Breast carcinoma developing in patients on hormone replacement therapy: a histological and immunohistological study

Isobel Fitzgerald O’Connor, Madhuri V Shembekar, Sami Shousha

Abstract

Aim—To study the histopathological features of breast carcinoma developing in postmenopausal patients on hormone replacement therapy (HRT).

Methods—The sample comprised 60 patients with invasive breast carcinoma including 31 who had received HRT at or shortly before presentation, and 29 who had not. Details concerning their tumour size, histological type and grade, lymph node status, and oestrogen and progesterone receptor status were compared. Immunoperoxidase staining for Bcl-2, p53, and E-cadherin was carried out on paraffin sections of all 60 patients. The results were then statistically analysed.

Results—Tumours detected in HRT patients were significantly smaller (mean 17 mm vs 25 mm; p = 0.0156) and of a lower histological grade (p = 0.0414) than those detected in non-HRT patients. The incidence of invasive lobular carcinoma was slightly higher in HRT patients (19% vs 14%). Immunohistologically, 87% of HRT tumours were Bcl-2 positive (compared with 79% in the control group), 29% were p53 positive (45% in the control), and 48% were E-cadherin positive (72% in the control group). Although the differences were not statistically significant there was a trend towards higher incidence of p53 negative and E-cadherin negative tumours in HRT patients.

Conclusions—Breast carcinomas detected in patients on HRT have a significantly higher incidence of two favourable prognostic features (small size and a low histological grade). They also show a trend, statistically not significant, of being p53 negative and E-cadherin negative; this may be related to the slightly higher incidence of invasive lobular tumours in these patients.

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Keywords: breast carcinoma; hormone replacement therapy; Bcl-2; p53; E-cadherin

Hormone replacement therapy (HRT) is being increasingly prescribed for the relief of postmenopausal symptoms and to protect patients against coronary heart disease and osteoporosis. The intake of HRT is probably associated with only a slightly increased risk of developing breast carcinoma, but as the number of women taking HRT increases, there is an increasing number of patients presenting with breast carcinoma who are on HRT. Our aim in this study was to determine whether breast carcinoma developing in patients on HRT has any particular histopathological features. Various histological and immunohistological indices were used to study and compare tumours from two groups of patients, one with and one without a history of HRT.

Methods

Sixty patients with primary invasive breast carcinoma were included in the study. These patients were selected consecutively from the department of histopathology archives on fulfilling specific criteria. These included age between 48 and 60 years, the availability of patients’ notes and tumour paraffin blocks, and whether it was clearly indicated in the patients’ notes either that they were definitely on HRT, or that they were, or definitely had been, on HRT. All patients’ notes were obtained from the medical records department at Charing Cross Hospital and individually reviewed.

Information about each patient’s histological diagnosis, tumour size and grade, axillary lymph node status, and oestrogen and progesterone receptor status—as determined immunohistochemically—was obtained from the histopathology reports. Almost all the cases included in this study were originally seen and reported by one of us (SS).

Tumour size was represented by the largest diameter of the tumour on macroscopic examination, unless it was less than 10 mm, when the largest diameter was determined by microscopic examination. Tumour grading was carried out using the grading system of Elston and Ellis.

Each patient’s collection of archival haematoxylin and eosin stained sections was then reviewed to confirm the histological diagnosis, and a section representative of the tumour was selected. The corresponding paraffin block was identified and five 5 μm thick sections were cut. These were then stained for the tumour markers Bcl-2, p53, and E-cadherin, using the avidin–biotin complex (ABC) immunoperoxidase technique. Endogenous peroxidase was blocked by a 10% solution of 30% hydrogen peroxide. An antigen retrieval step using a pressure cooker was then carried out. The specific primary antibodies used included mouse Bcl-2 monoclonal antibody (Dako) diluted 1/200 in Tris buffered saline (TBS); mouse monoclonal antibody NCL-p53-D07 (Novocastra Laboratories) diluted 1/200 in TBS; and E-cadherin mouse monoclonal anti-
Table 1 Histological features of breast carcinomas in patients who had a history of hormone replacement therapy (HRT; 31 cases) and those who did not (29 cases)

<table>
<thead>
<tr>
<th>Histological feature</th>
<th>HRT (%)</th>
<th>Non-HRT (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean size (mm)</td>
<td>17</td>
<td>25</td>
<td>0.0156</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal, including mixed</td>
<td>19 (61)</td>
<td>22 (76)</td>
<td></td>
</tr>
<tr>
<td>Invasive lobular</td>
<td>6 (19)</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>Tubular/cribriform</td>
<td>5 (16)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Medullary</td>
<td>1 (3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>grade 1</td>
<td>12 (39)</td>
<td>4 (14)</td>
<td>0.0014</td>
</tr>
<tr>
<td>grade 2</td>
<td>13 (42)</td>
<td>16 (55)</td>
<td></td>
</tr>
<tr>
<td>grade 3</td>
<td>6 (19)</td>
<td>9 (31)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The findings of this study suggest that exogenous oestrogens in the form of HRT are associated with changes in some histopatho-
logical features of malignant breast lesions in postmenopausal women.

A history of HRT intake was associated with a significantly higher incidence of smaller tumours. This may simply be a reflection of more rigorous breast screening in these women—for example, Magnusson et al have pointed out that women on HRT have more intense surveillance of their breasts, which would lead to early detection of any tumours that may develop. However, as most women above the age of 50 in the United Kingdom now undergo regular mammographic breast screening whether they are on HRT or not, the possibility of a direct slowing effect of HRT on tumour growth ought to be considered and investigated.

A higher incidence of non-ductal carcinoma was noted in HRT patients (39% v 24% in the control group, table 1), and a statistically significant difference was found in relation to tumour grade (table 1). Fisher's exact test gives a two sided p value of 0.0414, which indicates that HRT patients have more lower grade tumours than non-HRT patients. This is in agreement with the findings of Bonnier et al, who also reported that grade 1 tumours and lobular carcinomas were more frequent in patients on HRT than in their counterparts who had not received HRT. Although this reduced risk of developing high grade tumours may also be the result of greater breast surveillance, it is possible that HRT has a direct action on replication and cellular differentiation in these tumours.

Although there was no statistically significant difference in the prevalence of axillary lymph node metastases in patients who underwent axillary dissection in the two groups (p = 0.1636), there was a trend towards a lower incidence of node metastases in HRT patients (26% v 41%, table 2). We should add here that the reason why axillary dissection was not carried out in eight HRT patients, in contrast to only two non-HRT patients, was that they were considered to be at a low risk of developing lymph node metastasis because of the tumour's small size or low malignancy grade. Our data suggest that HRT has no effect on the expression of oestrogen receptor and progesterone receptor by breast cancer cells (table 2). This contrasts with the findings of Bonnier et al of a higher percentage of hormone receptor negative cases in HRT patients, perhaps because those investigators used a biochemical assay method rather than an immunohistochemical method as used in our study. Biochemical assays may sometimes give false negative results—particularly in small tumours—owing to sampling errors, and immunohistochemical assessment of receptor status is considered more accurate in these lesions.

There was no significant difference between Bcl-2 expression in the two groups of tumours examined (p = 0.5). The incidence of B cl-2 positivity in both groups was relatively high, but was even higher in tumours from the HRT group (87% v 79%, table 2). In this respect, it has recently been reported that B cl-2 positivity is significantly associated with small tumour size, low tumour grade, non-ductal morphol- ogy, oestrogen receptor positivity, and p53 negativity in node negative breast carcinomas. In our series, most of these features were more common in HRT tumours. In another study, Bcl-2 protein expression was significantly associated with the lobular histological type of breast carcinoma. In our study all 10 invasive lobular carcinoma (table 1) were Bcl-2 positive. In addition, all eight cases of tubular and cribriform type were also strongly Bcl-2 positive.

Similarly, there was no statistically significant difference between p53 immunostaining of the two tumour groups (p = 0.2848), but here there was a more obvious trend towards a much lower incidence of p53 positive tumours in the HRT group (29% v 45%, table 2). This is not surprising in view of the known close association between p53 positivity and higher tumour grade.

Most previous studies have found an inverse relation between E-cadherin expression and histological grade of tumour, and that most invasive lobular carcinomas are E-cadherin negative. Of the 334 primary tumours examined by Sitonen et al, 148 (44%) were E-cadherin positive, 129 (39%) had reduced E-cadherin expression, and 56 (15%) were negative. The corresponding results in all our cases combined were 31% E-cadherin ++, 31% E-cadherin+, and 37.9% negative. The higher negative rate in our study is probably a reflection of the relatively large numbers of invasive lobular carcinomas examined. The lower prevalence of E-cadherin positive tumours in our HRT patients is probably also related to the slightly higher incidence of invasive lobular carcinomas in these patients.

While these results are far from conclusive, it is also not clear how many of the HRT patients who have developed breast cancer would have done so if they had not been on HRT. Future investigations could be made into the family history and genetic status of HRT patients with breast carcinoma, to help determine the probability of their developing breast cancer in the absence of HRT. However, as most recent studies indicate that HRT is not associated with a significantly increased risk of developing breast carcinoma, the findings in our investigation tend to suggest that hormone supplementation may provide a beneficial modifying effect on some of the characteristics of tumours developing in patients taking HRT who are destined to develop breast carcinoma. Further investigations are needed to correlate these histopathological results with later outcome and to determine whether molecular mechanisms are involved in these HRT related effects.

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1 Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological


