

RESEARCH ARTICLE

Differential Association of the Lifestyle-Related Risk Factors Smoking and Obesity with Triple Negative Breast Cancer in a Brazilian Population

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Abstract

Background: A longer lifespan and changing lifestyle-related and reproductive risk factors have led to an increased incidence of breast cancer in Brazil. There have been few studies about associations of specific risk factors with molecular subtypes of the disease. The aim of the present study was to identify factors that modulate the risk of triple negative breast cancer. **Materials and Methods:** A case-case analysis was performed. Data for 236 breast cancer patients from two reference centres in North-eastern Brazil were applied to assess the association of risk factors with triple negative breast cancer relative to the luminal A subtype. Molecular subtypes were defined by expression status of hormone receptors and amplification of HER2. Nominal logistic regression was used to estimate odds ratios and to generate a model of independent variables. **Results:** Smoking and body mass index were differentially associated with likelihood of triple negative breast cancer compared to the Luminal A subtype ($p=0.013$; $p=0.004$): Women who ever smoked some time in their lives were 4.016 (OR= 0.249; CI 95%: 0.09- 0.71) times less likely to have triple negative breast cancer. Obese and overweight patients, respectively, were 4.489 (CI 95%: 1.32- 15.28) and 1.340 (CI 95%: 0.38- 4.69) times more likely to have triple negative breast cancer. **Conclusions:** Case-case analysis with the Luminal A subtype as the reference group indicated that smoking and body mass index are differentially associated with risk of triple negative breast cancer.

Keywords: Breast cancer- molecular subtypes- triple negative tumours- risk factors- obesity- smoking

Asian Pac J Cancer Prev, **18** (6), 1585-1593

Introduction

Breast cancer is the most frequently diagnosed cancer among females and a leading cause of death worldwide (Torre et al., 2015). The burden of the disease is progressively shifting from developed to developing countries of Africa, Asia and Latin America, where breast cancer incidence and mortality are increasing (Torre et al., 2015). In Brazil, the estimated risk increased from 52.00 to 56.20 new cases between 2006 and 2016, respectively, per 100,000 women (INCA 2006 and 2016). In North-eastern Brazil, within the same time period of 10 years, the increase of risk from 27.00 to 38.74 new cases per 100,000 women, was more prominent compared to Southern regions of the country (INCA, 2006 and 2016).

Breast cancer is not considered anymore as a single disease, but a heterogeneous group of diseases, with different molecular and cellular characteristics (Rivenbark et al., 2013). Based on global gene expression analysis, four main molecular subtypes were defined (Perou et al., 2000; Sorlie et al., 2001). These molecular subtypes are characterized by the expression status of hormone

receptors (HR), estrogen (ER), progesterone (PR) and amplification status of the human epidermal growth factor receptor-2 gene (HER2; Perou et al., 2000; Sorlie et al., 2001). Triple negative breast cancer (TNBC) is characterized by the negative expression status of both HR and it does not exhibit amplification of the HER2 gene (Palma et al., 2015; Joyce et al., 2016; Rody et al., 2017). Due to the lack of therapeutic opportunities and its aggressive nature, characterized by high graded tumours and high relapse rate, TNBC has the worst prognosis and lowest overall survival rate of all molecular subtypes (Palma et al., 2015; Joyce et al., 2016; Rody et al., 2017).

In Brazil, the frequency of TNBC varied between 14.00% and 20.30% in the south-eastern and northern regions, respectively (Carvalho et al., 2014). Two studies have indicated TNBC frequencies of 17.10% and 17.40% in the North-eastern region (Andrade et al., 2014; Carvalho et al., 2014). Most TNBC cases (67.39%) were identified in the group of postmenopausal women aged 50 years or older (Andrade et al., 2014). It is well established in literature that specific reproductive and lifestyle-related factors can increase the risk of TNBC

(Redondo et al., 2012; Tamimi et al., 2012; Martinez et al., 2013; Ritte et al., 2013; Ambrosone et al., 2014; Anderson et al., 2014; Nishino et al., 2014; Lambertini et al., 2016; Ma et al., 2017). There are no Brazilian studies about the possible association of lifestyle-related and reproductive risk factors with determined molecular subtypes of breast cancer. Increasing incidence of breast cancer in North-eastern Brazil underlines the importance to understand the association between risk factors and TNBC. The aim of the present study was to identify factors that are heterogeneously associated with chance of TNBC relative to the Luminal A molecular subtype in a population in North-eastern Brazil.

Materials and Methods

Study population

The sampling protocol was reviewed and approved by the Brazilian National Research Ethics Committee (CAAE plataforma Brasil: 22358113.1.0000.5187). Written informed consent was obtained from each participant to participate in this study. Data from breast cancer patients were sampled in two reference centres for breast cancer treatment in the state of Paraíba, North-eastern Brazil: The “Fundação Assistencial da Paraíba” public hospital (FAP) in Campina Grande and the “Hospital Napoleão Laureano” (HNL) in João Pessoa. Both hospitals together treat most breast cancer patients of the state. Patients seeking for treatment may come from regions as far as 600 km from the reference centre. João Pessoa, capital of the state of Paraíba, has about 800.000 inhabitants and is located on the coast (IGBE, 2017). Campina Grande, with about 400.000 inhabitants, the second most populated urban centre in Paraíba, is located about 120 km away from the capital in the inland of the state (IGBE, 2017). Like other states of North-eastern Brazil, Paraíba has mixed-ethnicity population composed of Indigenous, African and European ancestry.

Data sampling

No significant differences were observed between patients of both reference centres. Clinical and histopathological data were obtained from medical records. Molecular subtypes were defined as follows: Luminal A: ER positive (ER+) and/or PR positive (PR+), HER2 negative (HER2-); Luminal B: ER positive (ER+) and/or PR positive (PR+) and HER2 positive (HER2+), HER2 subtype: HER2 positive (HER2+), ER negative (ER-) and PR negative (PR-); Triple negative (TN): ER negative (ER-), PR negative (PR-) and HER2 negative (HER2-). Height and weight were also obtained from medical records. Height and weight had been measured when patients entered the hospital, before any therapeutic treatment. Body mass index (BMI) was defined according to the World Health Organization (WHO, 2015): Underweight: $< 18.5 \text{ kg/m}^2$; normal weight: $18.5\text{--}24.99 \text{ kg/m}^2$; overweight: $25.0\text{--}29.99 \text{ kg/m}^2$; obesity $\geq 30.0 \text{ kg/m}^2$.

All data about risk factors with exception of BMI, were obtained by interviewing participants. Interviews were performed within the chemotherapy and radiotherapy

units of both hospitals. A questionnaire was developed and tested successfully in a previous case-control study (Almeida et al., 2015). In the present study, a modified version of this original questionnaire was applied. The questionnaire was subdivided into three sections about socio-economic background and life-style related, respectively, reproductive risk factors. Breast cancer patients were interviewed between March 2016 and January 2017.

Recruitment of participants was randomly. Authors asked women directly and explained content and objective of the study. Participation rate varied between 90% and 95% in both hospitals. Time between diagnosis and recruitment was in 72 (30.51%) and 110 (46.61%) cases ≤ 12 and ≤ 24 month, respectively. In 54 (22.88%) cases, it varied between 24 and 36 month. Minimum wage and multiple values were used to characterize income. This is a popular and well-known method to define economic level among low- and middle-class subjects. Information about ethnic origin was obtained by self-information of participating women when asked during the interview.

Participants were eligible if diagnosed within 36 months from recruitment with invasive breast cancer and aged 18 years or older. Of 257 patients, 21 were excluded from the study, because of incomplete information about HR and HER2 expression status. The study included finally 236 patients with invasive operable breast cancer, diagnosed and treated between 2013 and 2016. Of all 236 patients 106 (55.08%) and 130 (44.92%) were from HNL and FAP hospitals, respectively.

Statistical analysis

ANOVA was applied to compare mean age and mean age of diagnosis. Pearson's Chi-Square (χ^2) test was applied to compare categorized variables. Molecular subtypes served as dependent variables and categorized data of risk factors as independent variables of regression analysis. Results were presented as adjusted odd ratios (OR), 95% confidence interval (CI) and p-value. P values of regression analysis were calculated using likelihood ratio tests (PLRT). Significant variables of univariate regression analysis were used for regression modelling. To quantify associations between risk factors and TNBC, multiple nominal logistic regression was applied. Luminal A subtype served as reference group. Variables with significance level of PLRT < 0.2 in the univariate analysis were entered into the model. The final model was tested for fitness using the likelihood ratio test. All statistical analyses were performed using the SPSS STATISTICS™ software (SPSS; IBM company; version 17).

Results

The mean age of women was $55.14 (\pm 12.33)$ years and 101 (51.80%) out of 236 were married (Table 1). Of all women, 35 (15.49%) and 92 (40.71%) informed African and mixed ethnicity, respectively, whereas 99 (43.80%) informed Caucasian ethnicity (Table 1). Altogether, 146 (64.03%) women had income of one or less than one minimum wage and 114 (58.46%) had completed elementary school or had no school degree (Table 1). The

majority of 189 (84.38%) women had no private health insurance (Table 1).

Overall, 134 (56.78%) tumours were Luminal A, 47 (19.92%) were Luminal B, 38 (16.10%) had TNBC and 17 (7.20%) were HER2 (Table 2). There was no significant difference among subtypes and association with mean age of patients and their mean age at diagnosis (Table 2).

Age at menarche in contrast, was significantly different among subtypes ($p=0.023$; Table 2). Of 205 parous women, 177 (86.34%) had performed breastfeeding. Parity and breastfeeding did not differ significantly among molecular subtypes ($p=0.303$; $p=0.586$; Table 2). There was no significant difference of anatomic stage or histological type of tumours among subtypes (Table 2). Of 38 triple negative tumours, 18 (48.65%) were grade three tumours ($p=0.021$; Table 2).

Results of univariate and multiple nominal regression analysis were summarized in Table 3. In univariate analysis, obesity (OR=2.780; CI 95%: 1.00-7.72) and early age at menarche (OR= 3.890; CI 95%: 1.61-9.38), were positively associated with TNBC compared to Luminal A subtype (Table 3). In contrast, smoking (OR=0.339; CI 95%: 0.14-0.79) and alcohol consumption (OR=0.567; CI 95%: 0.26-1.23), were negatively associated with TNBC (Table 3). A model was formed to evaluate the associations between risk factors and TNBC ($p=0.035$; Table 3). In the adjusted model of multiple analysis, BMI and smoking remained significant ($p=0.004$; $p=0.013$): Compared to normal weight and underweight patients, obese and overweight patients were 4.489 (CI 95%: 1.32- 15.28) and 1.340 (CI 95%: 0.38- 4.69) more likely of having TNBC compared to Luminal A subtype (Table 3). There was no significant association of obesity and overweight with TNBC if data were stratified by menopause status. Of 74 premenopausal and 158 postmenopausal women with known BMI, 25 (33.78%) and 62 (39.24%), respectively, were obese ($p=0.505$). The mean BMI of premenopausal and postmenopausal women with Luminal A subtype breast cancer was 27.63 (± 0.812) kg/m² and 28.28 (± 0.503) kg/m², respectively ($p=0.935$). Mean BMI of premenopausal and postmenopausal women with TNBC was 30.40 (± 2.015) kg/m² and 30.23 (± 1.073) kg/m², respectively ($p=0.935$). Compared to the mean BMI of 28.09 kg/m² for women with Luminal A breast cancer, BMI was increased to 30.29 kg/m² for women with TNBC ($p=0.024$). Women who did not perform any physical exercise were 2.843 (CI 95%: 1.06- 7.64) more likely of having TNBC compared to Luminal A subtype (Table 3).

Of all 236 women, 95 (40.25%) smoked some time in their lives. Smoking was negatively associated with TNBC: Women who smoked some time in their lives were 4.016 (OR= 0.249; CI 95%: 0.09- 0.71) times less likely of having TNBC compared to Luminal A subtype (Table 3). If data were stratified by menopause status, the odds ratio for premenopausal and postmenopausal women who ever smoked was 0.897 (CI 95%: 0.04-18.47; $p=0.268$) and 0.203 (CI 95%: 0.06-0.67; $p=0.012$), respectively. Results did not indicate significant association between subtype and amount of smoked cigarettes.

Regarding reproductive risk factors, the likelihood of TNBC was 4.235 times higher among women who had

menarche <12 years (CI 95%: 1.45- 12.42; Table 3). If data were stratified by menopause status, the odds ratio for premenopausal and postmenopausal women who had menarche <12 years was 0.956 (CI 95%: 0.03-33.46; $p=0.974$) and 6.500 (CI 95%: 1.83-23.07; $p=0.020$), respectively.

Discussion

To the best of our knowledge, this is the first Brazilian study that associated specific risk factors with molecular subtypes of breast cancer. Data of case- case analysis indicated that lifestyle-related risk factors smoking and obesity were heterogeneously associated with chance of TNBC compared to Luminal A subtype.

Increased BMI is a well- established lifestyle-related risk factor of breast cancer (Yang et al., 2011; Rose et al., 2015; Kerlikowske et al., 2016; Li et al., 2016). It was postulated that overweight and obesity might be associated with adipose tissue inflammation, favouring malignant cell transformation in the breast (Rose et al., 2015). This process can act on breast tissue, regardless of expression of hormone receptors in postmenopausal women (Rose

Table 1. Sociodemographic Characteristics of the 236 Patients

	Mean age (years)	N (%)
	55.14 \pm 12.34	
Marital status		
Married		101 (51.80)
Single		53 (27.18)
Divorced		18 (9.23)
Widow		23 (11.79)
Missing		41
Ethnic origin		
Afro descendent		35 (15.49)
Caucasian		99 (43.80)
Mixed ethnicity		92 (40.71)
Missing		10
Income		
No one		3 (1.32)
≤ 1		146 (64.03)
2-3		64 (28.07)
4-6		13 (5.70)
7-10		2 (0.88)
Missing		8
Health insurance		
Yes		35 (15.62)
No		189 (84.38)
Missing		12
Education		
\leq Elementary school		114 (58.46)
High school		50 (25.64)
Colleges		31 (15.90)
Missing		41

Table 2. Clinical and Histopathological Characteristics of 236 Breast Cancer Patients

	Luminal A N= 134	Luminal B N= 47	HER2+ N= 17	TNBC N= 38	P
Mean age (years)	55.47 ±1.03	54.11±1.70	53.41±2.72	56.00±2.40	0.817
Age at diagnosis	54.42±1.07	52.30±1.70	52.71±2.74	55.32±2.54	0.667
Age at menarche	13.27±0.15	13.37±0.29	13.35±0.28	12.29±0.31	0.023
	N (%)	N (%)	N (%)	N (%)	
Parity					
No	23 (16.42)	5 (10.64)	1 (5.89)	3 (7.89)	0.303
Yes	112 (83.58)	42 (89.36)	16 (94.11)	35 (92.11)	
Breastfeeding of parous women (N= 205)					
No	13 (11.61)	8 (19.05)	3 (18.75)	4 (11.43)	0.586
Yes	99 (88.39)	34 (80.95)	13 (81.25)	31 (88.57)	
Anatomic stage (TNM)					
I	16 (14.55)	1 (2.78)	1 (8.33)	2 (6.67)	0.493
II	42 (38.18)	12 (33.33)	4 (33.34)	12 (40.00)	
III	40 (36.36)	19 (52.78)	6 (50.00)	15 (50.00)	
IV	12 (10.91)	4 (11.53)	1 (8.33)	1 (3.33)	
Missing	24	11	5	8	
Tumour grade					
I	8 (6.30)	0 (0.00)	0	0	0.021
II	88 (69.29)	28 (63.64)	7 (50.00)	19 (51.35)	
III	31 (24.41)	16 (36.36)	7 (50.00)	18 (48.65)	
Missing	7	3	3	1	
Histological type					
Ductal invasive	107 (79.85)	43 (91.49)	14 (82.35)	32 (84.21)	0.681
Lobular invasive	7 (5.22)	0 (0.00)	0 (0.00)	1 (2.63)	
Mucinous invasive	4 (2.99)	0 (0.00)	1 (5.88)	1 (2.63)	
Others	16 (11.94)	4 (8.51)	2 (11.77)	4 (10.53)	

et al., 2015). Present results indicated that obese women were about 4.5 times more likely to have TNBC compared to Luminal A subtype. However, several studies attributed an increased chance of TNBC only to premenopausal women but not to postmenopausal women: A Turkish study that included 3767 breast cancer patients indicated that obese premenopausal women were more likely to have TNBC compared to Luminal A subtype (Sahin et al., 2016). In a study of the Breast Cancer Surveillance Consortium, increased BMI had a positive association with ER- negative breast cancer in pre/perimenopausal women only, but not in postmenopausal women (Kerlikowski et al., 2016). Similarly, a meta-analysis based on 11 original articles indicated an overall positive association between TNBC and obesity, which remained significant only for premenopausal women after stratification based on menopause status (Pierobon and Frankenfeld, 2013). The finding that data of 34 studies indicated an increased risk of TNBC for obese premenopausal women aged ≤ 50 compared to ER+/PR+ tumours, corroborates these results (Yang et al., 2011). Other Chinese studies also indicated that premenopausal obese and overweight women tended to have TNBC, whereas post- menopause

status was associated with Luminal subtypes (Chen et al., 2013; Li et al., 2016). Furthermore, in a Norwegian study that included more than 18.000 postmenopausal women, high BMI was associated with luminal but not triple negative subtypes (Horn et al., 2014). Similarly, a study on African American women has shown that obese postmenopausal patients had a decreased risk of having TNBC and an increased risk of having ER+ breast cancer (Bandera et al., 2015). These results are in contrast to a recent Chinese study, in which the risk of Luminal subtypes and TNBC were both equally positively associated with increased BMI in premenopausal women (Li et al., 2016). In another Chinese study with 444 premenopausal and 290 postmenopausal TNBC patients without stratification for menopause status, overweight and obesity were associated with Luminal A subtype and not to TNBC (Song et al., 2013).

According to the United Nations Organization (UNO), in the years between 2010 and 2014 overweight and obesity increased from 51.10% to 54.10%, respectively, from 17.80% to 20.00% among Brazilian adults (UNO, 2017). Furthermore, according to the Brazilian Society of Obesity and Metabolic Syndrome (ABESO), of all

Table 3. Associations Between Risk Factors and TNBC. Luminal A Served as Reference Group

	Luminal A (N= 134)	TNBC (N= 38)	Odds ratios (OR), confidence intervals (95% CI) and significance level of likelihood ratio tests (PLRT)			
	N (%)	N (%)	OR _{CRUDE} (95% CI)	PLRT	OR _{ADJUSTED} (95% CI) ¹	PLRT
Age categories						
20-29	1 (0.75)	2 (5.26)	6.800 (0.59-78.13)	0.448		
30-39	10 (7.46)	5 (13.16)	1.700 (0.53-5.43)			
40-49	38 (28.36)	6 (15.79)	0.537 (0.20-1.41)			
≥ 50	85 (63.43)	25 (65.79)	1			
Ethnic origin						
Other one	72 (55.38)	19 (54.29)	0.957 (0.45-2.02)	0.855		
Caucasian	58 (44.62)	16 (45.71)	1			
Family history						
Yes	92 (70.77)	25 (67.57)	0.861 (0.39-1.88)	0.191	0.904 (0.37- 2.23)	0.825
No	38 (29.23)	12 (32.43)	1			
Missing	4	1				
BMI						
Obesity	41 (32.28)	19 (54.29)	2.780 (1.00-7.72)	0.033	4.489 (1.32- 15.28)	0.004
Overweight	51 (40.16)	10 (28.57)	1.176 (0.39-3.52)		1.340 (0.38- 4.69)	
Normal ⁴	35 (27.56)	6 (17.14)	1			
Missing	7	3				
Physical activity						
No	79 (58.96)	26 (68.42)	1.508 (0.70-3.24)	0.055	2.843 (1.06- 7.64)	0.107
Yes	55 (41.04)	12 (31.58)	1			
Smoking						
Ever	59 (44.03)	8 (21.05)	0.339 (0.14-0.79)	0.012	0.249 (0.09- 0.71)	0.013
Never	75 (55.97)	30 (78.95)	1			
Alcohol consumption						
Yes	56 (41.79)	11 (28.95)	0.567 (0.26-1.23)	0.041	0.709 (0.28- 1.80)	0.22
No	78 (58.21)	27 (71.05)	1			
Age at menarche						
< 12	14 (10.61)	12 (31.58)	3.890 (1.61-9.38)	0.028	4.235 (1.45- 12.42)	0.069
≥ 12	118 (89.39)	26 (68.42)	1			
Missing	2	0				
Lifetime Breastfeeding ³						
≤ 12	50 (50.51)	17 (54.84)	1.275 (0.49-3.31)	0.064	1.210 (0.41- 3.55)	0.128
13-24	19 (19.19)	6 (19.35)	1.184 (0.35-3.94)		1.240 (0.31- 4.89)	
> 24	30 (30.30)	8 (25.81)	1			
Menopausal status						
Pre	39 (29.10)	13 (34.21)	1.267 (0.58-2.72)	0.906		
Post	95 (70.90)	25 (65.79)	1			
Age at menopause						
< 50	58 (61.05)	13 (52.00)	0.691 (0.28-1.67)	0.931		
≥ 50	37(38.95)	12 (48.00)	1			
Age at first life birth						
< 20	33 (28.95)	13 (37.14)	1.838 (0.45-7.47)	0.291		
20-29	67 (58.77)	19 (54.29)	1.323 (0.34-5.09)			
≥ 30	14 (12.28)	3 (8.57)	1			
Reproductive period ²						
≤ 5	29 (32.23)	8 (30.77)	0.713 (0.25-1.99)	0.459		
6-10	30 (33.33)	6 (23.08)	0.517 (0.17-1.55)			
≥ 11	31 (34.44)	12 (46.15)	1			

Table 3. Continued

	Luminal A (N= 134)	TNBC (N= 38)	Odds ratios (OR), confidence intervals (95% CI) and significance level of likelihood ratio tests (PLRT)			
	N (%)	N (%)	OR _{CRUDE} (95% CI)	PLRT	OR _{ADJUSTED} (95% CI) ¹	PLRT
Number of children						
0	22 (16.42)	3 (7.89)	0.409 (0.10-1.54)	0.345		
1-2	61 (45.52)	18 (47.37)	0.885 (0.41-1.89)			
> 2	51 (38.06)	17 (44.74)	1			
Oral contraceptive use						
Yes	88 (66.17)	23 (60.53)	0.784 (0.37-1.64)	0.124	0.664 (0.25- 1.75)	0.804
No	45 (33.83)	15 (39.47)	1			
Missing	1	0				

1, Adjusted for age categories and menopause status; 2, Between first and last full term pregnancy in years; 3, Total lifetime breastfeeding of parous women in months; 4, Including two cases of underweight.

Brazilian women in 2013 aged 18 years or older 24.40% were obese (ABESO, 2013). The latter study emphasized that the frequency of obesity increased with age and is more common among women aged 50 years and older (ABESO, 2013). In the present study, mean BMI of women with TNBC was increased if compared to Luminal A subtype. The positive association of obesity and TNBC was mainly detected among postmenopausal patients aged 50 years and older. In the study population, obesity could therefore exceed its effect on formation of TNBC mainly in postmenopausal women.

Present results indicated that women who did not perform any physical exercise had an increased chance of TNBC. However, physical exercise did not contribute significantly to the model. In a recent study the protective effect of physical exercise was more pronounced for HR+ and HER2+ breast cancer (Lope et al., 2017). This indicates that physical exercise, like BMI and smoking, can also contribute to heterogeneity of risk among molecular subtypes.

A recent meta-analysis, including 44 and 27 retrospective and prospective studies, respectively, indicated a moderate increase of breast cancer risk for women who smoke (Macacu et al., 2015). Present data indicated that women who smoked some time in their lives were 3.8 times more likely of having Luminal A breast cancer compared to TNBC. This result is in agreement with several previous studies: The Carolina Breast Cancer study identified an increased risk of Luminal breast cancer compared to triple negative basal subtype and this effect was more prominent among Afro-American women (Butler et al., 2016). Similarly, a study performed in the metropolitan area of Seattle reported an increased risk of ER+ breast cancer for women who smoked, whereas the risk of TNBC did not increase (Kawai et al., 2014). A recent study of Park and colleagues that included 5791 Afro-American women with breast cancer, reported a positive association of smoking with ER+ breast cancer among postmenopausal women (Park et al., 2016). In contrast, other recent studies did not indicate that cigarette smoking was differentially associated with molecular subtypes: In a Japanese case-control study, no increased risk due to cigarette smoking was found for any subtype (Nishino et al., 2014). Furthermore, in a recent Lithuanian

case-control study, passive smoking did not increase the risk of hormone-receptor positive breast cancer compared to other subtypes (Strumylaite et al., 2017). Furthermore, studies also revealed positive associations between Luminal A subtype and smoking duration, respectively, amounts of smoked cigarettes (Kawai et al., 2014; Butler et al., 2016).

Between 2006 and 2015, the number of active smokers in Brazil decreased about 30.70% and in 2015 about 9.00% of all adult women were smoking (Ministério da Saúde, 2015). However, in the present dataset, more than 40.00% of patients smoked some time in their lives and the majority were low-income women. This could mean that the number of active and former smokers is unusually high among breast cancer patients of this study.

Present data indicated that all reproductive risk factors, with the exception of age at menarche, did not increase the chance of TNBC compared to Luminal A breast cancer. On the one hand, this is not in agreement with some previous studies, which associated risk of TNBC differentially with parity and age at first birth (Millikan et al., 2008; Phipps et al., 2011; Li et al., 2013; Martinez et al., 2013). On the other hand, in agreement with present results, most previous studies also did not identify specific associations of TNBC with age at first birth, parity, menopause status and age at menopause (Islam et al., 2012; Redondo et al., 2012; 2Horn et al., 2014; Lambertini et al., 2016; Li et al., 2016; Sisti et al., 2016; Ma et al., 2017).

Multiple regression analysis showed borderline significance of early age at menarche. This could indicate a possible association of TNBC with early age at menarche. This is in contrast to most previous studies that identified early age at menarche as risk factor for Luminal A breast cancer and did not attribute increased risk to TNBC (Song et al., 2013; 2Horn et al., 2014; Song et al., 2014). Furthermore, several studies carried out in China, Japan, Korea, Germany and the USA, indicated a positive association between hormone receptor positive breast cancer and early age at menarche (Setiawan et al., 2009; Xing et al., 2010; Bao et al., 2011; Tamimi et al., 2012; Chung et al., 2013; Ritte et al., 2013; Warner et al., 2013). Alternatively, there may be a real positive association between early age at menarche and TNBC in the population of the present study: Menarche ≤ 12 years

also increased the risk of basal-like breast cancer in the Carolina Breast Cancer study (Millikan et al., 2008). In a study carried out in Atlanta including Afro-American and Caucasian women, Trivers (2009) reported that ER-PR- breast cancer was more frequent among women who had menarche ≤ 11 years.

In contrast to other reproductive risk factors, which in most cases are neither positively nor negatively associated with TNBC, it is well established in literature that breastfeeding can reduce its risk (Millikan et al., 2008; Phipps et al., 2008; Redondo et al., 2012; Tamimi et al., 2012; 2Horn et al., 2014; Sisti et al., 2016; Ma et al., 2017). Furthermore, breastfeeding reduced the risk of ER- breast cancer of parous women (Li et al., 2013; Ambrosone et al., 2014). Work and colleagues (2014) reported that parity \geq three children without breastfeeding, was associated with increased frequency of ER-PR- tumours (Work et al., 2014). In contrast, a study of 1041 women with Mexican ancestry indicated that breastfeeding > 12 month increased the risk of TNBC twice compared to Luminal A breast cancer (Martinez et al., 2013). A recent meta- analysis of 15 studies indicated that breastfeeding had a protective effect on both, TNBC and luminal subtype breast cancer (Lambertini et al., 2016). A recent study performed in North-eastern Brazil indicated that breastfeeding $24 \geq$ month decreased the risk of breast cancer (Almeida et al., 2015). However, present data did not indicate a specific protective effect of breastfeeding on TNBC compared to Luminal A subtype. The possibility that a larger dataset could reveal a protective effect of breastfeeding on TNBC in the population of the present study cannot be ruled out.

The present study had several limitations: Participants of the study were from a limited geographic area. Therefore, present results are not necessarily representative for other Brazilian populations. The most severe limitation was the low number of data. This may have obscured associations of risk factors with TNBC, caused a low data resolution and may have also caused biases. Present study did also not include all life style related risk factors. Participants were randomly selected. However, a selection bias, caused by not- participating patients and exclusion of participants with incomplete information about HR and HER2 expression status, cannot be excluded. Furthermore, as time span between diagnosis and recruitment was long, recall bias cannot be excluded, leading to incomplete or false information about risk factors. The study did not include age or time-related and detailed quantitative information about smoking. Furthermore, data sampling did not include information about other important anthropometric measures like central obesity and waist-to-hip ratio.

In Conclusion, present results indicated that smoking and obesity were differentially associated with TNBC and the Luminal A subtype: Obesity was positively associated with TNBC, whereas smoking was negatively associated with TNBC, respectively, if compared to Luminal A subtype. It will be important to elucidate in case control studies if smoking and obesity generally increase the risk of breast cancer in the population of North-eastern Brazil. Data amplification will be necessary to elucidate

in more detail the effects of smoking, BMI and a possible protective effect of breastfeeding on molecular subtypes. Data about age at initiation of smoking, time interval of smoking and amounts of smoked cigarettes will be necessary to obtain more detailed information about the association of smoking with Lumina A subtype. It will be also important to include additional anthropomorphic data about central obesity and waist-to-hip ratio. As in Brazil overweight and obesity frequently start at an early age, it will be important to elucidate possible different effects on breast cancer risk of this physical condition at distinct periods of life. It is important to point out that obesity is a growing problem in Brazil not only among adults, but also among children and adolescents. As overweight and obesity are mainly associated with modifiable lifestyle-related behaviour, it is of special interest to understand the association of this risk factor with aggressive TNBC in more detail.

Conflict of interest

There is no conflict of interest.

Acknowledgements

This work was funded by the Brazilian “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)”. We would like to thank participating women and supporting staff of the “Fundação Assistencial da Paraíba” public hospital and “Hospital Napoleão Laureano”.

References

- Almeida GS, Almeida LAL, Araujo GMR, Weller M (2015). Reproductive risk factors differ among breast cancer patients and controls in a public hospital of Paraíba, Northeast Brazil. *Asian Pac J Cancer Prev*, **16**, 2959-65.
- Ambrosone CB, Zirpoli G, Ruszczyk M, et al (2014). Parity and Breastfeeding among African-American Women: Differential effects on breast cancer risk by estrogen receptor Status in the Women’s circle of health study. *Cancer Causes Control*, **25**, 259–65.
- Anderson KN, Schwab RB, Martinez ME (2014). Reproductive risk factors and breast cancer subtypes: A review of the literature. *Breast Cancer Res Treat*, **144**, 1-10.
- Andrade ACM, Ferreira Júnior CA, Guimarães BD, et al (2014). Molecular breast cancer subtypes and therapies in a public hospital of Northeastern Brazil. *BMC Women’s Health*, **14**, 110.
- Bandera EV, Chandran U, Hong CC, et al (2015). Obesity, body fat distribution, and risk of breast cancer subtypes in African American women participating in the AMBER Consortium. *Breast Cancer Res Treat*, **150**, 655-66.
- Bao PP, Shu XO, Gao YT, et al (2011). Association of hormone-related characteristics and breast cancer risk by estrogen receptor/progesterone receptor status in the shanghai breast cancer study. *Am J Epidemiol*, **174**, 661–71.
- Brazilian society of obesity and metabolic syndrome (ABESO), accessed on 15/03/2017: <http://www.abeso.org.br/noticia/quase-60-dos-brasileiros-estao-acima-do-peso-revela-pesquisa-do-ibge>.
- Butler EN, Tse CK, Bell ME, et al (2016). Active smoking and risk of Luminal and Basal-like breast cancer subtypes in the Carolina breast cancer study. *Cancer Causes Control*, *Asian Pacific Journal of Cancer Prevention*, Vol 18 **1591**

- 27, 775-86.
- Carvalho FM, Bacchi LM, Pincerato KM, et al (2014). Geographic differences in the distribution of molecular subtypes of breast cancer in Brazil. *BMC Women's Health*, **14**, 102.
- Chen FY, Ou HY, Wang SM, et al (2013). Associations between body mass index and molecular subtypes as well as other clinical characteristics of breast cancer in Chinese women. *Ther Clin Risk Manag*, **9**, 131-7.
- Chung S, Park SK, Sung H, et al (2013). Association between chronological change of reproductive factors and breast cancer risk defined by hormone receptor status: results from the Seoul breast cancer study. *Breast Cancer Res Treat*, **140**, 557-65.
- Health Ministry, accessed on 10/03/2017: <http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/17921-numero-de-fumantes-no-brasil-cai-30-7-nos-ultimos-nove-anos>.
- 1Horn J, Alsaker MDK, Opdahl S, et al (2014). Anthropometric factors and risk of molecular breast cancer subtypes among postmenopausal Norwegian women. *Int J Cancer*, **135**, 2678-86.
- 2Horn J, Opdahl S, Engstrøm MJ, et al (2014). Reproductive history and the risk of molecular breast cancer subtypes in a prospective study of Norwegian women. *Cancer Causes Control*, **25**, 881-9.
- Brazilian institute of geography and statistics (IGBE, 2010), accessed on 15/03/2017: <http://cidades.ibge.gov.br/painel/painel.php?codmun=250400>.
- Islam T, Matsuo K, Ito H, et al (2012). Reproductive and hormonal risk factors for luminal, HER2-overexpressing, and triple-negative breast cancer in Japanese women. *Ann Oncol*, **23**, 2435-41.
- Joyce DP, Murphy D, Lowery AJ, et al (2016). Prospective comparison of outcome after treatment for triple-negative and non-triple-negative breast cancer. *Surgeon*, S1479-666X(16)30094-4. doi: <http://dx.doi.org/10.1016/j.surge.2016.10.001>.
- Kawai M, Malone KE, Tang MT, Li CI (2014). Active smoking and the risk of estrogen receptor-positive and triple-negative breast cancer among women ages 20 to 44 years. *Cancer*, **120**, 1026-34.
- Kerlikowske K, Gard CC, Tice JA, et al (2016). Risk factors that increase risk of estrogen receptor-positive and -negative breast cancer. *J Natl Cancer Inst*, **109**.
- Lambertini M, Santoro L, Del Mastro L, et al (2016). Reproductive behaviors and risk of developing breast cancer according to tumor subtype: A systematic review and meta-analysis of epidemiological studies. *Cancer Treat Rev*, **49**, 65-76.
- Li CI, Beaber EF, Tang MTC, et al (2013). Reproductive factors and risk of estrogen receptor positive, triple negative, and HER2-neu overexpressing breast cancer among women 20-44 years of age. *Breast Cancer Res Treat*, **137**, 579-87.
- Li H, Sun X, Miller E, et al (2016). BMI, reproductive factors, and breast cancer molecular subtypes: A case-control study and meta-analysis. *Int J Epidemiol*, **30**, 1-9.
- Lope V, Martín M, Castelló A, et al (2017). Physical activity and breast cancer risk by pathological subtype. *Gynecol Oncol*, **144**, 577-85.
- Ma H, Ursin G, Xu X, et al (2017). Reproductive factors and the risk of triple-negative breast cancer in white women and African-American women: a pooled analysis. *Breast Cancer Res*, **19**, 6.
- Macacu A, Autier P, Boniol M, Boyle P (2015). Active and passive smoking and risk of breast cancer: a meta-analysis. *Breast Cancer Res Treat*, **154**, 213-24.
- Martinez ME, Wertheim BC, Natarajan L, et al (2013). Reproductive factors, heterogeneity, and breast tumor subtypes in women of Mexican descent. *Cancer Epidemiol Biomarkers Prev*, **22**, 1853-61.
- Millikan RC, Newman B, Tse CK, et al (2008). Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat*, **109**, 123-39.
- National institute of cancer (INCA, 2006). Accessed on 15/03/2017: http://bvsms.saude.gov.br/bvs/publicacoes/versaofinal_estimativa2006.pdf.
- National institute of cancer (INCA, 2016). Accessed on 15/03/2017: <http://www.inca.gov.br/estimativa/2016/>
- Nishino Y, Minami Y, Kawai M, et al (2014). Cigarette smoking and breast cancer risk in relation to joint estrogen and progesterone receptor status: a case-control study in Japan. *Springer Plus*, **3**, 65.
- Palma G, Frasci G, Chirico A, et al (2015). Triple negative breast cancer: looking for the missing link between biology and treatments. *Oncotarget*, **29**, 26560-74.
- Park SY, Palmer JR, Rosenberg L, et al (2016). A case-control analysis of smoking and breast cancer in African American women: findings from the AMBER Consortium. *Carcinogenesis*, **37**, 607-15.
- Perou CM, Sorlie T, Eisen MB, et al (2000). Molecular portraits of human breast tumours. *Nature*, **406**, 747-52.
- Phipps AI, Malone KE, Porter PL, Daling JR, Li CI (2008). Reproductive and hormonal risk factors for postmenopausal luminal, HER-2-overexpressing, and triple-negative breast cancer. *Cancer*, **113**, 1521-6.
- Phipps AI, Chlebowski RT, Prentice R, et al (2011). Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. *J Natl Cancer Inst*, **103**, 470-7.
- Pierobon M, Frankenfeld CL (2013). Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat*, **137**, 307-14.
- Redondo C, Gago-Domínguez M, Ponte SM, et al (2012). Breast feeding, parity and breast cancer subtypes in a Spanish cohort. *PLoS One*, **7**, e40543.
- Ritte R, Tikk K, Lukanova A, et al (2013). Reproductive factors and risk of hormone receptor positive and negative breast cancer: a cohort study. *BMC Cancer*, **13**, 584.
- Rivenbark AG, O'Connor SM, Coleman WB (2013). Molecular and cellular heterogeneity in breast cancer: challenges for personalized medicine. *Am J Pathol*, **183**, 1113-24.
- Rody A, Cornelia L (2017). Neoadjuvant therapy for patients with triple negative breast cancer (TNBC). *Rev Recent Clin Trials*, doi: 10.2174/1574887112666170307095945.
- Rose DP, Gracheck PJ, Vona-Davis L (2015). The interactions of obesity, inflammation and insulin resistance in breast cancer. *Cancers*, **7**, 2147-68.
- Sahin S, Erdem GU, Karatas F, et al (2016). The association between body mass index and immunohistochemical subtypes in breast cancer. *Breast*, **16**, 30182-5.
- Setiawan VW, Monroe KR, Wilkens LR, et al (2009). Breast cancer risk factors defined by estrogen and progesterone receptor status: the Multiethnic Cohort study. *Am J Epidemiol*, **169**, 1251-9.
- Sisti JS, Collins LC, Beck AH, et al (2016). Reproductive risk factors in relation to molecular subtypes of breast cancer: Results from the nurses' health studies. *Int J Cancer*, **138**, 2346-56.
- Song Q, Huang R, Li J, et al (2013). The diverse distribution of risk factors between breast cancer subtypes of ER, PR and HER2: A 10-year retrospective multi-center study in China. *PLoS One*, **8**, e72175.
- Song N, Choi JY, Sung H, et al (2014). Heterogeneity of epidemiological factors by breast tumor subtypes in Korean

- women: a case-case study. *Int J Cancer*, **135**, 669-81.
- Sorlie T, Perou CM, Tibshirani R, et al (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*, **98**, 10869-74.
- Strumylaite L, Kregzdyte R, Poskiene L, et al (2017). Association between lifetime exposure to passive smoking and risk of breast cancer subtypes defined by hormone receptor status among non- smoking Caucasian women. *PLoS One*, **12**, e0171198.
- Tamimi RM, Colditz GA, Hazra A, et al (2012). Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. *Breast Cancer Res Treat*, **131**, 159-67.
- Torre LA, Bray F, Siegel RL, et al (2015). Global cancer statistics, 2012. *CA Cancer J Clin*, **65**, 87-108.
- Trivers KF, Lund MJ, Porter PL, et al (2009). The epidemiology of triple-negative breast cancer, including race. *Cancer Causes Control*, **20**, 1071-82.
- United nations organization (UNO), accessed on 05/03/2017: <https://nacoesunidas.org/aumentam-sobrepeso-e-obesidade-no-brasil-aponta-relatorio-de-fao-e-opas/>.
- Warner ET, Colditz GA, Palmer JR, et al (2013). Reproductive factors and risk of premenopausal breast cancer by age at diagnosis: are there differences before and after age 40? *Breast Cancer Res Treat*, **142**, 165-75.
- Work ME, John EM, Andrulis IL, et al (2014). Reproductive risk factors and oestrogen/progesterone receptor-negative breast cancer in the breast cancer family registry. *Br J Cancer*, **110**, 1367-77.
- World health organization, accessed on 10/03/2017: <http://www.who.int/mediacentre/factsheets/fs311/en/>.
- Xing P, Li J, Jin F (2010). A case- control study of reproductive factors associated with subtypes of breast cancer in Northeast China. *Med Oncol*, **27**, 926-31.
- Yang XR, Chang-Claude J, Goode EL, et al (2011). Associations of breast cancer risk factors with tumor subtypes: A pooled analysis from the breast cancer association consortium studies. *J Natl Cancer Inst*, **103**, 250-63.