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Genotoxic effects of occupational exposure among gas station attendants in Santarem, Para, Brazil

Avaliação dos efeitos genotóxicos da exposição ocupacional em frentistas atuantes em postos de gasolina no município de Santarém, Pará

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ABSTRACT | Background: Gas station attendants are at high risk of poisoning due to continuous exposure to fuel fumes. Benzene, present in gasoline, is considered a carcinogen and harmful to the health of gas station attendants. **Objective**: To investigate genotoxic effects on exfoliated oral mucosa cells in association with occupational exposure among gas station attendants in Santarem, Para, Brazil, and to establish the rate of adherence to personal protective equipment (PPE). **Methods**: The results of the micronucleus test performed with oral mucosa cells stained by means of the Feulgen and Fast Green methods were compared between 126 exposed gas station attendants distributed across six groups and controls. **Results**: The frequency of micronuclei was higher among gas station attendants compared to the group not exposed to benzene ($p<0.01$). The frequency of micronuclei was significantly higher ($p<0.01$) among the gas station attendants who reported to drink alcohol compared to non-drinkers. None of the participants (0%) used PPE. **Conclusion**: The frequency of micronuclei in exfoliated oral mucosa cells was higher among gas station attendants compared to controls; frequency was even higher for the gas station attendants who reported to consume alcohol often.

Keywords | occupational risks; genotoxicity; micronuclei, chromosome-defective; genomic instability; benzene.

RESUMO | Introdução: Profissionais frentistas encontram-se em situação de risco tóxico pela exposição constante aos vapores dos combustíveis. O benzeno, presente na gasolina, é considerado cancerígeno e prejudicial à saúde desses trabalhadores **Objetivo**: Avaliar os efeitos genotóxicos decorrentes da exposição ocupacional em células esfoliativas da mucosa oral de frentistas do município de Santarém, Pará; e determinar a taxa de adesão ao uso de equipamentos de proteção individual por esses colaboradores. **Métodos**: Um total de 126 frentistas, distribuídos em 6 grupos contendo indivíduos expostos foram comparados ao grupo controle utilizando a técnica do ensaio de micronúcleos em células da mucosa oral coradas com as técnicas de Feulgen/*Fast-green*. **Resultados**: Os frentistas apresentaram maior frequência de micronúcleos em relação ao grupo não exposto ao benzeno ($p<0,01$). Frentistas com hábito etílico apresentaram aumento significativo de micronúcleos ($p<0,01$), quando comparados aos frentistas abstêmios. Verificou-se que 100% dos profissionais entrevistados não fazem uso dos equipamentos de proteção. **Conclusão**: Constatou-se um aumento na presença de micronúcleos em células esfoliativas de frentistas em comparação ao grupo controle. Esse aumento é ainda maior quando o frentista ingere frequentemente bebidas alcoólicas.

Palavras-chave | riscos ocupacionais; genotoxicidade; micronúcleos com defeito cromossômico; instabilidade genômica; benzeno.

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INTRODUCTION

Fossil, petroleum-derived fuels are increasingly used, as e.g. gasoline, which consists of a complex and highly toxic mixture of paraffin hydrocarbons, naphthalene and phenols. These compounds pose serious occupational risks to several categories of workers during the extraction, refining, transport and distribution of fuels[1].

Gas station attendants are exposed to several ergonomic, physical and chemical hazards, including gasoline fumes, which toxic effects have been poorly studied and treated. Gasoline fumes are mainly composed of aliphatic and polycyclic aromatic hydrocarbons (PAH), which are low-density, instable and volatile molecules. Benzene, toluene, ethylbenzene and xylene (BTEX) are the most toxic among these compounds and have been associated with carcinogenesis[2].

Benzene (C_6H_6) is particularly toxic and lacks safe exposure limits. Nevertheless, it can be found in a wide variety of products of daily use, from cigarettes to plastic. When mixed into fossil fuels such as gasoline and diesel, its light, unstable and volatile nature makes it evaporate rapidly[2].

Benzene vapor easily enters the body of exposed individuals through the oral and nasal mucosa, which epithelium is very thin and thus increases the permeability of benzene and its diffusion into the bloodstream, where it binds to plasma proteins to then accumulate in fatty tissues, and organs such as the kidneys, lungs (inhalation) and mainly the liver, where it is metabolized[3].

Continuous exposure to considerable amounts of benzene is associated with several harmful effects to humans, including carcinogenesis, blood, bone marrow and nervous system toxicity[2].

Having similar molecular, toxicokinetic and toxicodynamic characteristics, also the action of the other BTEX members is similar to that of benzene. In addition to organ-specific damage, some studies showed that prolonged exposure is associated with central nervous system depression and bone marrow hypoplasia, leading to cytopenia, i.e. leukopenia, thrombocytopenia and anemia[4-6].

The main routes of occupational exposure to BTEX among gas station attendants are direct skin contact while handling fuels, which causes local irritation, urticaria and burns, and vapor inhalation while pumping gas, which facilitates absorption and thus has more systemic effects[2,7].

The damage caused by BTEX at the cell level is related to their ability to cross cell and nuclear membranes and interact with the DNA nitrogen bases weakening their bonds, which might result in gene mutations[8,9].

These facts point to the need to monitor populations exposed to these chemicals, for which purpose biological parameters are used, as e.g. cytogenetic tests, to detect damage and elucidate the mutagenic effects of exposure[10].

The micronucleus test is used to monitor genotoxic and cytotoxic damage. This assay is useful to screen for genotoxicity based on the presence of micronuclei and other nuclear abnormalities in interphase cells[3].

Micronuclei are indicators of mutagenicity derived from genotoxic damage to epithelial cells and precede actual carcinogenesis. They consist in chromatin fragments originated in aberrant mitosis which remain close to the cell nucleus. Their frequency is used to estimate the degree of genotoxic damage to animal cells[11].

Assessing the frequency of micronuclei in oral mucosa cells enables identifying groups at high risk for respiratory cancer associated with exposure to potentially carcinogenic compounds, as was demonstrated for workers exposed to wood dust and welding fumes and gas station attendants[12-14].

The aims of the present study were to assess genotoxic damage to exfoliated oral mucosa cells in association with exposure to benzene present in gasoline fumes among gas station attendants in Santarem, Para, Brazil, and to establish the latter's rate of adherence to personal protective equipment (PPE).

METHODS

STUDY DESIGN

The present explanatory and experimental study with quantitative analysis sought to establish the frequency of micronuclei, by means of the micronucleus test, in exfoliated oral mucosa cells of gas station attendants exposed to gasoline fumes.

The micronucleus test was selected due to its higher sensitivity and specificity for monitoring individuals at high risk for carcinogenesis by comparison to other biological assays. High frequency of micronuclei in oral mucosa cells is indicative of high rates of mutations and has been associated with occurrence of carcinoma[15].

PARTICIPANTS

The sample comprised 147 participants, being 126 attendants at 11 gas stations of different oil companies in the urban area of Santarem, and 21 controls. The study was performed in July 2016.

Following analysis of data collected in interviews, in which a questionnaire was administered, the sample was divided into seven groups with 21 participants each: G1, G2 and G3—gas station attendants with 0 to 5 years in the job, being non-drinkers, drinkers and mouthwash users, respectively; G4, G5 and G6—gas station attendants with 6 to 10 years in the job, aged 18 to 36 years old, being non-drinkers, drinkers and mouthwash users, respectively; G7 was the control group (non-exposed individuals).

Gas station attendants were categorized according to length of exposure to gasoline fumes and alcohol consumption/use of mouthwash to establish whether length of exposure to BTEX, alcohol and mouthwash use were associated with higher frequency of micronuclei among the participants with more years in the job.

The results of the micronucleus test were compared between gas station attendants and controls. We also analyzed adherence to PPE among gas station attendants.

The participants were allocated to the aforementioned groups following structured interviews conducted by the investigators to collect data on age, sex, occupation, years in the job, exposure to mutagenic agents, such as smoking, alcohol and x-rays, use of orthodontic appliances, mouthwash and medication.

We included individuals who met any the following criteria: gas station attendants with 1 month to 10 years in the job; gas station attendants who consumed 14 doses of alcohol weekly, on average; and gas station attendants who used mouthwash every day. The control group was selected based on occupational considerations to control for confounding factors, and comprised unemployed university students, university professors and health workers not exposed to genotoxic agents.

All the participants signed an informed consent form as indicated in the National Health Council Resolution no. 466/2012. The study was approved by the research ethics committee of State University of Para, ruling no. 1,827,532.

MICRONUCLEI ASSESSMENT: SAMPLE COLLECTION, PREPARATION AND ANALYSIS OF SLIDES

The micronuclei test was performed following the method described by Souto et al.[16].

Sample collection was performed after the participants responded a 10–question structured interview. The oral mucosa cells were collected with Cytobrush® brush and spread on slides previously cleaned with 99.5% alcohol. Smears were prepared at room temperature, then fixated with cytology fixative spray. Cytokinesis was not blocked.

The samples were subjected to DNA acid hydrolysis with 10% hydrochloric acid solution for 2 minutes at room temperature, next they were heated on water bath at 60ºC and then immediately left at room temperature.

The slides were stained with basic fuchsine solution for 12 minutes away from light, next slightly rinsed with water to remove excess dye. The slides were then counterstained with Fast Green solution for one minute, then rinsed with 70% alcohol.

Micronuclei were counted on 2,000 cells per participant, blindly to avoid bias, under optical microscope with 100x oil immersion lens. The parameters selected for micronucleus counting were: diameter less than a third of that of the associated nucleus, chromatin texture and staining pattern identical to those of the nucleus and absence of bridge to the nucleus (Figure 1).

STATISTICAL ANALYSIS

The frequency of micronuclei was expressed as median and mean and standard deviation of the mean (SD). Means between groups were compared with ANOVA and Tukey's test. The significance level was set to $p<0.05$. Analysis was performed with software BioEstat version 5.3.

RESULTS

One hundred and nineteen gas station attendants were male. None of the gas station attendants (n=126, 0%) used PPE in their daily work.

The frequency of micronuclei was higher among gas station attendants exposed to gasoline fumes, those who reported to consume alcohol and used mouthwash compared to controls ($p<0.01$). This finding indicates that genotoxic agents present in gasoline, such as BTEX, and the other analyzed variables contributed to increase the frequency of micronuclei in the analyzed cells of gas station attendants (Graphic 1).

The frequency of micronuclei was also significantly higher ($p<0.01$) among the gas station attendants who reported to consume alcohol compared to non-drinkers. This finding indicates that consuming alcohol possibly contributed to increase the frequency of micronuclei. There was not significant difference in the frequency of micronuclei between the gas station attendants who consumed alcohol and those who used mouthwash (Table 1).

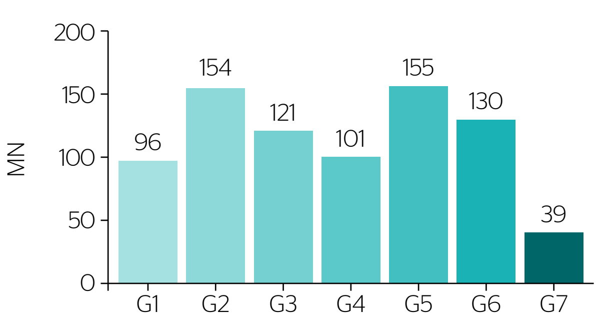
DISCUSSION

The results relative to micronuclei frequency indicate higher genotoxicity among gas station attendants compared to controls. This finding is related to their occupational exposure to gasoline fumes at the time of pumping gas.

A study previously performed in Brazil found that gas station attendants are at high risk for mutagenesis as a result of the synergic effects of methanol and other compounds present in fuels, which was evidenced by higher frequency of micronuclei at three different time-points[17].

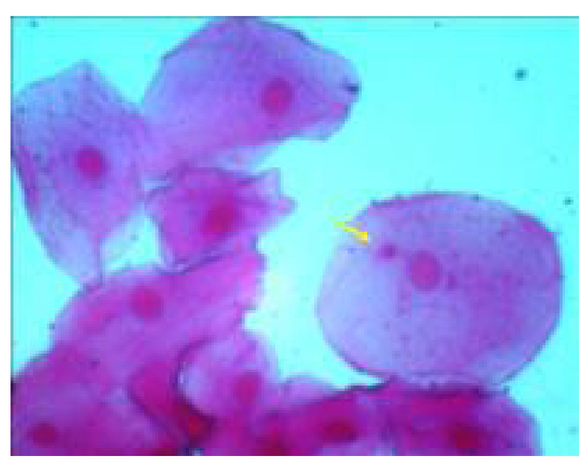
Another study that investigated micronuclei and other nuclear abnormalities in exfoliated oral mucosa cells of gas station attendants in a municipality in southern Brazil found higher frequency of micronuclei and abnormalities such as binucleated cells and broken egg structures in this group compared to non-exposed individuals[14]. The authors of a study performed in India that investigated genotoxicity among gas station attendants described the micronucleus test as a relevant biomarker for assessment of damage among workers exposed to carcinogenic substances. This method evidenced higher cytotoxic risk for the gas station attendants[18]. A study conducted in Egypt found higher rates of DNA fragmentation, higher frequency of micronuclei and dead cells percent (peripheral leukocytes) among gas station attendants exposed to benzene compared to controls[3].

Oral mucosa cells are susceptible to the genotoxic effects of alcohol, which increases the frequency of micronuclei in exfoliated cells. Damage is greater among individuals who consume alcohol compared to non-drinkers[19,20]. In the present study, mutagenicity was more frequent among the gas station attendants who reported to consume alcohol compared to the ones who did not. This finding indicates that alcohol consumption contributed to increase the frequency of micronuclei. In their study, Faria and Braga analyzed the genotoxic potential of alcohol for the oral epithelium among university students. The frequency of micronuclei was significantly higher among those who reported to consume alcohol compared to controls[21].



G1: gas station attendants with 0 to 5 years in the job who did not consume alcohol (n=21); G2: gas station attendants with 0 to 5 years in the job who reported to consume alcohol (n=21); G3: gas station attendants with 0 to 5 years in the job who reported to use mouthwash (n=21); G4: gas station attendants with 0 to 5 years in the job who did not consume alcohol (n=21); G5: gas station attendants with 6 to 10 years in the job who reported to consume alcohol (n=21); G6: gas station attendants with 6 to 10 years in the job who reported to use mouthwash (n=21); G7: controls (n=21).

Graphic 1. Frequency of micronuclei on the micronucleus test per group, Santarem, Para, Brazil, 2016 (n=147).



Arrow: micronucleus.

Figure 1. Microphotograph of an oral epithelial cell with a micronucleus, Santarem, Para, Brazil, 2016.

As concerns length of exposure, the frequency of micronuclei was not significantly higher among the gas station attendants with 6 to 10 years in the job compared to those with 0 to 5 years. Yet the frequency of micronuclei was higher in both groups by comparison to the non-exposed controls. Also Salem et al. found that length of exposure did not significantly influence genotoxicity among gas station attendants. These findings indicate that despite the proven cytotoxicity of benzene, the rate of damage remains constant even when the duration of exposure varies[3].

In the present study we found that factors alcohol consumption and mouthwash use considerably increased the frequency of micronuclei among gas station attendants continuously exposed to benzene. There was no significant difference in the frequency of micronuclei between the gas station attendants who reported to consume alcohol and those who used mouthwash.

The frequency of micronuclei was higher among the participants exposed to genotoxic substances. This finding indicates that the micronucleus test has adequate efficacy and sensitivity to measure genetic abnormalities among individuals exposed to carcinogens.

Gas station attendants are directly exposed to compounds present in fuels through inhalation of fumes or direct skin contact. Gasoline is particularly harmful due the presence of benzene, a highly dangerous chemical that causes cancer[22]. Benzene becomes toxic through products derived from biotransformation, to wit, benzene epoxide, which is highly reactive and unstable, and 1,4-benzoquinone, which possibly accounts for myelotoxicity[23]. This aromatic hydrocarbon is able to bind to the DNA originating adducts, which cause mutations through base substitution, which might lead to error in transcription, or small deletions or insertions; a large number of adducts might break the DNA molecule, resulting in loss of genetic material[24]. Occupational exposure to benzene is associated with higher risk of genotoxic damage among individuals exposed to gasoline[25].

Our results evidenced low rates of PPE use among the analyzed gas station attendants, which make them more susceptible to the genotoxic action of fuel components. The main reasons the participants mentioned not to use PPE were unavailability/lack of supply by the employer, discomfort during the performance of tasks and lack of experience in the handling of this equipment[5].

Adherence to PPE is low among gas station attendants nationwide[26]. None of the participants in study that analyzed occupational exposure to gasoline among 20 gas station attendants in a municipality in the Northeast region of Brazil reported to use PPE[10], which agrees with the results of the present study. In their study with 32 gas station attendants in Santarem, Portela et al.[27] found that gloves, glasses or masks were not routinely used, but only during specific tasks, such as internal fuel reservoir unloading. Uniforms and boots were the most frequently used pieces of PPE.

Low adherence to PPE increases the exposure of gas station attendants to carcinogenic agents in gasoline, and thus contributed to the higher frequency of genotoxic abnormalities found among the exposed groups in the present study.

Table 1. Statistical analysis of the distribution of the frequency of micronuclei among the analyzed groups according to variation in means and standard deviations on ANOVA and Tukey's test, significance level p<0.05, Santarem, Para, Brazil 2016 (n=147).

Median	number	Median	Mean±standard deviation
Gas station attendants, non-drinkers, 0 to 5 years	21	5 .0	4 .5±1 .2*
Gas station attendants, drinkers, 0 to 5 years	21	7 .0	7 .3±2 .6*#
Gas station attendants, mouthwash users, 0 to 5 years	21	6 .0	5 .7±1 .8*
Gas station attendants, non-drinkers, 6 to 10 years	21	5 .0	4 .8±1 .6*
Gas station attendants, drinkers, 6 to 10 years	21	7 .0	7 .3±2 .2*#
Gas station attendants, mouthwash users, 6 to 10 years	21	6 .0	6 .1±2 .0*
Controls	21	2 .0	1 .8±1 .3

*Gas station attendants exposed to gasoline fumes, alcohol and mouthwash compared to the control group; there was significant increase in the frequency of micronuclei, p<0.01; #gas station attendants who reported to consume alcohol versus non-drinkers; there was significant increase in the frequency of micronuclei, p<0.01.

CONCLUSION

The results of the present study indicate that the frequency of genotoxic damage to oral epithelial cells was significantly higher among gas station attendants exposed to gasoline fumes, those who reported to consume alcohol and use mouthwash compared to controls. Damage was constant among non-drinkers, even between those with different length of exposure. The frequency of genotoxic damage was higher among the gas station attendants who reported to consume alcohol, but not significantly different by comparison to mouthwash users. Finally, the participants reported not to use PPE frequently.

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