INTRODUCTION

Breast cancer (BC) is considered to be the most frequent cancer in women. The literature risk is found to be 1 in 8 in the northern and western European population (1) and is the second major cause of death in women. In spite of high incidence of BC, absence of clear genetic data, including the cytogenetic changes all reflect the difficulties of procedures performing on the tumours. However, more investigations have been carried out on the basis of fluorescence in situ hybridization (FISH). This technique was also named interphase molecular cytogenetics which facilitates the study of numerical chromosomal changes in the interphase of tumour cells. These data revealed the variation of signals count with a heterogeneous distribution over the nuclei of chromosomal region (cen). Although there is no consistent involvement of specific chromosome alterations in breast tumours, but aberration of chromosome 3 is found to be one of the common ones (5,6,7,8). Therefore, it is important to investigate and detect the genetic alterations in the primary tumours of BC. The present data is a part of our initial oncogenic investigation from Iran (9).

MATERIALS AND METHODS

The fresh primary tumours of 6 patients with infiltrating ductal carcinoma (IDC) were directly cultured post-surgery and the cell preparations were made (Table 1).

The fluorescence in situ hybridization (FISH) was performed with application of DNA-Probe for centromere of chromosome 3 (3 cen) (2,3). FISH-technique was applied according to the following procedures:
- Slides were denatured with formamide at 75°C, then dehydrated in 70%, 90% and 100% ethanol.
- Hybridization in Hybridol and biotinylated probe (overnight incubation at 37°C).
- Slides were rinsed in formamide.
- Detection and amplification of the signals were performed with Avidin/HTC and goat-anti-Avidin.
- Staining with propidium iodide/DAPI.

The distribution of signals is presented on the basis of signal analysis in 613 cells (Table 1) and lymphocytes were used as quality control.

RESULTS

Table 1 presents tumours distribution according to the pathologic classification and the signals-count in individual and the total of tumours. The number of signals ranged between 1-6 and more than 6 (Fig 1). In the total analysis, the majority of interphases revealed to present 1 signal (33.6%) followed by 3 signals (27.8%), 2 signals (16.8%) and 4 signals (15.5%). Only 2 tumours (33.3%) showed the presence of more than 6 (up to 10) signals.
Table 1. Distribution of hybridization - signal of DNA - Probe 3 cen in the tumours of 6 breast cancer with infiltrating ductal carcinomas (IDC)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>&gt;6</th>
<th>Total number of cells</th>
</tr>
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<tbody>
<tr>
<td>20</td>
<td>52.2%</td>
<td>56.4%</td>
<td>5.6%</td>
<td>1.0%</td>
<td></td>
<td></td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>58.4%</td>
<td>81.7%</td>
<td>6.4%</td>
<td>3.7%</td>
<td></td>
<td></td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>56.5%</td>
<td>31.5%</td>
<td>33.3%</td>
<td>20.7%</td>
<td>5.2%</td>
<td>18.3</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>43.9%</td>
<td>68.6%</td>
<td>60.6%</td>
<td>10.2%</td>
<td></td>
<td></td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>58.8%</td>
<td>45.5%</td>
<td>17%</td>
<td>1.1%</td>
<td></td>
<td></td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>39.6%</td>
<td>56.4%</td>
<td>43.1%</td>
<td>17.2%</td>
<td>1.7%</td>
<td>0.8%</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.6%</td>
<td>56.4%</td>
<td>27.8%</td>
<td>13.5%</td>
<td>1.0%</td>
<td>0.4%</td>
<td>6%</td>
<td>613</td>
</tr>
</tbody>
</table>

* 7-10 signals
** 9 signals

DISCUSSION

In BC, a preferential loss of chromosome 3p was observed. The distribution of signal count per nucleus was very heterogeneous which indicates a large variability in the tumour karyotype, especially in ductal carcinomas. One of the advantages of FISH is that all cells population of tumours could be analysed. Finally, the concordance of the numerical involvement of chromosome 3 was confirmed. Most frequent signal count revealed to be one spot, either individually (12.7%-58.4%) or totally (33.6%) which followed by 2 and 4 signals (Table 1, Fig. 1).

The presence of 2 signals which could be reflective of diploid mode, was observed in only one of 4 tumours (ID 16.6%). The remaining 6 tumours (93.3%) presented 1 spot (Fig. 1) as the most frequent signal-count.

In two tumours (ID 27, ID 33, 28.5%), the most frequent signal-count was 3 (33.1%) followed by 4 and more than 6 signals (25.5% and 18.3% respectively) in tumour 27, and 3 signals (43.1%) followed by 1 and 4 signals (30.1% and 17.2% respectively) in tumour 33.

However, the present data could be considered an initial step for onco-genetic investigation on solid tumours, specifically BC, which only reflects a primary and partial report of our series of patients under genetic investigations in Iran. In conclusion, the application of fluorescence in situ hybridization (FISH) is considered as a powerful technique for the chromosome numerical alterations, including chromosome 3 cen in tumour cells of BC patients.

Acknowledgment

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REFERENCES


