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Association Between Smoking and a Molecular Profile of Breast Cancer

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ABSTRACT

Background: Female breast cancer is one of the causes of the highest cancer mortality and morbidity in the world. It is already known that there is a strong association between smoking and breast cancer. However, the association between smoking and tumor severity is not very clear. The objective of this study was to assess the severity of the breast tumor using the tumor's molecular classification as a tool according to its immunohistochemical profile in smoking and nonsmoking women.

Methods: This is a longitudinal study in which 208 women with a diagnosis of breast cancer were followed for 17 months, 80 of whom were smokers, and all underwent anatomopathological diagnosis by core biopsy and subsequent immunohistochemistry, followed by treatment indicated according to the type and the clinical staging of the tumor. The severity of the tumor was assessed by its molecular classification according to its immunohistochemical profile.

Results: Smoking was associated with higher mortality. The tumor with the most severe immunohistochemical profile was found in younger smokers. Overall, 19.7% of smokers and 10% of non-smokers had a triple-negative tumor. The age of female smokers with triple-negative was 48.2 years, and that of nonsmoking women was 52.6 years (P=0.005). In 17 months of follow-up, mortality among smokers was 39.5%, and for nonsmokers, 20%. Survival was statistically significantly lower among the group of smokers (P=0.01).

Conclusion: Smoking is associated with greater breast cancer severity, as the risk for cancer severity was 5.5 times higher for the smoking group, and survival was statistically significantly lower among the smoking group.

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INTRODUCTION

Breast cancer is one of the causes of the highest cancer mortality and morbidity in the world.[1] It is more prevalent in women, being the major cause of cancer death among them. The profile of women's lifestyle has changed in recent years amid the social transformation occurring in these decades, as well as

sentinel lymph node (SLN) biopsy is a standard procedure for patients with clinically node-negative (cN0) breast cancer, playing a crucial role in determining the need for axillary lymph node dissection (ALND). ALND is unnecessary not only in patients with negative SLNs[1,2], but also in those with one or two positive SLNs undergoing breast- the prevalence of diseases that affect women. In this context, the new lifestyle adopted by today's women, including smoking, explains part of the emergence of certain diseases.[2,3] For Brazil, according to the National Cancer Institute[1], which is the national reference government agency for the treatment of this disease, 59,700 new cases of breast cancer are expected, with an estimated risk of 5.6, 33 cases per 100,000 women.

Breast cancer is a chronic disease characterized by disordered cell growth, resulting from genetic mutations. It is estimated that 90–95% of them are sporadic (non-familial) and result from somatic mutations, damage to genetic material that accumulates throughout life. It is also estimated that 5–10% are hereditary (family members) due to the inheritance of a germline mutation at birth, which gives these women a greater susceptibility to breast cancer during their lifetime.[4,5] The modern Western model of life produces and reproduces risk factors that directly influence carcinogenesis.[6]

Some studies have shown that the carcinogenic agents present in tobacco, for example, are polycyclic aromatic hydrocarbons, aromatic amines, and N-nitrosamines.[3,5,7] They can be transported through the bloodstream to the mammary glandular tissue, initiating the neoplastic process by damaging the nuclear DNA content with these agents.[8] However, it is not entirely clear whether these lesions in the cell DNA caused by these agents make the tumor more aggressive.

A study published in the scientific journal Cancer, from the American Cancer Society, reported the link between tobacco consumption and the most common type of breast cancer in young women. In the study, researchers concluded that patients under 44 years of age who smoke at least 1 pack of cigarettes a day for at least ten years have a 60% higher risk of developing tumors, but it is not yet known whether tobacco is related to the severity of the disease.[9]

Given this, the objective of this study is to compare the severity of breast cancer according to the molecular classification.[3,4] According to its immunohistochemical profile (luminal A, luminal B, hybrid luminal, *HER2* overexpression, and triple negative),[5,6] clinical staging (early encompassing stages from 0 to IIB, and late encompassing stages from III to IV), and survival, among a group of smoking and nonsmoking women.

METHODS

This is a longitudinal study with a quantitative approach, involving 208 women with breast carcinoma undergoing treatment at the mastology service of 2 public hospitals in the State of São Paulo. Among them, 80 were smokers, and 128 were non-smokers. These were all breast cancer patients treated during the study period. Smokers were considered those who regularly used at least one cigarette per day.

Follow-up was carried out by visits every 15 days during the chemotherapy period, and every 30 days after this period. After 17 months, data from these consultations were not included in this study.

This study included women aged 25 to 65 years who, at the time of breast cancer diagnosis, had a history of full-term pregnancy (38 to 41 weeks of gestational age) with breastfeeding exercise of at least one month.

The study did not include women with other types of previous cancer, men with breast cancer, or any other condition of autoimmune diseases or immune deficiency (Figure 1).

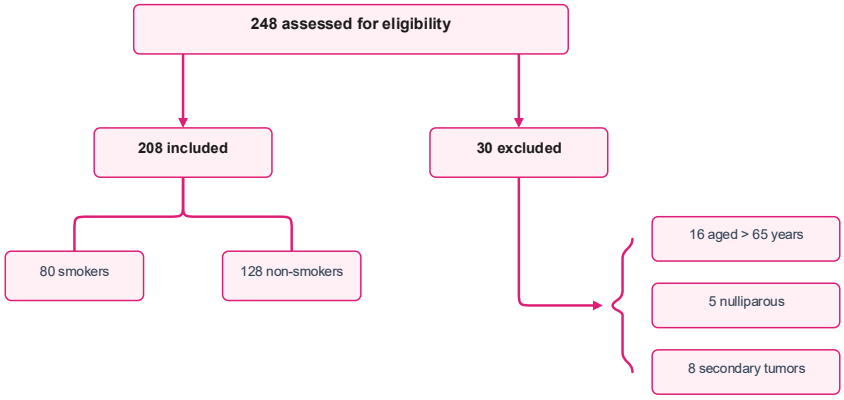

Figure 1. Patient Randomization Flowchart

The diagnosis of breast cancer was made using pathological and immunohistochemical exams.[10,11] Smokers were those who smoked at least one cigarette per day.

The sample was of a convenience nature, from patients who sought the Unified Health System (SUS) service, scheduled at random by the "State Regulation System" (SISREG), for the specialty of mastology. The concomitance of the diagnosis of breast cancer and the pregnant puerperal period was considered positive if the diagnosis of breast cancer occurred during pregnancy or in the year after delivery. This protocol was approved by the institutional ethics committee under number 1,064,564. All patients signed a free and informed consent form.

The molecular classification[3,4] of the tumor followed pre-established criteria that define distinct subtypes according to the molecular studies of breast carcinoma based on the identification of the gene expression profile using the microarray cDNA.[12] The subtypes were: Luminal A, Luminal B, Luminal Hybrid, *HER2* overexpression, and triple-negative. The clinical staging of the tumors followed the criteria defined by the tumor, node, metastasis (TNM)[14] system of the International Union against Cancer.

The immunohistochemical diagnosis was performed by histological sections, with the respective positive and negative controls undergoing immunohistochemical examination in an automated system with antigen recovery in PTLink (Dako) and incubation, development, and counterstaining in AutoStainer Link, using highly sensitive polymer and ready-to-use FLEX antibodies. The calculation of mortality was established from the moment of diagnosis until 17 months after.[12,13]

The statistical analysis was based on the Kolmogorov-Smirnov normality test to assess the distribution of data in relation to normality. Data were presented as means and standard deviations; the odds ratio test was used for categorical variables of severe or non-severe cancer (smoker vs non-smoker) between the groups. The variables related to the results of exams were evaluated by means of analysis of variance. Survival between smokers and non-smokers was analyzed using the Kaplan-Meier test. $P<0.05$ was considered statistically significant.

RESULTS

Of 208 women with breast cancer undergoing treatment, 30 were excluded. Overall, 80 women reported smoking, and 128 were classified as non-smokers. No difference was found between the smoking and nonsmoking groups in the anthropometric data and age at diagnosis; however, in the nonsmoking group, there was a higher proportion of women with arterial hypertension, as shown in Table 1.

Table 1. Distribution of patients with breast cancer according to smoking

Variables	Smoking n=80	Nonsmoking n=128	*P*
	mean±SD	mean±SD	
Age, y	52.4±7.3	54.5±8.1	0.85
BMI, kg/m^2	28.1±4.7	29.4±4.9	0.67
Number of children	2.5±1.4	2.5±1.5	0.99
Age of diagnosis, y	51.3±8.6	53.2±8.4	0.61
Age of first pregnancy, y	23.5±5.7	24.7±6.6	0.58
	%	%	P
Family history	25.3	14.8	0.04
Arterial hypertension	59.0	74.2	0.02
Diabetes	23.0	22.6	0.86

Chi-square test

In general, the molecular profile and gene expression of cancer most frequently found were Luminal B, followed by Luminal A, triple-negative, Luminal Hybrid, and *HER2* overexpression (Figure 2). However, it was seen that for the group of nonsmoking women, there was a higher proportion of women with Luminal A and B types, while among smokers, there was a higher proportion of women with Luminal B, Luminal A, and triple-negative tumors, according to Table 2.

For the sample studied, the clinical stage most frequently found was I, followed by IIB, IIA, and IIIA

at the same frequency, IIIB, and IV. No difference was found for the groups in relation to clinical staging, as shown in Table 3.

Table 2. Distribution of the Molecular Profile and Gene Expression of the Tumor in Patients with Associated Breast Cancer and Smoking

Variables Molecular Profile)	Smoker n=80	Nonsmoker n=128	*P*
Luminal A, %	24.0	35.9	0.035
Luminal B, %	31.3	35.9	0.246
Luminal hybrid, %	14.4	11.7	0.281
HER2 overexpression, %	7.2	6.3	0.391
Triple-negative, %	19.0	10.1	0.030
Others, %	4.1	0.1	0.85
Total, %	100	100	-

Chi-square test

Figure 3 presents the results of the comparative analysis of the risk for the severity of cancer (neoadjuvant chemotherapy groups) for the smoking and nonsmoking groups. The analysis showed that the risk of more severe cancer is 5.5 times higher for smokers compared to the group of nonsmoking patients.

Table 3. Distribution of the Clinical Stage of the Tumor in Patients with Associated Breast Cancer and Smoking

Variables clinical stage	Smokers n=80	Nonsmokers n=128	*P*
I, %	36.1	35.9	0.45
IIA, %	15.6	25.0	0.09
IIB, %	19.2	15.6	0.12
IIIA, %	14.4	13.2	0.85
IIIB, %	13.2	10.3	0.45
IV, %	1.5	0.5	0.84
Total	100	100	

Chi-square test

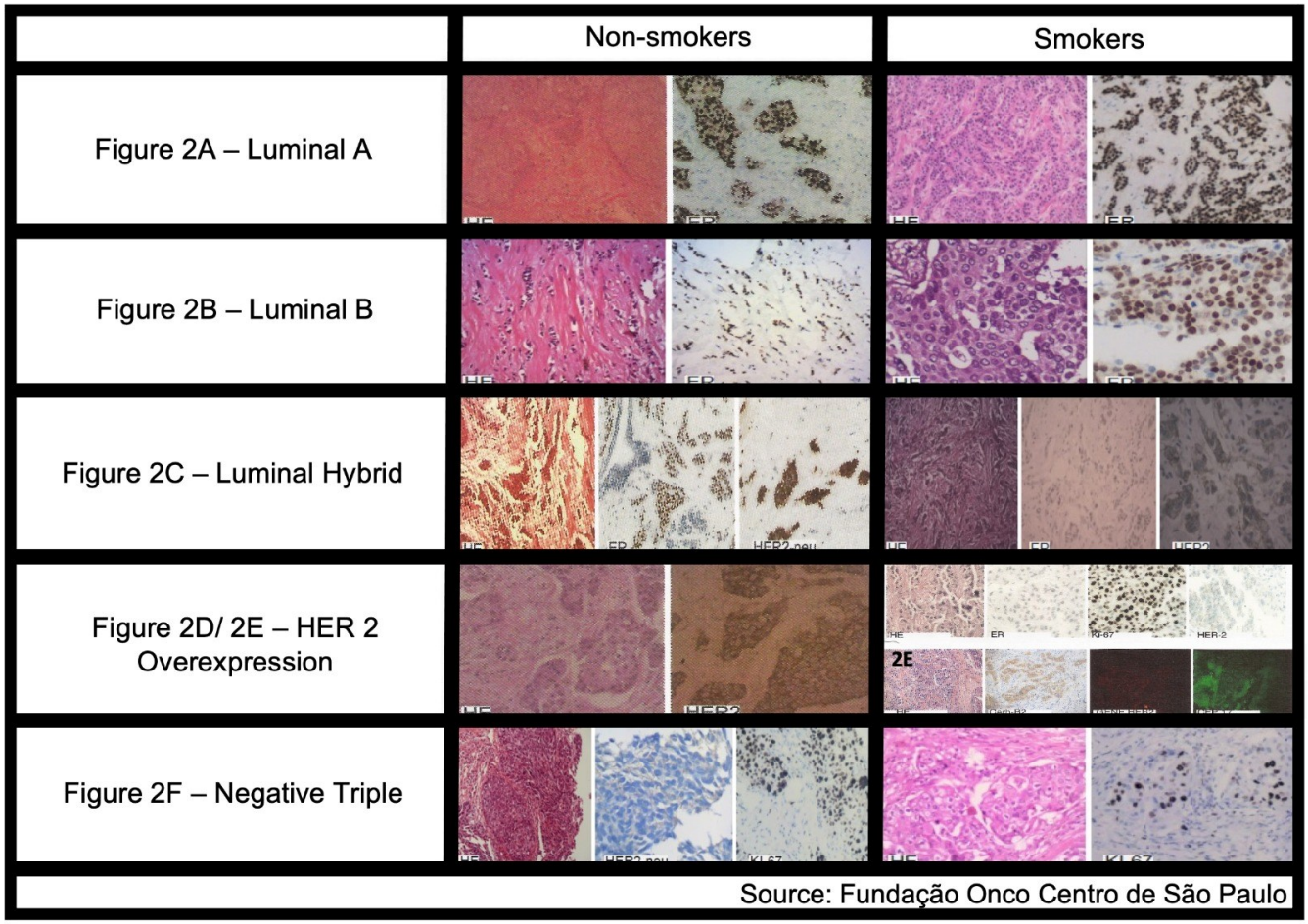

Figure 2. Immunohistochemical profiles of breast cancer molecular subtypes. The figure displays representative images of different subtypes in both non-smoking and smoking patients. Panels show: (A) Luminal A, (B) Luminal B, (C) Luminal Hybrid, (D/E) HER2 Overexpression, and (F) Triple-Negative breast cancer. Stains include Hematoxylin and Eosin (H&E) and immunohistochemistry for key markers like Estrogen Receptor (ER), HER2, and Ki-67. (Source: Fundação Onco Centro de São Paulo).

Figure 4 shows the results of the assessment of survival among patients with breast cancer, smokers, and nonsmokers, during a 17-month follow-up period. It was seen that the survival of the nonsmoking group was significantly higher than that of the smoking group (P=0.01). On average, the survival of nonsmoking patients was 240 days. It was also found that the risk of death for these patients was 2.2 (95% CI, 1.19–4.58).

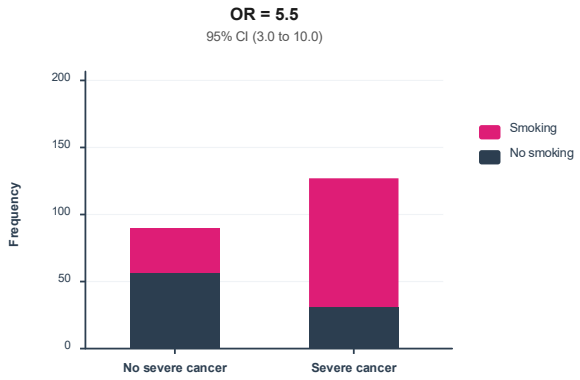

Figure 3. Risk of Severe Cancer in the 2 Groups

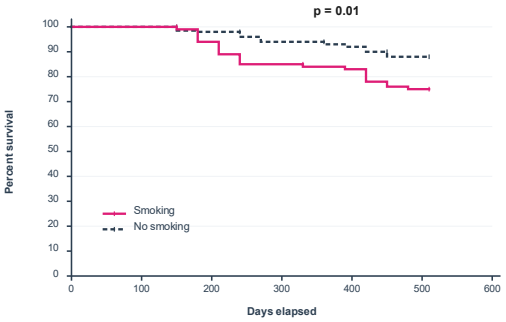

Figure 4. Survival of the 2 Groups in 510 Days of Follow-Up

DISCUSSION

The main objective of this study was to assess whether smoking is related to the severity of breast cancer. Among the most important results demonstrated are the association between smoking and the histological severity of cancer, and that smoking anticipates the appearance of breast cancer by at least 3 years.

It has been seen that younger patients and smokers have histologically more severe breast cancer. Young patients, under the age of 35, have a higher incidence of high histological grade tumors (undifferentiated cells), high cell proliferation with a high mitotic index, marked cell pleomorphism, high cytoplasm-to-nucleus ratio, and higher proportions of tumors with negative estrogen receptors and/or overexpression of *HER2*.[14]

The 2013 PLATINO Study[15] showed that approximately 14% of adult smokers in Brazil. The profile of women with breast cancer studied showed a high number of cases associated with tobacco use between the two groups.

In this study[16], the prevalence of the most serious type of cancer, i.e., triple negative, was almost double in smokers compared to nonsmokers, and was also associated with younger women. In a case-control study, it was found that younger age at smoking initiation is related to younger age at diagnosis. Smokers were diagnosed on average 10 years earlier than non-smokers, and the incidence of the invasive carcinoma subtype increased with the intensity and duration of smoking. Di Cello *et al.*[14] suggested that cigarette smoke promotes epithelial-to-mesenchymal transition, producing a more aggressive breast cancer phenotype in vitro.

In a study of 73,388 women, of whom 3721 patients had invasive breast cancer identified during an average follow-up of 13.8 years, the incidence was higher among current smokers and ex-smokers (hazard ratio [HR]=1.13) than among those who never smoked. It was also seen that women who started smoking before menarche (HR=1.61) or before the first pregnancy (HR=1.45) were at higher risk.[17] In this study, mortality was higher among female smokers. This was possibly due to the molecular changes caused by smoking, which enable the development of more invasive tumors with a greater probability of manifestation in other organs. The risk of progression and death from oropharyngeal cancer increases directly as a result of exposure to tobacco at the time of diagnosis and during therapy and is independent of tumor status and treatment.[18]

Tobacco is one of the risk factors for the development of cancer of the respiratory and non-respiratory pathways through carcinogens in its composition, such as polycyclic aromatic hydrocarbons, aromatic amines, and N-nitrosamines.[3,5,7] Tobacco carcinogens cross the alveolar membrane and go into the bloodstream, where they are transported to the breast tissue via plasma lipoproteins. These carcinogenic agents can be stored in the fatty tissue of the mammary gland and metabolized and activated by the epithelial cells of the breast.[18,19]

According to the prospective Women's Health Initiative Observational study, conducted by the Health Partners Research Foundation, Minneapolis, USA, from 1993 to 1998 to determine links between smoking and breast cancer, where 80,000 women were analyzed and followed for 10 years, 3250 cases of invasive breast cancer were identified. The results showed that women who smoke after menopause are 16% more likely to develop breast cancer than women

who have never smoked in their lives, and ex-smokers have a 9% chance. There is a greater danger among those who smoke after 50 years of age, or those who start smoking in adolescence, periods considered to be a "risk window" for breast cancer. The risk of developing cancer persists until twenty years after the woman stops smoking.[20,21]

Therefore, the relationship between histologically more severe breast cancer in younger patients and smoking has distinct biological characteristics for worse prognosis and shorter survival. Very consistent data, such as the California Teachers Study[22], where more than 116,000 women were followed for 5 years, confirm the importance of smoking in the risk of breast cancer, stressing that women with no family history of the disease have a higher risk of cancer when they are smokers. Other studies show that smoking is an independent risk factor for severe skin reactions due to adjuvant radiotherapy for breast cancer.[7]

The limitations of this study include the fact that the follow-up was not carried out until 5 years, which could have better shown the outcome of all women. However, this study has important clinical implications. The main implication that makes this study unique is that smoking is related to the severity of the disease. This information is important in terms of health promotion in encouraging women to avoid smoking, and also to relate the prevalence based on the number of women who smoke.

CONCLUSION

This study showed an association between smoking and breast cancer, with a more severe molecular profile for women who smoke, and a strong relationship was also seen between early smoking initiation and higher mortality. The molecular subtype with triple-negative gene expression had a frequency of 19.0% in women who smoked and only 10.1% in women who did not smoke. The mean age of triple-negative smoking women was 48.2 years, and the age of nonsmoking women was 52.6 years (P=0.005). In 17 months of follow-up, mortality among smokers and among non-smokers was 39.5% and 20%, respectively.

ETHICAL CONSIDERATIONS

This protocol was approved by the institutional ethics committee under number 1,064,564. All patients signed a free and informed consent form.

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CONFLICT OF INTEREST

The authors have no relevant financial or nonfinancial interests to disclose.

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