Comparison of Stromal CD10 Expression in Benign, Borderline and Malignant Phyllodes Tumors

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Abstract

Phyllodes tumors are group of biphasic fibroepithelial tumors of the breast of varying malignant potential, ranging from benign tumors to fully malignant sarcomas. According to the Egyptian National Cancer Institute, female malignant cases showed appreciable increase in the recent time period for breast cancer with the malignant phyllodes tumors representing 0.41% of cases in the year 2003-2004. This is an immunohistochemical study to compare CD10 expression in benign, borderline and malignant phyllodes tumors, in order to highlight its diagnostic and prognostic values. This study conducted 34 Egyptian female cases of phyllodes tumors of different grades to be studied histologically and immunohistochemically using antibodies against CD10. The Chi square test was used to determine differences in CD10 expression between benign, borderline and malignant tumors. One way ANOVA test was used to determine whether the difference was significant. Significance was established at $p<0.05$. In the 24 cases of benign phyllodes tumors, only 4 cases (16.7%) showed positive CD10 reactivity. Three cases (60%) out of 5 borderline phyllodes tumors showed positive CD 10 reactivity, while 4 (80%) out of 5 cases of malignant phyllodes tumors showed positive CD10 staining. In conclusion, from these highly significant results this study suggests that there is a strong correlation between CD 10 expression and tumor grade, which could be an important observation that may have both diagnostic and prognostic implications as well as promising potential target for development of novel therapies.

Key Words: CD10 – Immunohistochemistry – Phyllodes – Stromal breast tumors.

Material and Methods

Introduction

PHYLLODES tumors of the breast have varying malignant potential, ranging from completely benign tumors to fully malignant sarcomas [1].

Phyllodes tumors are classified as benign, borderline or malignant based upon histological criteria of which stromal overgrowth is the most important [2]. However, while histological features are helpful, they are not accurate predictors of tumor behavior, and no single parameter is reliable [3]. Because of the limited data available, the percentage of benign versus malignant phyllodes tumors is not well defined. Reports suggest, however, that about 85-90% of phyllodes tumors are benign and that approximately 10-15% are malignant [1].

Surgery with margin negative excision is the primary mode of management in both benign and malignant tumors; however radiotherapy is beneficial as adjuvant modality after surgery in borderline and malignant phyllodes tumors, which emphasizes the importance of distinguishing the benign from the malignant tumors [2].

The ability of immunohistochemical studies to distinguish benign from malignant phyllodes tumors have been questioned in this study, through evaluating the stromal CD10 expression in different grades of breast phyllodes tumors.

This current study tried to prove that the degree of CD 10 expression in the stromal cells of phyllodes tumors was related to the tumor grade.

This is a preliminary study that consisted of the paraffin blocks of 34 Egyptian female cases of phyllodes tumors of different grades.

Phyllodes tumors were histologically classified as benign, borderline and malignant based on: 1- Stromal cellularity and overgrowth, that is absent epithelial elements within a low power field. 2- Mitotic activity (benign $\leq 2$, borderline 3-4, and malignant $>5$ mitotic figures per 10 high power fields). 3- Microscopic tumor border (circumscribed or infiltrating). 4- Nuclear pleomorphism [4].
Depending on the criteria described in the previous section, benign phyllodes tumors were classified when there was no extensive stromal overgrowth, mitotic figures were 2 or less per 10 high power fields, well circumscribed border and absence of nuclear pleomorphism (Fig. 1). On the other hand, malignant phyllodes tumors were diagnosed when they showed extensive stromal overgrowth, 5 or more mitotic figures per 10 high power fields, infiltrating border and marked nuclear pleomorphism (Fig. 2). Borderline phyllodes tumors were more atypical than the benign but did not fulfill all the criteria of malignancy.

Each paraffin block was re-cut by rotatory microtome at 4 microns thickness then mounted on glass slides to be stained by hematoxylin and eosin (H & E) for routine histopathological examination and on charged slides for immunostaining using standard immunoperoxidase method.

For the assessment of CD 10 expression, a representative slide from each case was stained using an antibody against CD 10 (CD 10/CALLA, Ab-2, mouse monoclonal, antibody, clone 56C6, dilution 1: 50). As positive control for cases stained for CD 10, a section of tonsil was employed. The negative control was carried out by omitting the primary antibody in each case.

Every section was carefully examined at power magnification (x100) for the presence of tumor stromal immunostaining using Olympus microscope CX21.

The CD 10 immunostaining was scored as negative when there was no tumor stromal staining, weak when there was either diffuse weak staining or weak or strong focal staining in less than 30% of tumor stromal cells and strong when there was strong staining in 30% or more of tumor stromal cells [5].

Statistics:

The Chi square test was used to determine differences in CD 10 expression between benign, borderline and malignant tumors. One way ANOVA test was used to determine whether the difference was significant. Significance was established at $p < 0.05$. Data was collected, coded and analyzed by SPSS software version (9) under windows XP.

Results

This study included 34 cases of phyllodes tumors classified as 24 cases of benign phyllodes tumors (70.6%), 5 cases of borderline phyllodes tumors (14.7%) and 5 cases of malignant phyllodes tumors (14.7%).

The patients’ ages ranged from 17 years up to 65 years and the tumor sizes ranged from 2cm up to 31 cm in maximal diameters.

In the 24 cases of benign phyllodes tumors, the patients’ ages ranged from 17 years up to 55 years (mean $32.5\pm 10.7$) and the tumor sizes ranged from 2cm up to 8cm in maximal diameters (mean $2.8\pm 1.3$). In the 5 cases of borderline phyllodes tumors, the patients’ ages ranged from 37 years up to 65 years (mean $49.2\pm 12.1$) and the tumor sizes ranged from 5cm up to 15.5cm in maximal diameters (mean $8.1\pm 4.4$). As for the remaining 5 cases of malignant phyllodes tumors the patients’ ages ranged from 35 year up to 63 years (mean $50.2\pm 11.6$) and the tumors sizes ranged from 9cm up to 3 1 cm in maximal diameters (mean $16\pm 8.8$). These results showed highly significant correlation between the patients’ ages ($p < 0.0001$) and the tumor sizes ($p < 0.005$) on one hand and the tumor grade on the other hand. These results were summarized in (Tables 1,2) respectively.

Another highly significant correlation was found between CD 10 expression and the tumor grade ($p < 0.0001$). In cases of benign phyllodes tumors, only 4 cases (16.7%) showed positive CD 10 reactivity, distributed as 3 (12.5%) weakly stained and only one (4.2%) strongly stained, while 20 cases were negatively stained for CD10 (83.3%). Three (60%) of the borderline phyllodes tumors showed positive CD10 reactivity, distributed as 2 (40%) weakly stained (Fig. 3) and only one (20%) strongly stained, while the remaining 2 (40%) showed negative CD10 staining. As for cases of malignant phyllodes tumors, the majority of cases showed positive CD 10 staining constituting 4 cases (80%), distributed as only one (20%) weakly stained case and 3 (60%) strongly stained cases (Fig. 4), while the remaining one (20%) was CD 10 negative. These results were summarized in (Table 3).

<table>
<thead>
<tr>
<th>Table (1): Relation between patients’ age and tumor grade.</th>
</tr>
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<tbody>
<tr>
<td>Youngest</td>
</tr>
<tr>
<td>Benign phyllodes tumors</td>
</tr>
<tr>
<td>Borderline phyllodes tumors</td>
</tr>
<tr>
<td>Malignant phyllodes tumors</td>
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</tbody>
</table>

* Year.
# Standard deviation.
$p < 0.0001$.
Table (2): Relation between tumor size and tumor grade.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Smallest size (cm)</th>
<th>Largest size (cm)</th>
<th>Mean±SD#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign phyliodes tumors</td>
<td>2</td>
<td>8</td>
<td>2.8±1.3</td>
</tr>
<tr>
<td>Borderline phyliodes tumors</td>
<td>5</td>
<td>15.5</td>
<td>8.1±4.4</td>
</tr>
<tr>
<td>Malignant phyliodes tumors</td>
<td>9</td>
<td>31</td>
<td>16±8.8</td>
</tr>
</tbody>
</table>

# Standard deviation.

Table (3): Relation between CD10 expression and tumor grade.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>CD10 positive</th>
<th>CD10 negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign phyliodes tumors</td>
<td>4 (16.7%)</td>
<td>20 (83.3%)</td>
<td>24 (100%)</td>
</tr>
<tr>
<td>Borderline phyliodes tumors</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>Malignant phyliodes tumors</td>
<td>4 (80%)</td>
<td>1 (20%)</td>
<td>5 (100%)</td>
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</tbody>
</table>

p<0.0001.

Discussion

Phyllodes tumor includes a group of biphasic fibroepithelial tumors of the breast of varying malignant potential, ranging from completely benign tumors to fully malignant sarcomas [1].

According to the Egyptian National Cancer Institute, female malignant cases showed appreciable increase in the recent time period for breast cancer with the malignant phyllodes tumors representing 0.41% of cases in the year 2003-2004 [6].

Although the benign tumors do not metastasize, they have a tendency to grow aggressively and can recur locally. Similar to other sarcomas, the malignant tumors metastasize hematogenously. Unfortunately, the pathologic appearance of a phyllodes tumor does not always predict the neoplasm’s clinical behavior or outcome; in some cases, there-
fore, there is a degree of uncertainty about the lesion's classification [7].

CD 10 is a zinc-dependent peptidase (metalloproteinase), which degrades a variety of bioactive peptides. Earlier studies suggested that CD 10 expression in tumor stroma is associated with biological aggressiveness of the tumor. So CD10 constitutes a clinically important prognostic marker and a potential target for development of novel therapies. In fact, CD10 has been used as a diagnostic marker to differentiate endometrial stromal sarcoma from leiomyoma [8].

Unfortunately CD10 expression is not well documented in the breast, to my knowledge, there are only very few reports on its expression in myoepithelial cells and its use as an aid to the diagnosis of problematic lesions. Studies of CD10 expression in stroma of invasive breast cancer and its use as a possible predictor of clinical outcome are even rarer. Similarly, CD10 expression in mammary stromal neoplasms, most notably phyllodes tumors, had not been well documented, to my knowledge only very few exist and none regarding the African, nevertheless the Egyptian population [4].

This study shows that CD10 expression strongly correlates with the phyllodes tumor grade, which can help in the differentiation between benign and malignant variants. This is evidenced by the fact that CD 10 immunoreactivity was seen in only 16.7% of the benign phyllodes tumors, 60% of the borderline phyllodes tumors and 80% of the malignant phyllodes tumors submitted in this study (Table 3). This may be attributed to the fact that CD 10 belongs to the metalloproteinase family, so it provides tumors with the capacity to invade blood vessel walls, facilitating its metastatic potential [4].

Reports on CD10 expression in the breast are scarce, but it has been reported in different other cell types. One of the few studies done on CD10 expression in breast tumors, was that conducted by Tse et al., [4] showing the same conclusion as this current study. Their study consisted of 102 benign phyllodes tumors showing 96% negative staining and only 5.9% positive CD10 reactivity, 51 cases of borderline phyllodes tumors showing 68.6% negative staining and 31.4% positive CD10 immunoreactivity and 28 malignant phyllodes tumors showing 50% positive and 50% negative CD 10 staining. These results showed high significance. In another small series study done by Mechtersheimer et al., [8] 3 benign phyllodes tumors were studied showing weak CD 10 staining, while the only malignant phyllodes tumor case conducted showed very intense staining.

Conclusions:

This study concluded that CD10 expression is higher in borderline and malignant phyllodes tumors. This could be an important observation that may have both diagnostic and prognostic implications as well as promising potential target for development of novel therapies, a subject for further investigations.

References