity (Fig 3).

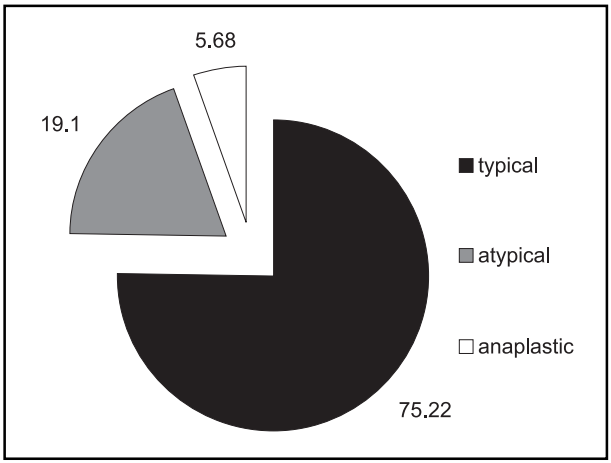

Fig 1. Meningiomas: frequency of different types (%).

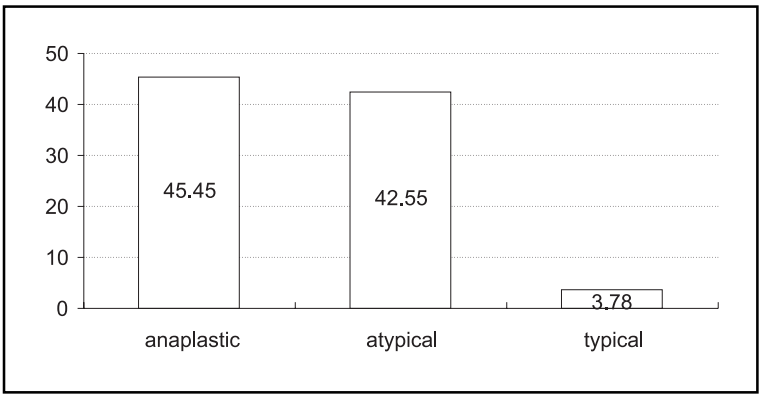

Fig 2. Recurrency among meningiomas.

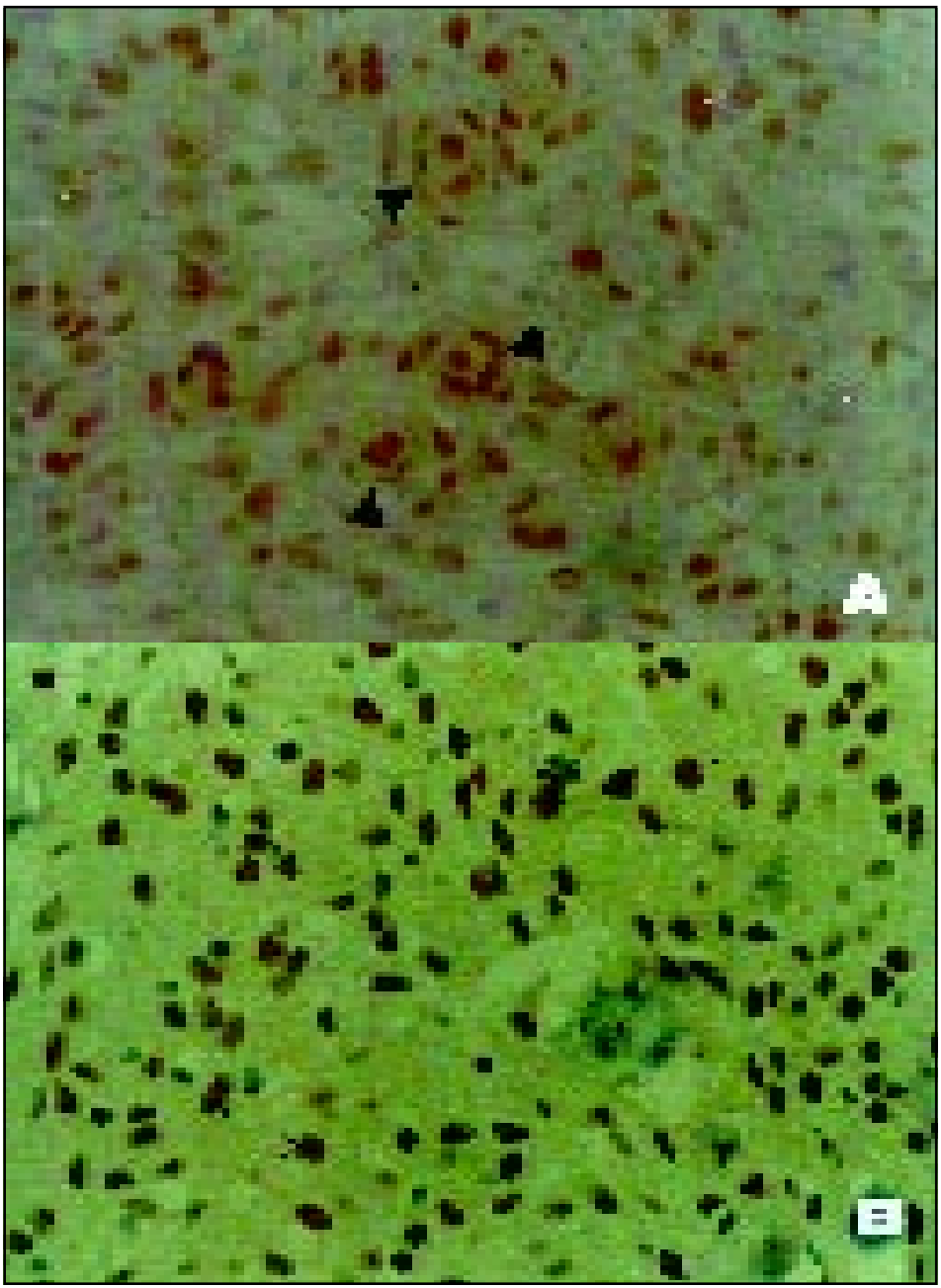

Fig 3. A - Immunoreactivity to progesterone receptors in typical meningiomas. Nuclear positivity is showed in tumor with numerous concentric whorl; B - Atypical meningioma positive to progesterone receptors (obj. 40X).

Table 1. Correlation between progesterone receptor status and selection criteria for non-typical meningiomas.

Selection criteria	Value of p
hipercelullarity	p=0.4
high mitotic level	p=0.18
necrosis	p=0.12
brain invasion	p=0.27
brain invasion+necrosis	p=0.016*
brain invasion+ high mit.	p=0.038*
necrosis+high mit.	p=0.57
brain invasion+necrosis+high mit.	p=0.04*

Statistical analysis: X2 and Fisher's test; *p<0.05

Table 2. Immunoreactivity for progesterone receptors in meningiomas.

Tumor Grade	PR positive	PR negative
typical	35	25
atypical	23	23
anaplastic	4	6
Total	62	54

Among typical tumours, 35 cases (58.3%) were positive for PR while 27 (48.2%) were positive among non-typical (Table 2).

All 116 tumours analyzed for ER were negative, both typical and non-typical meningiomas.

Patients age, gender and histological subtypes did not correlate with PR status. There was not statistical significance between PR status and tumor grade, neither atypical (p=0.73) nor anaplastic (p=0.23).

Analysing each criteria of selection, none was statistically significant alone. Otherwise, those tumors which display brain invasion plus necrosis or higher mitotic rate were lacking PR more frequently (p=0.01 and p=0.03). When all three criteria were present, again the difference was significant (p=0.04) (Table 1).

Among non-typical tumors, the recurrence was higher when meningioma was negative than when it was positive for PR, although without statistical significance (p=0.33).

DISCUSSION

Meningioma is generally benign, well circumscribed and a slow growth tumor. It can be surgically removed in most cases and surgery has been the only available treatment. However, some tumors are inoperable because of the poor clinical condition of very old patients or due to invasion of bone and/or blood vessels[20-22]. They may also show atypical or anaplastic features[13,22,25]. The reported percentage of more malignant tumors varies, according to investigator's criteria, from 0.9%[23] to 24%[24].

Rates of recurrence for atypical and anaplastic tumors, as well as benign ones following incomplete surgical resection are high. We have a recurrence rate of 13%, higher in atypical (42.5%) and anaplastic (45.5%) than in typical (3.78%). These data are in agreement with other authors[21,22,25].

The presence of hormonal receptors in meningiomas has been the issue of many papers since the first description made by Donnel et al. in 1979[8]. The majority of studies used receptor binding assays to detect the presence of ER and PR[5,6,10,14]. It has produced widely varied and confusing results, possible due to differences in the handling of tissue, variations in techniques, and criteria used in determining the receptor status based on levels of cytosolic receptor binding sites. In recent years, monoclonal antibodies are available and made possible the utilization of immunohistochemical techniques[7,16,17]. It offers a rapid and reliable method to assess receptor status with the advantage of showing the presence and localization of hormonal receptors directly into the tissue. It also permits retrospective studies with stored material and the detection of receptors even with small amount of tissue.

The ER is undetectable in almost all of studies, using different techniques[6,10,16,17]. It appears that ER is actually devoid in this kind of tumors. In our study of 116 meningiomas, none of them display ER immunoreactivity. On the other hand, we find that the majority of meningiomas is immunoreactive for PR, in agreement with other authors[5-8,16,17]. The presence of PR and lack of ER has led to believe that these PRs are estrogen independent, unlike PRs present in other hormone-regulated tumors, such as breast and uterus.

The significance of these receptors in the growth of meningiomas remains unclear. *In vitro* studies have contradictory results. Adams et al.[26] suggest that PRs are not involved in growth of meningiomas, while Jay et al.[11] show that hormonal manipulation may modify the growth in some tumors.

Koper et al.[12] suggested that the presence of progesterone in the culture medium increases the sensitivity of meningioma cells to mitogenic stimuli with epidermal growth factor and other growth factors, whereas mifepristone (progesterone receptor blocking agent) can counteract the stimulating effects of progesterone.

The correlation of hormonal receptors with tumor grade and outcome of patient has received little attention in meningiomas with contradictory results. Ironside et al.[10] describe 45 meningiomas with 3 malignant tumors, all of them without progesterone receptors, but the group was small to get conclusions.

Piquer et al.[27] found low levels of PR in meningiomas with necrosis and high mitotic rate. Kostron et al.[13] demonstrated higher recurrence and short-disease-free interval among non-typical meningiomas without PR.

Perrot-Applanat et al.[17] were unable to find correlation between hormonal receptor status and proliferative index detected by Ki-67 immunoreactivity. Brandis et al.[28] studied 53 typical tumors and 8 non-typical and found differences only for men. Recently, Hsu et al.[9] suggested that absence of PR, high mitotic index and higher tumor grade were significant factors for shorter disease-free interval and that the presence of PR, even in a small number of tumor cells, is a favorable prognostic factor for meningiomas.

In our study, 58.3% of typical meningiomas displayed PR immunoreactivity, while 48.2% of non-typical tumors do it. Although without statistical significance, if we take tumors with some criteria such as brain invasion plus increased mitotic rate and/or necrosis, the lack of PR in this group has statistical significance compared with typical ones.

The detection of PRs in most meningiomas also arises the possibility of medical management in meningiomas with antiprogesterone agents. Mifepristone, a progesterone antagonist, has demonstrated to be benefical in treating breast cancer patients and may be useful in meningiomas. Two studies were done with mifepristone in inoperable meningiomas. In 13 patients studied by Grunberg et al.[29], 5 tumors reduced, 3 improved without reduction of tumor and three enlarged regardless treatment. Two of three which failed were malignant. Lamberts et al.[30] also used mifepristone in 10 pacients with inoperable meningiomas and 4 failed. The search for PR receptor was done in none of them. Maybe the lack of response is due to treatment of tumors without receptors for progesterone.

CONCLUSION

We conclude that the majority of meningiomas show progesterone receptors and lack estrogen receptors. The PR status may be a useful prognostic tool if associated with other histological features. Otherwise, the clinical approach with hormonal therapy may play a role in management of meningiomas and needs further investigation.

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