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Introduction

Breast cancer is the most common tumor in women in the Eastern world. Their different incidence among different geographic areas, suggests that certain environmental or lifestyle factors may be involved in its etiology. Among the known risk factors that can be controlled include the smoking habit, weight, diet, exercise, alcohol consumption, exposure to estrogen, recent oral contraceptive use, stress and anxiety. In this regard, it is believed that modifying certain lifestyle (smoking, body mass index (BMI), alcohol consumption, fruit and vegetable consumption, and physical activity) could reduce a significant number of tumors, highlighting the 6.3% (0.5-12.1%) of postmenopausal breast cancer [1].

The smoking habit has been subject of intense study since its modifiable factor character. We know that there is a higher risk of benign tumors, preferably fibroadenomas, in current smokers, but without statistically significant [2,3] and that tobacco smoke has been implicated in various human disease conditions and the International Agency for Research on Cancer identified tobacco smoking as the cause of cancer at more organ sites that any other human carcinogen [4]. In relation to breast cancer, some groups have denied such a relationship [5-9]; others have shown a positive discreet relationship especially in premenopausal women [10-13], others an inverse relationship [14,15], and finally some suggest an increased risk associated with time, quantity and age of onset of the habit [12-16]. Xue et al. [17] observed that breast cancer incidence was associated with a higher quantity of current and past smoking, younger age at smoking initiation, longer duration of smoking and more pack-years of smoking. Premenopausal smoking was associated with a slightly higher incidence, while

Conclusion: Our results led us to the following: 1) Women with IDCs and smoking habit had lower age and the tumors were more frequently bcl2 positive than never smoking habit; 2) age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in nonsmoking habit subgroup (p: 0,003). Smokers women showed more frequently history of contraceptives intake (26,4% vs 7,8%; p<0,001). When we considered the molecular subtypes of IDCs, we not observed any statistically difference; nevertheless, when we analyzed current and quit versus never smoking patients, we noted that luminal B subtype was less frequent in those (p: 0,068) and the tumors were more frequent bcl-2 positive (p:0,048) than never smoking subgroup.

Results: IDCs of smoking habit subgroup had lower age than nonsmoking habit subgroup and were more frequently bcl-2+ (p:0,083). There were not differences in the other clinico-biological parameters as well as in the follow-up considering recurrences and deaths due to the tumor. Likewise, age of ex-smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in nonsmoking habit subgroup (p: 0,003). Smokers women showed more frequently history of contraceptives intake (26,4% vs 7,8%; p<0,001). When we considered the molecular subtypes of IDCs, we not observed any statistically difference; nevertheless, when we analyzed current and quit versus never smoking patients, we noted that luminal B subtype was less frequent in those (p: 0,068) and the tumors were more frequent bcl-2 positive (p:0,048) than never smoking subgroup.

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Abstract

Introduction: The aim of this study was to analyse the possible associations between smoking habit and some clinico-biological parameters of breast infiltrating ductal carcinomas (IDCs).

Material and Methods: The study group included included 291 females with IDC who had undergone no prior treatment. Out of them, 48 were current smoking, 11 quit smoking and 232 never smoking. All were studied at the same Breast Cancer Unit. Age, tumor size, axillary lymph node involvement (N), distant metastasis (M) and histological grade (HG), as well as the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), K67, p53, bcl2 and androgen receptor (AR) were analyzed. Also, we dose the serum levels of CEA and CA15.3. We can follow up 276 patients during a period of time which ranged between 8 and 240 months (83,3+/51,8; median 84 months).

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Research Article

Smoking Habit and Clinico-Biological Parameters of Breast Cancer

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postmenopausal smoking was inversely correlating with the breast cancer risk. Braithwaite et al. [18] noted that women who were current smokers had a two-fold higher rate of dying from breast cancer and an approximately four-fold higher rate of dying from non-breast causes. Daniell et al. [19] have observed that breast tumors with four or more positive nodes were more frequently associated with larger primary tumors, obesity and smoking habit. Likewise, they found little evidence of an association between former smoking and breast cancer mortality.

Illic et al (20) have found that the breast cancer risk was significantly increased in those who quit smoking at ≤50 years of age and in those who quit smoking less than 5 years before diagnosis of the disease. Pierce JP et al. [21] compared with never smokers, former smokers with less than 20 pack-years of exposure had no increased risk of any outcome. However, former smokers with 20 to less than 34.9 pack-years of exposure had a 22% increased risk of breast cancer recurrence. For former smokers with 35 or more pack-years of exposure, the probability of recurrence increased by 37% and breast cancer mortality increased by 54%. Current smoking increased the probability of recurrence by 41% and increased breast cancer mortality by 60%. In meta-analyses, current and former smoking were weakly associated with risk while a stronger association was observed in women who initiated smoking before first birth [22].

Regarding passive smoking, it has been seen that there exist significant interactions between certain types of passive smoke exposure and genetic variants in CYP2E1, NAT2 and UGT1A7 [23], and that those have higher risk, especially if it comes to premenopausal women [24,25].

In last years, prognostic value of preoperative CEA and CA15-3 levels in breast cancer has more important. Study has shown that levels of CEA combined with CA15-3 give us important information for diagnosis and treatment of breast cancer [26]. Accordingly, the European Group on Tumor Markers has recommended the CEA and CA15-3 levels be used for assessing prognosis, the early detection of disease progression, and treatment monitoring in breast cancer [27].

In this study we aimed to analyze several factors, such as the histological grade (HG), ER status, PgR status, Ki67 (proliferation marker), p53, bcl2 and AR in order to identify biological characteristics of breast carcinomas in smokers, ex-smoker, and non-smoking women. Smokers, ex-smoker, and non-smoking women.

Material and Methods

Our study group included 291 females with Infiltrating Ductal Carcinomas (IDC) who had undergone no prior treatment. Out of them, 48 were current smoking, 11 quit smoking and 232 never smoking. Everyone were studied at the same Breast Cancer Unit. Age, tumor size, axillary lymph node involvement (N), distant metastasis (M) and histological grade (HG), as well the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PgR), Ki67, p53, bcl2 and androgen receptor (AR) were analyzed. Serum carcinoembryonic antigen (CEA) and CA15.3 were determined by an electrochemiluminescence immunoassay (ECLIA) from Roche (Swiss), and an electro-chemo luminescence assay (ECLIA- Elecsys 170 Roche) respectively. We can follow up 276 patients during a period of time which ranged between 8 and 240 months (83,3+/−51,8; median 84 months).

Immunohistochemical staining on tissue sections of 4–5 microns was done by the EnVision method with a heat-induced antigen retrieval step. Sections were immersed in boiling 10 mmol/l sodium citrate at pH 6.5 for 2 minutes in a pressure cooker. ER and PgR were determined by mAbs ER/PgR phramDx (clones dID5 (0,1mg/mL) and ER–2123 (0,5 mg/ml) for ER and PgR) 1294 for the PgR. P53 (DO–7, Dako, (335 mg/L) dilution 1/50), Ki67 (MIB-1, Dako, (80mg/L) dilute 1/200), bcl2 (Biogenex (10mg/ml), dilution 1/150) and Androgen receptor (AR441, Dako, dilution 1/150) were used in this study. Dako goat polyclonal biotinylated secondary antibody was used. The ER and PgR were assessed according to the Allred score in negative (scores 0–2) and positive (score 3–8) and the thresholds of positivity for p53, Ki67 were 20% and 15 % respectively. AR were classified as positive or negative without any score, and bcl2 as negative (-: <10% stained cells), weakly positive (+: 10–20%) and strong positive (++: >30%).

Data obtained were evaluated using the SPSS 15.0 software for Windows (SPSS, Chicago, IL. USA). Parameters that did not follow a normal distribution, values were presented as range, 25th percentile, 75th percentile and median. We used the Chi square test with Yates correction, if necessary, for qualitative variables comparison and the Mann Whitney test for continuous ones. A p-value ≤ 0.05 was considered as statistically significant.

Results

As shown in Table 1, the IDCs of smoking habit subgroup had lower age than non-smoking habit subgroup (55,0 +/- 6,6 vs 60,7 +/- 8,0; p <0,00001) and were more frequently bcl-2+ (40/45 vs 161/208) near statistical significance (p=0,083). In the other parameters we do not find statistically significant differences, nor in the subsequent outcome considering recurrences and/or deaths from the tumor. Likewise, age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in non-smoking habit subgroup (54,7 +/- 5,3 vs 60,7 +/- 8,0; p=0,003). Smokers women showed more frequency background of contraceptives intake (26,4% vs 7,8%; p<0,001. Data not shown). When considering molecular types of IDCs, we found no statistically significant differences between different subtypes; however, by grouping smokers and ex-smokers women, we found that luminal B subtype (ER+ and PgR+/−, HER2−, Ki67 >/=14%) was less frequent in this patient group compared to women who did not smoke (7/50 vs 53/202; p=0,068) (Table 2).

When comparing non-smokers subgroup against active and former smokers, we found, as shown in Table 3, that the different immunohistochemical expression of bcl-2 reached
statistical significance (p=0.048), being lower in non-smoking habit subgroup. We did not observe differences in the other parameters analyzed among smokers and non-smokers women.

**Discussion**

Tobacco causes approximately 25% of all cancers in men and 4% in women and has been associated with increased mortality following diagnosis of a variety of cancers as prostate, colorectal, leukaemia, malignant melanoma, ... etc [18]. Likewise, in women over the age of 50, smoking (>15 cigarettes either currently or in the past) was the only factor associated with cancer (breast 31.9% or colorectal 12.7%) [12]. Several reports have studied smoking habit as a risk factor for breast cancer and the results have been inconclusive. Currently it seems accepted that breast cancer incidence is associated with a higher quantity of current and past smoking, younger age at smoking initiation, longer duration of smoking and more pack-years of smoking [17].

We know that the phenotypical alterations induced by cigarette smoke are accompanied by numerous changes in gene expression that are associated with epithelial to mesenchymal transition and tumorigenesis [28]. Recent studies evaluating the possible modifying role of polymorphisms in genes involved in the metabolism of tobacco products, particularly NAT2, have contributed another dimension to these assessments, although to date that evidence remains equivocal [29-32]. Likewise, other genes related with inflammation, DNA repair, apoptosis, signal transduction, metabolism, cell cycle, cell proliferation and transcription related genes are involved also. Carcinogens in tobacco pass through the alveolar membrane and enter the bloodstream transported to mammary tissue through plasma lipoproteins. Furthermore, because these breast carcinogens are lipophilic, they may be stored in breast adipose tissue and metabolized and activated by mammary epithelial cells. Also, in breast cancer patients with smoking habit there is a significant rise in oxidative stress and low levels of antioxidants. Free radicals facilitate the progression of breast cancer [33]. Smoking affects circulating hormone levels [33]; so, the current smoking and increasing amount of daily smoking is associated with high testosterone levels [34] and postmenopausal current smokers had lower IGF-1 and IGFBP-3 levels [35]. Salem et al. [36], have observed that cigarette smoke exposure is indeed sufficient to drive the onset of the cancer-associated fibroblast phenotype via the induction of DNA damage, autophagy and mitophagy in the tumor stroma. These fibroblasts increased breast cancer tumor growth in vivo up to 4-fold. When comparing smokers and non-smokers women subgroups, we found that those showed breast carcinomas a much younger age (55 vs 60.7 years), confirming highly statistically significant differences.

**Table 1:** Distribution of different parameters in the subgroups of patients included in the study.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>SH+Ex-SH</th>
<th>Non-SH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41-68 (55.0+/-6.6)</td>
<td>37-88 (60.7+/-8.0)</td>
<td>46-64 (54.7+/-5.3)</td>
</tr>
<tr>
<td>Size</td>
<td>0.3-7.0 (1.7+/-1.2)</td>
<td>0.2-8 (1.5+/-0.9)</td>
<td>0.2-3.0 (1.2+/-0.7)</td>
</tr>
<tr>
<td>CEA</td>
<td>0.1-1.5 (0.5)</td>
<td>0.1-1.8 (0.9)</td>
<td>ng/ml</td>
</tr>
<tr>
<td>CA15.3</td>
<td>1-33.9 (19.3)</td>
<td>0.1-162 (17.2)</td>
<td>9.2-28 (20.1)</td>
</tr>
<tr>
<td>N+</td>
<td>14/48</td>
<td>60/232</td>
<td>0/11</td>
</tr>
<tr>
<td>N+&gt;3</td>
<td>4/48</td>
<td>12/232</td>
<td>0/11</td>
</tr>
<tr>
<td>N+&gt;10</td>
<td>1/48</td>
<td>2/232</td>
<td>0/11</td>
</tr>
<tr>
<td>M+</td>
<td>1/48</td>
<td>5/232</td>
<td>0/11</td>
</tr>
<tr>
<td>HG3</td>
<td>12/48</td>
<td>63/232</td>
<td>0/11</td>
</tr>
<tr>
<td>ER+</td>
<td>42/48</td>
<td>192/232</td>
<td>0/11</td>
</tr>
<tr>
<td>PgR+</td>
<td>33/48</td>
<td>142/232</td>
<td>0/11</td>
</tr>
<tr>
<td>AR+</td>
<td>39/45</td>
<td>160/200</td>
<td>0/11</td>
</tr>
<tr>
<td>PS3+</td>
<td>7/48</td>
<td>47/232</td>
<td>0/11</td>
</tr>
<tr>
<td>Bcl2+</td>
<td>40/45</td>
<td>161/208</td>
<td>0/11</td>
</tr>
<tr>
<td>Ki67+</td>
<td>23/48</td>
<td>120/232</td>
<td>0/11</td>
</tr>
</tbody>
</table>

Abbreviations: SH: Smoking Habit; N: axillary lymph Node involvement; M: distant Metastasis; HG: Histological Grade; ER: Estrogen Receptor; PgR: Progesterone Receptor and AR: Androgen Receptor.

**Table 2:** Molecular subtypes and smoking habit (SH).

<table>
<thead>
<tr>
<th>TYPE</th>
<th>SH+Ex-SH</th>
<th>Non-SH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>27/39</td>
<td>117/202</td>
<td>ns</td>
</tr>
<tr>
<td>Luminal B</td>
<td>6/39</td>
<td>52/202</td>
<td>ns</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>3/39</td>
<td>16/202</td>
<td>ns</td>
</tr>
<tr>
<td>Her2+</td>
<td>3/39</td>
<td>16/202</td>
<td>ns</td>
</tr>
</tbody>
</table>

Abbreviation: SH: Smoking Habit.

**Table 3:** Clinical and biological differences between current and ex smoking habit (SH) and non-smoking habit groups.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>SH+Ex-SH</th>
<th>Non-SH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N+</td>
<td>16/59</td>
<td>60/232</td>
<td>ns</td>
</tr>
<tr>
<td>N+&gt;3</td>
<td>4/59</td>
<td>12/232</td>
<td>ns</td>
</tr>
<tr>
<td>N+&gt;10</td>
<td>1/59</td>
<td>2/232</td>
<td>ns</td>
</tr>
<tr>
<td>M+</td>
<td>1/59</td>
<td>5/232</td>
<td>ns</td>
</tr>
<tr>
<td>HG3</td>
<td>16/59</td>
<td>63/232</td>
<td>ns</td>
</tr>
<tr>
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<td>192/232</td>
<td>ns</td>
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<td>PgR+</td>
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<td>142/232</td>
<td>ns</td>
</tr>
<tr>
<td>AR+</td>
<td>47/59</td>
<td>160/200</td>
<td>ns</td>
</tr>
<tr>
<td>PS3+</td>
<td>11/59</td>
<td>47/232</td>
<td>ns</td>
</tr>
<tr>
<td>Bcl2+</td>
<td>50/56</td>
<td>161/208</td>
<td>0.048</td>
</tr>
<tr>
<td>Ki67+</td>
<td>26/59</td>
<td>120/232</td>
<td>ns</td>
</tr>
<tr>
<td>CK5/6</td>
<td>2/30</td>
<td>5/57</td>
<td>ns</td>
</tr>
<tr>
<td>Deaths</td>
<td>1/55</td>
<td>6/221</td>
<td>ns</td>
</tr>
<tr>
<td>Recurrences</td>
<td>1/55</td>
<td>6/221</td>
<td>ns</td>
</tr>
</tbody>
</table>

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Statistically significant differences: Age SH vs Non-SH: p<0.00001; Age Non SH vs Ex-SH: p=0.003; bcl-2 SH vs Non-SH: p= 0.083; CEA SH vs CEA ex-SH: p= 0.004.
The same fact was observed among former and non-smokers females, which leads to believe that smoking (current or former) is associated with mammary tumors at earlier ages.

Clinically and biologically, mammary tumors did not differ between the two groups (smokers and non-smokers patients), except in the immunohistochemical expression of bcl–2, which was more frequent in the smokers group near patients, except in the immunohistochemical expression of bcl–2 reached statistical significance (p=0.048), being lower in non-smoking habit subgroup, all of which support the positive association between the expression of bcl–2 and smoking habit, whether active or not.

The bcl2 gen encodes for a mitochondrial protein which prevents apoptosis and prolongs cell survival, thus antagonizing the effects of the p53 protein [37, 38]. This suggest the possibility that increased expression of bcl–2 in mammary tumors from smokers’ women determine a worse prognosis by preventing apoptosis [39] or be accompanied by a better prognosis as have been shown in these malignant neoplasms [40]. It could also be that defined a poorer response to certain chemotherapy as demonstrated Samanta et al. [41] in non–small cell lung cancer. Cucima y cols. [42] have observed that nicotine, contained in cigarette smoking, stimulates cell proliferation and suppress physiological apoptosis in colon cancer cells. Nicotine induced a statistically significant increase in the expression of PI3K and P-Akt/Akt ratio as well as in the expression of PKC, ERK1/2, surviving and P–bcl2 (Ser70; 70 phosphorylated bcl–2) in colon cancer cells. These findings support that we detect higher expression of bcl–2 in smokers’ women group.

Many apoptosis related genes are deregulated with smoking habit. One is the bcl2 associated anathogene (BAG1), novel cytoplasmic binding partner membrane form of heparin-binding EGF–like growth factor [43], which is down–regulated. BAG1 is a membrane protein that blocks a step in a pathway leading to apoptosis or programmed cell death. The protein encoded by this gene binds to bcl2 and enhances the anti-apoptotic effects representing a link between growth factor receptors and anti–apoptotic mechanisms. The protein was found at high levels in several types of human tumor cell lines among leukaemia, prostate, breast, etc. Likewise, high levels have been implicated as a prognostic indicator in breast cancer [44]. Also, BAG1 is implicated, among several other functions, in the response to nicotine. Over–expression of BAG1 significantly inhibited p53 induced growth arrest in some tumor cell lines [44] and this is consistent with our findings, since from the 40 cases bcl–2+ in the smoker group, 36 were p53 negative, not appreciating differences in the number of cases with lymph node involvement, distant metastasis, histological grade and cell proliferation (Ki67) between the two subsets of tumors. Some authors shows transformed phenotype in normal breast epithelial cell line (MCF10A) associated with Bcl–xL mRNA increase in a dose–dependent manner whereas mRNA level of Bcl–2 remained unchanged [45], this Bcl–xL increase is regulated by C/EBPbeta in MCF10A cells in response to cigarette smoke condensate (CSC) treatment suggesting this as a potential target for chemotherapy [46].

Considering the molecular types of IDCs [47], we observed that the luminal B subtype was less frequent in the group of smokers (6/39 vs 52/202), near statistical significance (p=0.099), relationship increased when compared non-smoker subgroup with active and former smokers subgroups together (7/50 vs 53/202; p=0,068). Although several groups have described that some of the substances found in cigarette act like estrogens, smoking has been postulated to have anti-estrogenic effect and this would explain our findings also that smoking is associated with an increased occurrence of hormone–receptor negative tumors [48], especially when the patients starting to smoke at an early age of <= 19 years [49, 50].

Finally, we observed higher concentrations of serum CEA in smokers (See Table 1), an association known for years [51] and while not in serum CA15.3.

Our results led us to the following: 1) Women with IDCs and smoking habit had lower age and the tumors were more frequently bcl2 positive than never smoking habit; 2) age of ex smoking habit subgroup was similar to that observed in active smoking habit subgroup, and lower than that observed in non-smoking habit subgroup; 3) in smoking/ex–smoking women vs non–smoking women, luminal B molecular subtype was less frequent than in non–smoking women.

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References


