

Brief Communication

Health Survey and Assessment of the Polymorphisms *BRCA1/P871L*, *BRCA1/Q356R*, and *BRCA2/N372H* in Female Gas Station Workers in Rio de Janeiro

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Gas station workers are exposed to chemicals known to be carcinogenic, especially benzene. The objective was to analyze the health problems of female gas station workers by means of sociodemographic and clinical questionnaires, and laboratorial exams. We performed the genotyping of the polymorphisms *BRCA1/P871L* and *BRCA1/Q356R* by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, and of variant allele *BRCA2/N372H* through direct sequencing. The female workers showed a higher concentration

of monocytes ($P=0.039$); a greater number of spontaneous abortions ($P=0.025$, OR = 4.977, 95% CI = 1.135–30.669); higher tobacco consumption ($P=0.013$); and higher alcohol consumption ($P=0.05$). The statistical analysis of the polymorphisms associated with the variables monocyte concentration and miscarriage number did not reveal a significant relationship, and smoking and spontaneous abortion were not statistically associated either. Environ. Mol. Mutagen. 58:730–734, 2017. © 2017 Wiley Periodicals, Inc.

Key words: polymorphism; benzene toxicity; gas station; miscarriage; blood; occupational exposure

INTRODUCTION

Benzene, which was classified as carcinogenic by the International Agency for Research on Cancer 2010 (included in group A1), is a polycyclic aromatic hydrocarbon present in the gasoline mixture that is dispersed in the air through vehicle exhaust and cigarette smoke [WHO, 2010]. In Brazil, about 150,000 women work at gas stations for a living, according to the National Federation of Employees at the Petroleum Derivatives and Fuels Services Office [FEPOSPETRO, 2017]. These workers are constantly exposed to higher benzene and other aromatic compound concentrations during their work shift, unlike the general population [Carrieri et al., 2006].

The aims of this study were to evaluate the health of the women working at gas stations in Rio de Janeiro and to analyze whether the polymorphisms *BRCA1/P871L*,

BRCA1/Q356R, and *BRCA2/N372H* were related to health problems. *BRCA1* and *BRCA2* genes play a role in one of the DNA repair mechanisms. This is the first study devoted exclusively to female workers at gas stations, which is quite important in that gender influences the

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biological effects of occupational benzene exposure [Moro et al., 2017].

MATERIALS AND METHODS

The female gas station workers and the voluntary controls were recruited between July 2013 and July 2016. The workers group was comprised of 47 women, with a mean age of 32.2 years, SD 8.0. The control group consisted of 58 healthy women, with a mean age of 32.1 years, SD 8.5, who had no occupational exposure to gasoline or any other source of benzene.

Each participant was invited to respond to socio-demographic and clinical questionnaires. The data of the workers were compared with that of the women in the control group. Peripheral blood samples were collected for complete hemograms, biochemistry tests, and genotyping of all subjects. The study was approved by the local ethics committee of the hospital of the State University of Rio de Janeiro (UERJ/HUPE), number 34310014.9.0000.5259/14, and of the Brazilian National Cancer Institute (INCA), number 121/09.

Hematological and Biochemistry Analysis

The hematological analysis consisted of a complete hemogram. The biochemistry analysis consisted of measuring gamma glutamyl transpeptidase, alanine aminotransferase (ALT), glutamic pyruvic transaminase, bilirubin, and creatinine levels. All blood tests were performed in the routine laboratories of UERJ/HUPE and INCA according to standard hematological methods.

Genotyping

Genomic DNA from peripheral blood leukocytes was obtained by phenol–chloroform extraction and analyzed by polymerase chain reaction and restriction enzyme digestion (PCR-RFLP) assays for *BRCA1/P871L* and *BRCA1/Q356R* polymorphisms according to previous publications [Wenham et al., 2003; Zhou et al., 2009]. The PCR reactions were carried out in the Veriti[®] Thermal Cycler (Applied Biosystems) and all reactions were standardized in a single cycling with initial denaturation at 95°C for 3 min, and 30 cycles with denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec. PCR products were digested with *HpaII* (*BRCA1/P871L*) and *AlwNI* (*BRCA1/Q356R*) (New England Biolabs) for 2 hr, respectively, and then separated by electrophoresis in 10% polyacrylamide, and the digested/separated products were further visualized by silver staining. Positive and negative controls were used in all reactions. *BRCA2/N372H* polymorphism was determined by DNA sequencing [Wenham et al., 2003] carried out on the Sequencer 3130 Genetic Analyzer (Applied Biosystems), and the products were analyzed with the Sequencer Program (Gene Codes Corporation, Ann Arbor, MI).

Statistical Analysis

The Hardy–Weinberg (HW) equilibrium was tested using the Chi-Square (χ^2) statistic for the goodness-of-fit test for each polymorphism, and the differences in the allele and genotype frequencies between groups were analyzed using standard χ^2 or Fisher's exact tests. Statistical analysis was carried out with statistical program R (v.2.15.1). In the distributed variables, A nonparametric Mann–Whitney test was used for comparison of the distributed variables between groups using the Predictive Analytics Software – PASW (Version 18). The odds ratio (OR) was also calculated. For all statistical tests, P -value < 0.05 was considered significant. Inconclusive results concerning hemogram/biochemical/molecular analysis and uncertain questionnaire information were excluded from the statistical analysis and, consequently, from the tables.

TABLE I. Polymorphisms in the Study Groups

Polymorphisms	Workers (N = 47)	Controls (N = 58)
<i>BRCA1/P871L</i> (LL/LP) and <i>BRCA1/Q356R</i> (RR/QR) and <i>BRCA2/N372H</i> (HH/NH)	2	4
<i>BRCA1/P871L</i> (LL/LP) and <i>BRCA1/Q356R</i> (RR/QR)	7	7
<i>BRCA1/P871L</i> (LL/LP) and <i>BRCA2/N372H</i> (HH/NH)	12	21
<i>BRCA1/Q356R</i> (RR/QR) and <i>BRCA2/N372H</i> (HH/NH)	0	0
<i>BRCA1/P871L</i> (LL/LP)	23	22
<i>BRCA1/Q356R</i> (RR/QR)	0	1
<i>BRCA2/N372H</i> (HH/NH)	1	1
None	2	2

RESULTS

With regard to the socio-demographic data of the female workers, the average monthly income was R\$2,166.55, which was higher than the Brazilian average (R\$1,193.00). As for race, 60% (28/47) self-declared as brown, 19% (9/47) white, 15% (7/47) black, 4% (2/47) native Indians, and 2% (1/47) Asiatic. For education, most (36%) had finished high school, and 11% had completed higher education. Because of the wide range of exposure time the workers, they were divided into 2 groups: Group 1 (31/47), with shorter time exposure, up to 36 months with a mean exposure time of 8 months, SD 7.3, median = 4; and Group 2 (15/47) with longer time exposure, 36 months or more, mean exposure time of 79 months, SD 30.4, median = 72. One worker did not remember when she started working at the gas station.

The polymorphisms *BRCA1/P871L*, *BRCA1/Q356R*, and *BRCA2/N372H* did not show deviation from the HW Equilibrium in the workers group and in the controls ($P = 0.083$, $P = 1.00$, $P = 0.294$, respectively). The frequencies of the genotypes were compared between the female gas station workers and the control population, and no significant difference was detected, indicating that the two populations were equivalent. The polymorphisms in each group are shown in Table I.

In the workers group, the monocytes were found to be significantly higher, when compared with the control group ($P = 0.03$). However, the other blood variables were not statistically significant when compared with the controls. No statistically significant differences were found in the mean values of liver and kidney function tests among the exposed workers.

The comparative analysis between the groups for the variables mean age at menarche and the number of pregnancies showed significant P values for both variables related to the group of workers, according to the nonparametric Mann–Whitney test ($P = 0.008$ and $P = 0.001$, respectively). When we compared the variables of induced abortion, miscarriage, and family history of

cancer between the groups, we observed the higher prevalence of miscarriage among the workers, when compared to the control group (22%, $P = 0.025$) (Table II). The Chi-square tests applied to OR analysis adjusted for miscarriage revealed a significant effect on the workers compared to the control group (OR = 4.97, 95% CI, 1.135–30.66, $P = 0.025$). However, no other association could be identified between gas station workers and monocytosis ($P = 0.09$) (Table III).

When we associated the two significant variables in the study group, monocytosis ($P = 0.039$) and spontaneous abortion ($P = 0.025$, OR = 4.977, 95%CI = 1.135–30.669), with each category of genotypes studied, we did not find an association between the variables and the genotypes (Table IV). There was also no association between smoking and miscarriage in this population ($P = 0.172$), although the prevalence of tobacco smokers and alcohol consumption habits was observed among the case group ($P = 0.013$ and $P = 0.05$, respectively), (data not shown).

TABLE II. Fisher's Exact Test Analysis of the Association of History of Abortion and Cancer Among the Population Studied

Variables		Workers	Controls	<i>P</i> -value
Induced abortion	Yes	3 (7.3%)	3 (5.3%)	0.692
	No	38 (92.7%)	54 (94.7%)	
Miscarriage	Yes	9 (22%)	3 (5.3%)	0.025
	No	32 (78%)	54 (94.7%)	
Family history of cancer	Yes	21 (50.0%)	32 (59.3%)	0.412
	No	21 (50%)	22 (40.7%)	

TABLE III. Chi-Square Test of the Association Between Controls and Female Workers for Miscarriage and Monocytosis

Variables		Controls	Workers	OR = 95% CI	<i>P</i> -value
Miscarriage	No	54 (94.7%)	32 (78%)	4.977 (1.135–30.669)	0.025
	Yes	3 (5.3%)	9 (22%)		
Monocytosis (%)	Normal	54 (100%)	42 (93.3%)	∞^a (0.504– ∞)	0.090
	High	0 (0%)	3 (6.7%)		

^a ∞ = Infinite; Frequency of the group = zero (0); R software (version 2.13.0).

TABLE IV. Fisher's Exact Chi-Square Test to Associate Polymorphisms with Variables

Polymorphisms	Monocytosis			Miscarriage			
	Genotypes/ <i>P</i> -value			Genotypes/ <i>P</i> -value			
<i>BRCA1</i> (P871L)	LP	LL	LP+LL	<i>BRCA1</i> (P871L)	LP	LL	LP+LL
	0.608	0.242	0.191		0.277	0.260	1.000
<i>BRCA1</i> (Q356R)	QR	RR ^a	QR+RR	<i>BRCA1</i> (Q356R)	QR	RR ^a	QR+RR
	1.000	0	0		0.167	0	0
<i>BRCA2</i> (N372H)	NH	HH	NH+HH	<i>BRCA2</i> (N372H)	NH	HH	NH+HH
	1.000	1.000	1.000		0.410	0.679	1.000

^aRare allele, Wenhan et al. [2003].

DISCUSSION

Little information is available on female workers in gas stations, although they account for a third of the current labor force. In the present study, the participants in the workers group were young and, surprisingly, with education above the Brazilian average. Most of the female gas station workers self-declared as brown, which is a characteristic of the population of the city of Rio de Janeiro in the Southeast of Brazil, being different from the study conducted in the south of Brazil [Cezar-Vaz et al., 2012]. Knowing the social and the education profile of the female workers is important for drawing strategies aimed at their health care. Another surprising finding was that the average wage of the workers was higher than the Brazilian average [Brazil Agency, 2017]. Earning a higher wage over the Brazilian wage average may explain the increasing engagement of female workers in the gas station labor force, in spite of the health risk.

In contrast to our hematological and biochemical findings, some studies found statistically significant differences in many blood variables. A recent Brazilian study on metabolic polymorphisms reported significant changes in mean corpuscular volume and neutrophils [Mitri et al., 2015]. Although the study populations were from Rio de Janeiro, the exposure time to benzene may have been responsible for the divergence in hematological results between the studies. In our study the mean exposure was 8 months for Group 1, and 79 months for Group 2, while the previous study [Mitri et al., 2015] reported a longer exposure time (average 168 months). This difference emphasizes that the health problems and DNA damage

caused by the harmful gasoline compounds can continue for years or even decades in the occupationally exposed population. A study conducted by Yin et al. [1987], which evaluated hematological and biochemical parameters similar to ours in female solvent workers, found statistically significant associations for lymphocytes, eosinophils, and monocytes. In this study, the researchers evaluated groups of workers exposed to benzene, toluene, and the combination of both compared to a control group, demonstrating that on exposure to about 50 ppm benzene, about 40 ppm toluene, or the combination of about 20 ppm of benzene and about 20 ppm of toluene, there was no sign of pancytopenia, and levels of markers of renal and hepatic function remained unchanged. These findings were similar to ours, even though we did not accomplish the measurement of levels of these toxic compounds in the atmosphere of gas stations enrolled in our study.

Regarding biochemical findings, an Iranian cross-sectional study conducted by Neghab et al. [2015] evaluated the nephrotoxic and hepatotoxic potential of 200 employees at the Shiraz oilfield in the city of Shiraz exposed to BTX (mean exposure = 10.4 years) at varying concentrations of benzene dispersed in the air and compared these workers to a control group of 200 unexposed workers, and found statistical differences for the ALT, AST, and bilirubin levels, which were increased in the exposed group. In our study group no biochemical alterations were found, which can be explained by the shorter exposure time. D'Andrea and Reddy 2014 conducted a study with residents of areas affected by the emission of benzene in the air after a refinery explosion in Texas City, Texas, in 2010. In this study, hematological and biochemical tests were performed on 784 women, and significant differences were found in the serum levels of liver enzymes (ALP, AST, ALT, and creatinine), as well as the difference in mean leukocyte and platelet counts. Similar to our results, this Texas study did not show significant changes in the levels of hematocrit (%) and hemoglobin (g/dL) between the groups exposed and not exposed to benzene. The exposure time to benzene and other toxic compounds was 147 days up until the date of the exams, which can be considered a short time of exposure.

Breast cancer burden related to benzene and other urban contaminants should be considered by exposed women working in gas stations [Weiderpass et al., 2011; Garcia et al., 2015; Fenga, 2016]. Additionally, the polymorphisms *BRCA1/P871L*, *BRCA1/Q356R*, and *BRCA2/N372H* were associated with higher cancer risk [Wenham et al., 2003; Zhou et al., 2009; Xue et al., 2014; Su et al., 2015]. To our knowledge, there was no suspicious cancer case in our study group, although we recognize the limitation of not applying screening tests (e.g. mammogram). As we found differences in the monocyte and miscarriage rates, we tested if these differences could be related to

any pleiotropic effect of *BRCA1/P871L*, *BRCA1/Q356R*, and *BRCA2/N372H*.

An important result of our study was the high incidence of spontaneous abortions among women workers (22%, OR = 4.97). In comparison, Cecatti et al. [2010] evaluated the prevalence of spontaneous abortion in seven regions of Brazil in a population of 12,612 women of reproductive age, and reported the top rate of 14% in the Northeast of Brazil, which is the poorest region. One of the participants reported an abortion at 4 months of gestation and a fetus with congenital malformation (short stature); she justified the cause as the hormone replacement that she had been doing for 12 years. Santiago et al. [2017] described two cases of gas station workers with a history of miscarriage, which, in the chromosomal analysis, presented complex rearrangements and immunological changes involving NK (Natural Killer) cell counts.

In our study, we did not find a statistically significant association between smoking and abortion ($P = 0.172$), although the frequency of smoking was higher among female workers than controls (28.6% and 6.9%, respectively). Although our analyses did not show an association between smoking and abortion, it is known that tobacco smoke is strongly associated with failure of the mechanism of fetal neurodevelopment, low birth weight, preterm birth, and abortion [Barbara, 2009]. In a 2014 systematic review of journal articles, in which smoking and miscarriage were associated in 50 of the 98 related articles, smoking was associated with an increased risk of spontaneous abortion, and the relative risk increased 1% for each cigarette smoked per day. However, in the same article they also reported the increased risk of miscarriage in passive smokers by up to 11% [Pineles et al., 2014]. Tobacco smoking is a harmful factor during pregnancy, not only because of its effects on the reproduction process, but also because of its implication for the health of the offspring. In this study, it was not possible to calculate the smoking load, which limited the analyses on the relationship between smoking and the other variables, thus emphasizing the need to carry out future studies for better clarification.

Some actions have been recently taken to protect pregnant women at occupational risk in Brazil. In 2016, the law 13,287/16 was approved for removing pregnant and nursing workers from the workplace considered unhealthy with full payment of their wages. This measure was extended to the class of gas station workers, favoring 150,000 women, representing 30% of the class. This measure was very important and was celebrated.

CONCLUSION

This is the first study to be devoted exclusively to women exposed to benzene, associating occupational

exposure to benzene with a high risk of miscarriage in gas station workers in Brazil. The analysis of the polymorphisms *BRCA1/P871L*, *BRCA1/Q356R*, and *BRCA2/N372H* showed no statistical association between benzene exposure and increased monocyte concentration and the number of miscarriages. According to our study, these polymorphisms do not represent an additional risk to the health of female workers. However, there was a significant increase in monocyte concentration and miscarriages among these women, corresponding to a fourfold higher chance of abortion than in the nonexposed population. These data lead us to reflect on how much the genome of the female gas station workers and their offspring may be at risk due to occupational exposure to benzene. The increasing inclusion of female labor at gas stations is a relatively recent event, and this is why more long-term studies are needed to prove this hypothesis.

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