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CYP17, *CYP19*, and *NQO1* Genetic Polymorphisms and Breast Cancer Susceptibility in Young Women in Brazil

Sabrina S Santos[1], Guillermo Patricio Ortega Jácome[1], Rosalina Koifman[1] and Sergio Koifman[1*]

[1]National School of Public Health (ENSP), Oswaldo Cruz Foundation, Av. Leopoldo Bulhões, 1480, CEP 21041-210 - Rio de Janeiro, RJ. Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author SSS wrote the protocol, collected data, performed laboratory and statistical analysis and wrote the first draft of the manuscript. Author GPOJ participated in data and biological samples collection. Authors RK and SK designed the study and guided all the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Breast cancer is the most common cancer in women worldwide, being exposure to estrogens the acknowledged main risk factor. CYP17, CYP19 and NQO1 are enzymes involved in the estrogen metabolism, so their polymorphisms may be involved in breast carcinogenesis. The aim of this study was to determine the magnitude of the association between *CYP17 MspA1*, *CYP19* Arg^{264}Cys, and *NQO1* C^{609}T polymorphisms and breast cancer in young women.
Methods: This is a hospital-based case-control study carried out in Rio de Janeiro. Cases were 270 women with age range 18-35 years and a histopathological diagnosis of breast cancer between 1999-2009. Controls were 270 women without cancer at the same age range.
Results: An association between *CYP17 MspA1* or *CYP19* Arg^{264}Cys polymorphisms and breast cancer were not observed (OR = 1.02, 95% CI 0.72-1.44 for *CYP17* genotypes TC/CC and OR = 0.85, 95% CI 0.48-1.49 for *CYP19* genotypes CT/TT). However, a statistically significant increased risk estimate was identified in women who had at least one *NQO1* polymorphic allele (T), OR= 1.96, 95% CI 1.13-3.40 following

**Corresponding author: Email: koifman@ensp.fiocruz.br;*

adjustment for selected confounders.
Conclusion: This study suggests that the *NQO1* ^{609}T polymorphism may be a risk factor for breast carcinogenesis in women less than 36 years in Brazil.

Keywords: Breast cancer; NQO1; CYP17; CYP19; genetic polymorphism; young women; case-control study.

1. INTRODUCTION

Breast cancer (BC) is the most incident cancer among women worldwide [1]. Although the highest BC incidence occurs in women 50 years or older; an increase in its incidence and mortality in young women has been increasingly reported [2-4]. This rise might be explained either by an improvement of cases notification, or as a result of a change in the pattern of exposure to different environmental risks factors, which possibly turned this group of young women more susceptible to develop BC at an early age [5]. Hence, changes in women lifestyle and reproductive history occurring in the last decades could have modified the prevalence of known BC risk factors [6]. These factors include early menarche, delayed age at first pregnancy, nulliparity or reduced parity, reduced breastfeeding length, sedentary lifestyle, obesity, and increased alcohol consumption [7-8]. All these risk factors generate excessive estrogen exposure during a woman's life [9]. Another pathway of exposure to estrogens is the adoption of hormonal contraception, despite the fact that the association between hormonal contraceptives use and BC remains unclear [10-12].

Cytochrome P450c17α (CYP17) and aromatase (CYP19) are important enzymes in the biosynthesis of estrogen [13]. While CYP17 controls two successive early steps of endogenous estrogen biosynthesis by converting pregnenolone and progesterone to precursors of androgen and estrogen [14], aromatase catalyzes the conversion of androgens to estrogens [15].

CYP17 gene contains a single base pair polymorphism in the promoter region, which increases transcriptional activity (*MspA1* polymorphism) [16], and some studies have found an association between such polymorphism and BC [17-19]. However, other studies have not found such association, especially when pre and postmenopausal women were analyzed together [20-26].

CYP19 gene contains Arg^{264}Cys polymorphism [27], but its association with BC remains controversial [25,28-30].

Estrogens stimulate breast cell proliferation, which may leave cells more susceptible to genetic mistakes during DNA replication [31]. However, there is other mechanism by which estrogen may affect the risk of developing BC. Estrogens are first oxidized in the breast to catechols, and then to quinones that react with DNA to form adducts, which lead to mutations associated with BC [9,32-33]. NAD(P)H:quinone oxidoreductase 1 (NQO1) is an enzyme involved in the metabolism of endogenous and exogenous quinines and in other protective mechanisms against carcinogenesis [34]. This enzyme reduces quinones generated during estrogen metabolism back to catechols, and thus may be protective against BC [35].

NQO1 is a polymorphic enzyme, and studies showed that C^{609}T (Pro^{187}Ser) variant was a poor reducer of estrogen quinones, which cause increased formation of estrogen-DNA

adduct [36,37], thus increasing BC risk. Some molecular epidemiological studies were conducted to evaluate the association between *NQO1* $C^{609}T$ polymorphism and BC risk in diverse populations, but their results remain conflictive [38-41].

Considering the suggestive evidence on the increasing BC incidence rates among young women in different populations, and the relatively scarce data on single genetic polymorphisms involved in breast carcinogenesis at an early age, the goal of this study was to determine the magnitude of the association between *CYP17 MspA1* (*rs743572), CYP19* $Arg^{264}Cys$ (*rs700519*) and *NQO1* $C^{609}T$ *(rs1800566)* polymorphisms and BC under 36 years of age in women in Brazil.

2. METHODS

2.1 Study Population and Design

This is a hospital-based case-control study conducted in Rio de Janeiro, Brazil. The study population is composed of women at age range 18-35 years, living in the Metropolitan Region of Rio de Janeiro (Brazil). Cases were 270 women with a histopathological confirmed diagnosis of BC (ICD 10 50.0-50.9) at age range 18-35 years and referred to the Brazilian National Cancer Institute (INCA), the Oncology Reference Centre settled in the city of Rio de Janeiro, between 1999-2009. Controls were 270 women, at the same age range, enrolled among hospitalized patients with no-neoplastic diseases and also their visitants, in three public hospitals, Pro-Matre Hospital (Gynecological and Obstetric Center), Institute of Trauma Orthopedics (INTO), and the Lagoa Federal Hospital; all of them offering free care in Rio de Janeiro.

Between the eligible BC cases contacted by telephone and asked to come to the hospital to participate in the study, 49% were included at the final sample of 270 cases, 14.3% died subsequent to diagnosis at an advanced stage, 22.8% could not be contacted because of address or telephone number change, 6.7% did not show up in for the scheduled interview, 4.5% were not available as a consequence of ongoing treatment and 2.7% were not willing to participate or to donate a blood sample. Between controls approximately 66% of the invited women agreed to participate in the study.

After signing an informed consent, participants were in-person interviewed by trained interviewers, using a standardized questionnaire designed for the study, which included socio-demographic, lifestyle and medical antecedents [5]. Peripheral blood samples were collected in EDTA Vacutainer tubes and used for genomic DNA extraction following a standard protocol [42].

2.2 Genetic Analysis

Genetic polymorphisms were assessed as previously described PCR-RFLP protocols [43-45] with minor modifications. In brief, the amplification of target DNA was achieved by PCR optimized conditions as follows: a final reaction volume of 25 µL was composed of 100-200 ng of DNA, 0.2 mM of each dTNP (Invitrogen), 3 mM of $MgCl_2$, 0.75 U of Platinum Taq DNA polymerase (Invitrogen), 1× PCR buffer (Invitrogen), and 10 pmol of each primer of one pair, forward and reverse (*CYP17*: forward 5'cattcgcacctctgg3' and reverse 5'ggctcttggggtact3'. *CYP19*: forward 5' cgctagatgtctaaa3' and reverse 5' catatgtggcaatggg 3'. *NQO1*: forward 5'cctctctgtgctttctgtatcc3' and reverse 5'gatggacttgcccaagtgatg3'). The reaction conditions

used were a pre-denaturation at 94ºC for 5 min followed by 35 or 40 (*NQO1*) cycles with three steps each: 94ºC for 40 s, 62ºC or 60ºC (*NQO1*) for 40 s, and 72ºC for 30 s or 45 s (*CYP19* and *NQO1*); and a cycle of 7 min at 72ºC. Negative controls were included in every run, and the success of amplification was confirmed in agarose 1.5% gels, stained with Gel Red (Biotium), and visualized under ultraviolet light. Endonuclease digestions of *CYP17* were performed as follows: a final reaction volume of 20 µL composed of 3µL of *CYP17* PCR products, 5U of *MspA1I* restriction enzyme (BioLabs), 1× reaction buffer (BioLabs) and Bovine serum albumin (BSA - 100 µg/mL), using overnight 37ºC incubation conditions. Endonuclease digestions conditions of *CYP19* were: a final reaction volume of 20 µL composed of 5µL of *CYP19* PCR products, 5U of *LweI* restriction enzyme (Fermentas) and 1× reaction buffer (Fermentas), incubated overnight at 37ºC. Endonuclease digestions of *NQO1* were performed as follows: a final reaction volume of 20 µL composed of 3µL of *NQO1* PCR products, 5U of *HinfI* restriction enzyme (Fermentas), and 1× reaction buffer R (Fermentas), using overnight 37ºC incubation conditions. Determination of genotypes was performed in agarose 3% gels, visualized under ultraviolet light. Goodness-of-fit of genotype distribution to Hardy-Weinberg equilibrium was ascertained for controls, using R 2.15.2 software. As genotype distribution of *CYP19* polymorphism was not in Hardy-Weinberg equilibrium; the efficiency of our genotyping technique was confirmed in a sample of 94 patients by sequencing of the PCR products by the FIOCRZ Network Technology Platforms that includes a 48-capillary 3730 DNA Sequence Analyzer (Applied Biosystems).

2.3 Statistical Analysis

Continuous variables were expressed as means ± standard deviation (SD) and differences between them were analyzed using the Student t test. Categorical variables were expressed as percentages and Pearson chi-square was used to analyze differences between them.

Unconditional logistic regression models were used to calculate unadjusted and adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) to estimate the magnitude of association between BC and *CYP17*, *CYP19* and *NQO1* polymorphisms using STATA 10.0 software. A *P*-value <0.05 was used to ascertain the occurrence of statistical significance. All the confounders (age, skin color, education, pregnancy, age at menarche, hormonal contraceptives use and family history of breast and/or ovary cancer of first degree relatives) was tested in the logistic regression, and those that do not modify the association of BC and the genetic polymorphisms were eliminated of the final model.

With a sample size of 270 cases and 270 controls, considering a 95% significance level and a population prevalence of exposure to *CYP19* $Arg^{264}Cys$ polymorphism of 10%, this study has a power of 80% to detect an OR = 2.4 between such polymorphism and BC. According to *CYP17* polymorphism, a study with the sample size, a 95% confidence interval and a prevalence of 63% of *CYP17 MspA1*, the study has a power of 95% to detect an OR = 2.0 between such polymorphism and BC. This power remains the same for the association between *NQO1* $C^{609}T$ polymorphism (prevalence of 42%) and BC.

2.4 Ethical Aspects

All proceedings were approved by the Ethics Research Committees of all involved institutions (INCA, Pro-Matre Hospital, INTO, Lagoa Federal Hospital and ENSP). All participants signed a declaration manifesting their agreement to participate in the investigation.

3. RESULTS

Data of estrogen and progesterone receptor status of the tumor were collected from a sample of 132 of the 270 study cases. For these, 37.1% express estrogen and progesterone receptors, 25.0% express only estrogen receptor, 6.1% express only progesterone receptor and 31.8% do not express these hormonal receptors.

The distribution of BC cases and controls according to selected variables (age, skin color, education, pregnancy (yes or no), age at menarche, hormonal contraceptives use and family history of breast and/or ovary cancer of first degree relatives) are presented at Table 1. Age of BC cases was significantly higher than age of controls (*P* value <0.001). Regarding skin color, whites accounted for 30.0% of cases and 32.2% of controls, *P* = 0.58. Controls used to have a higher education than cases, and 61.8% of the former had studied more than 8 years, comparatively to 28.9% of the latter, *P* < 0.001. Pregnancy was significantly more frequent in controls than in BC cases (*P* = 0.02), and the mean age at menarche was 12.7 ±2.1 years among cases and 12.7 ±1.7 years among controls (*P* = 0.78). The mean time of hormonal contraceptives use was 5.5 ±4.5 years among cases and 5.3 ±5.1 years among controls (*P* = 0.61). Family histories of breast and/or ovary cancer, in first degree relatives (mother and sisters), were reported by 22.2% of cases and 12.2% of controls, *P* = 0.001.

The associations between *CYP17 MspA1*, *CYP19* $Arg^{264}Cys$, and *NQO1* $C^{609}T$ polymorphisms and BC are presented at Table 2. Data analysis did not show an association between the presence of at least one *CYP17* polymorphic allele (genotypes TC and CC) and BC in young women (OR = 1.02, 95% CI = 0.72-1.44). The adjusted OR for selected confounders revealed: OR = 1.09, 95% CI = 0.74-1.61 when adjusted for age, education, pregnancy (yes or no) and *CYP19* $Arg^{264}Cys$ polymorphism (data not showed); and OR = 1.02, 95% CI = 0.70-1.48 when adjusted for *CYP19* $Arg^{264}Cys$ and *NQO1* $C^{609}T$ polymorphisms, education (categorical), time of hormonal contraceptives use in months, and interaction between *NQO1* and time length of hormonal contraceptives use.

CYP19 $Arg^{264}Cys$ polymorphism (genotypes CT and TT) also was not associated to BC in young women according to the crude (OR = 0.85, 95% CI = 0.48-1.49) or adjusted ORs (OR = 0.89, 95% CI = 0.48-1.64, after adjustment for age, education, pregnancy, and *CYP17 MspA1* polymorphism, data not shown; OR = 0.85, 95% CI = 0.47-1.56, after adjustment by *CYP17 MspA1* and *NQO1* $C^{609}T$ polymorphisms, education, time length of hormonal contraceptives use in months and interaction between *NQO1* and time length of hormonal contraceptives use). Although, genotype distribution of *CYP19* polymorphism was not in Hardy-Weinberg equilibrium; the sequencing of a sample of PCR products confirmed that there were no methodology errors.

In relation to *NQO1*, the crude OR did not show an increase in risk of BC among women less than 36 years who had *NQO1* ^{609}T allele (OR = 1.15, 95% CI = 0.82-1.61 for CT or TT genotypes). However, the adjusted OR for selected confounders (time of hormonal contraceptives use, interaction between *NQO1* and hormonal contraceptives use time length, and education) revealed a statistically significant increase in risk of BC among women who had at least one *NQO1* polymorphic allele (T), (OR = 1.94, 95% CI = 1.12-3.36 - data not showed). Result of the final model is shown in Table 2 with the same variables and *CYP17 MspA1* and *CYP19* $Arg^{264}Cys$ polymorphisms (OR = 1.96, 95% CI = 1.13-3.40). In this model, the association between BC and the factor of interaction between *NQO1* $C^{609}T$ polymorphism and time of hormonal contraceptives use showed an OR = 0.99 (*P* = 0.02;

adjusted for *NQO1* C^{609}T, *CYP17 MspA1* and *CYP19* Arg^{264}Cys polymorphisms, education and time of hormonal contraceptives use).

Table 1. Distribution of breast cancer cases (N = 270) and controls (N = 270) according to selected variables, Rio de Janeiro, Brazil, 1999-2012.

Variables	Controls N (%)	Cases N (%)	Odds Ratio (95% Confidence interval)	*P* value
Age (yr.):				
mean ± SD	29.9±4.5	31.5±3.4	--	<0.001*
range:				
18–23	32 (11.8)	7 (2.6)	1.00	
24–29	65 (24.1)	59 (21.8)	4.15 (1.70-10.11)	
30–35	173 (64.1)	204 (75.6)	5.39 (2.32-12.52)	<0.001**
Skin color:				
White	87 (32.2)	81 (30.0)	1.00	
Non-White	183 (67.8)	189 (70.0)	1.11 (0.77-1.60)	0.577**
Education (yr.):				
>8	167 (61.8)	78 (28.9)	1.00	
8	58 (21.5)	102 (37.8)	3.77 (2.48-5.73)	
<8	45 (16.7)	90 (33.3)	4.28 (2.74-6.70)	<0.001**
Pregnancy:				
No	41 (15.2)	62 (23.0)	1.00	
Yes	229 (84.8)	208 (77.0)	0.60 (0.39-0.93)	0.021**
Age at menarche (yr.):				
mean ± SD	12.7±1.7	12.7±2.1	--	0.784*
range:				
>12	62 (23.0)	66 (24.5)	1.00	
12-14	172 (63.7)	167 (61.8)	0.91 (0.61-1.37)	
<14	36 (13.3)	37 (13.7)	0.97 (0.54-1.72)	0.899**
Contraceptives use (yr.):				
mean ± SD	5.3±5.1	5.5±4.5	--	0.609*
range:				
0-1	86 (31.8)	67 (24.8)	1.00	
>1-5	68 (25.2)	81 (30.0)	1.53 (0.97-2.41)	
>5	116 (43.0)	122 (45.2)	1.35 (0.90-2.03)	0.162**
Family history of breast/ovary cancer of first degree relatives:				
No	237 (87.8)	210 (77.8)	1.00	
Yes	33 (12.2)	60 (22.2)	2.05 (1.29-3.26)	0.001**

** Student t test; ** χ^2 test*

Table 2. Distribution of breast cancer cases (N = 270) and controls (N = 270) according to *CYP17*, *CYP19* and *NQO1* genotypes, Rio de Janeiro, Brazil, 1999-2012.

Variables	Controls N (%)	Cases N (%)	Crude odds ratio (95% confidence interval)	Adjusted odds ratio (95% confidence interval)	*P** value
CYP17					
TT	98 (36.30)	97 (35.93)	1.00	1.00	
TC	128 (47.41)	144 (53.33)	1.14 (0.79-1.64)	1.18 (0.79-1.75) [a]	
CC	44 (16.30)	29 (10.74)	0.67 (0.39-1.15)	0.62 (0.35-1.11) [a]	0.133
TC/CC	172 (63.70)	173 (64.07)	1.02 (0.72-1.44)	1.02 (0.70-1.48) [a]	0.929
CYP19					
CC	241 (89.26)	245 (90.74)	1.00	1.00	
CT	22 (8.15)	23 (8.52)	1.03 (0.56-1.90)	1.02 (0.54-1.95) [b]	
TT	7 (2.59)	2 (0.74)	0.28 (0.06-1.37)	0.30 (0.06-1.57) [b]	0.243
CT/TT	29 (10.74)	25 (9.26)	0.85 (0.48-1.49)	0.85 (0.47-1.56) [b]	0.566
NQO1					
CC	156 (57.78)	147 (54.44)	1.00	1.00	
CT	95 (35.19)	111 (41.11)	1.24 (0.87-1.77)	2.16 (1.21-3.85) [c]	
TT	19 (7.04)	12 (4.44)	0.67 (0.32-1.43)	1.27 (0.38-4.30) [c]	0.213
CT/TT	114 (42.22)	123 (45.56)	1.15 (0.82-1.61)	1.96 (1.13-3.40) [c]	0.435

* χ^2 test

Hardy-Weinberg: CYP17 P = 0.91; CYP19 P < 0.001; NQO1 P = 0.47

[a] Adjusted for CYP19 Arg^{264}Cys and NQO1 C^{609}T polymorphisms, education, time of hormonal contraceptives use in months, and interaction factor of NQO1 and time of hormonal contraceptives use.

[b] Adjusted for CYP17 MspA1 and NQO1 C^{609}T polymorphisms, education, time of hormonal contraceptives use in months, and interaction factor of NQO1 and time of hormonal contraceptives use.

[c] Adjusted for CYP17 MspA1 and CYP19 Arg^{264}Cys polymorphisms, education, time of hormonal contraceptives use in months, and interaction factor of NQO1 and time of hormonal contraceptives use.

4. DISCUSSION

Estrogen exposure represents the major known risk factor for development of BC in women [9]. Estrogens metabolism and biosynthesis involve a series of enzymatic steps regulated by genes for which some involved genetic polymorphisms have been described, that may be associated with BC risk. Among them are included *CYP17* and *CYP19*, involved in estrogen synthesis [46], and *NQO1* involved in the metabolism of exogenous quinones or quinines generated during estrogen metabolism and in other cancer protection mechanisms [34].

CYP17 MspA1 polymorphism is a transition from T to C ($T^{-34}C$), which creates an additional Sp-1 binding site (CCACC boxes) in the promoter region [47]. This results in an increased expression of CYP17 enzyme and consequently an increase in estrogen plasma concentrations [48]. So *CYP17 MspA1* polymorphism can hypothetically be associated with an increased BC risk. However, in this study *CYP17 MspA1* polymorphism is not associated with BC risk in young women. Similarly; other studies did not find an association between *CYP17 MspA1* polymorphism and BC, but all of them have combined pre and postmenopausal women in the analysis [20-26].

In a case-control study with women under 37 years old, Bergman-Jungeström and coworkers [19] found an association between *CYP17 MspA1* polymorphism and the BC risk (OR = 2.0, 95% CI = 1.1-3.5, for TC/CC genotypes). Others studies in premenopausal women also found a statistically significant association between *CYP17 MspA1* polymorphism and BC, one in nulliparous women, homozygous for this polymorphism (OR = 2.12, 95% CI = 1.04-4.32) [18], and other only in heterozygous women (OR = 1.62, 95% CI = 1.02-2.58) [17]. As a whole, these studies seem to suggest that *CYP17 MspA1* polymorphism may have an influence in breast carcinogenesis in young women. Nevertheless, we could not find this association in our study, which is in agreement with Samson et al who did not find a statistically significant association between this polymorphism and the risk of developing BC in premenopausal women [49].

CYP19 gene has four non-synonymous single nucleotide polymorphisms, however, in most populations, $Arg^{264}Cys$ (C to T substitution in exon 7) is the most prevalent [27]. The presence of at least one allele of this polymorphism was associated with an increased BC risk in Korean women, OR = 1.5, 95% CI = 1.1-2.2 [30]. However, in this study *CYP19* $Arg^{264}Cys$ polymorphism could not be associated with BC risk in young women. This result is in agreement with other studies that also did not find such association [17,25,28-29].

NQO1 $C^{609}T$ polymorphism results in the substitution of a proline to a serine at codon 187 of NQO1 protein ($Pro^{187}Ser$) [37]. According to in vitro tests, this variant results in an extremely low or undetectable enzyme activity in homozygous (TT) cells, and a twofold lower activity in heterozygote (CT) cells, compared to the wild type (CC) [50]. These differences can be partly due to a lower expression of polymorphic protein [50]. In our study *NQO1* $C^{609}T$ polymorphism was statistically associated with BC risk in young women, when OR was adjusted for time length of hormonal contraceptives use in months, the interaction between *NQO1* and hormonal contraceptives use time, and education. Others studies, performed in Caucasian, have found an association between the homozygous for the ^{609}T allele and risk of BC. One of these studies found an OR = 3.68 (95% CI = 1.41-9.62) [39], and other investigation found an OR = 3.80 (95% CI 1.73-8.34) [39]. However, two studies conducted in China [41,51] and one study in north Indian [52] did not find a statistically significant association between *NQO1* $C^{609}T$ polymorphism and the risk of BC. A nested case-control study carried out with post-menopausal North-American women also did not find an association [38]. Yuan and coworkers [53] conducted a meta-analysis on 3177 BC cases and 4038 controls from seven published case-control studies, and showed that the ^{609}T allele was not associated with a significantly increased BC risk for all combined groups. However, in the stratified analysis, *NQO1* $C^{609}T$ polymorphism was associated with increased BC risk in Caucasians women (OR = 1.15, 95% CI = 1.01-1.30, for genotypes CT/TT).

In our investigation, the crude OR did not show an increase in BC risk among women who had *NQO1* ^{609}T allele. This risk could only be verified by the OR adjusted for time of hormonal contraceptives use, in months, interaction factor of *NQO1* and hormonal contraceptives use time and education.

The association between hormonal contraceptives use and BC is controversial [10-12]. In the current study, women reporting hormonal contraceptives use have shown a higher risk estimate of developing BC than nonusers or those that did it less than one year. Nevertheless, these estimates were of reduced magnitude, being of borderline statistical significance for hormonal contraceptives use > 1-5 year, or no statistically significant for their use > 5 years. As occurring with endogenous estrogens, the exogenous estrogens

metabolism also generates quinines that may react with DNA and form adducts [9]. NQO1 metabolizes these quinines, protecting against DNA damage [35]. In a case-control study, Fowke and coworkers [41], despite finding not statistically significant results, suggested that the association between oral contraceptive use and BC risk in premenopausal women could depend on *NQO1* genotype. In their study, the use of oral contraceptives for more than 18 months was a BC risk factor (OR = 2.34, 95% CI = 0.92-5.99) in women with the CC genotype, and a protective factor in women with at least one polymorphic allele (genotype CT / TT; OR = 0.69, 95% CI = 0.38-1.25). The authors suggested that the metabolism of endogenous estrogens would generate more quinones than the metabolism of synthetic estrogens. Synthetic estrogens partially suppress endogenous estrogens release from the ovary and the metabolism of synthetic estrogens would produce less quinones than endogenous estrogens metabolism. So, women using hormonal contraceptives probably would produce less quinones than nonusers. This could represent a protection for breast carcinogenesis. However, this protection is most evident in women with polymorphic *NQO1*, because in women with the wild type genotype, NQO1 metabolism would be enough to eliminate this quinones excess. For this reason, the protective effect of the interaction between *NQO1* $C^{609}T$ polymorphism and the use of hormonal contraceptives is needed, to analyze the effect of NQO1 on breast carcinogenesis.

In relation to education (schooling years analyzed as a categorical variable), it probably modify the association between *NQO1* $C^{609}T$ polymorphism and BC in an indirect way. In our study, many of the participants had low educational level and have worked in unskilled manual jobs (maids and others), thus with a higher chance to have been occupationally exposed to several chemical substances, and/or previously migrated from rural areas, often exposed to pesticides. The degradation of some chemicals such as pesticides also generates quinones that are degraded by NQO1 [54]. However, the measurement of chemical exposure only through a personal interview is usually more imprecise, generating biases such as recall bias, than collecting data on participants' education.

Although a great effort was done in collecting patients of this age group, the limitation of our study is the sample size, so the role of chance cannot be excluded.

5. CONCLUSION

This study suggests that *CYP17 MspA1* and *CYP19* $Arg^{264}Cys$ polymorphisms are not associated with the risk of BC in young women. However, *NQO1* ^{609}T polymorphism may be a risk factor for BC development in young women when others risk factors like hormonal contraceptive use are considered.

CONSENT

All authors declare that 'written informed consent was obtained from all the patients.

ETHICAL APPROVAL

All authors declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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