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Micronuclei and other nuclear anomalies in exfoliated buccal cells of urban solid waste collectors and recyclers in southern Brazil

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HIGHLIGHTS

- Cytogenetic alterations were evaluated in workers handling urban solid waste.
- Studies using the MN assay in buccal cells in this group of workers are scarce.
- The frequencies of MN, binucleated cells and karyorrhexis were higher in the exposed workers.
- Urban solid waste collectors are exposed to cytotoxic and mutagenic contaminants.

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ABSTRACT

Workers involved in urban solid waste collection may be exposed to various environmental contaminants, including chemical pollutants, which might be mutagenic and increase the risk of diseases such as cancer. Evaluation of DNA damage in workers in this field are still scarce. This study aims to evaluate mutagenic and cytotoxic effects in workers involved in the collection and segregation of urban solid waste generated in southern Brazil. Municipal solid waste collectors were recruited in two municipalities of the state of Rio Grande do Sul. The control group was composed of workers of the education and commerce areas, with no exposure to known genotoxic agents. Slides of exfoliated buccal cells were analyzed to estimate the frequency of micronuclei (MN) as well as other nuclear abnormalities, such as broken-egg/bud, binucleation and karyorrhexis. The analyses of 44 workers and 45 control subjects have shown that the frequencies of MN, binucleated cells and karyorrhexis in the exposed workers were significantly higher than in the control group. In the exposed group, frequencies of MN and binucleated cells showed a significant positive correlation. The other cytogenetic parameters were not correlated among each other or with age and exposure time. These results indicate that the workers involved in urban solid waste collection are exposed to mutagenic and cytotoxic agents.

1. Introduction

According to the 2014 Panorama of Solid Waste of the Brazilian Association of Public Cleaning and Special Waste Companies (ABRELPE), the total solid waste generation in Brazil in 2014 was approximately 78.6 million tons. The Brazilian municipal solid waste is composed of organic matter (51.4%) followed by materials that can be recycled (31.9%), such as glass, plastics, paper, steel and aluminum (Massukado et al., 2013). In 1191 municipalities of the three States of the southern region of Brazil, 22.328 tons of waste was generated. Of these, 70.7% are destined for landfills, 18.3% for controlled landfills and 11% for rubbish dumping areas. In the state of Rio Grande do Sul, 8.643 tons of waste are generated per day and, in the region of the Sinos River Valley, about 450 tons per day.

Domestic solid waste management procedures in several countries are characterized by a dominance of manual handling tasks. Working procedures and waste properties expose persons involved in waste collection and recycling to a diversity of occupational health hazards (Bleck and Wettberg, 2012). Some studies indicate that health problems are associated with the entire process of handling, treatment and disposal of urban solid waste (Domingo and Nadal, 2009; Giusti, 2009). Municipal solid waste collectors are potentially exposed to a variety of bioaerosols and to a mixture of chemicals (Nielsen et al., 1998; Domingo and Nadal, 2009; Giusti, 2009), which have an unknown toxicological profile (Rushton,

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2003). According to Giusti (2009), the main emissions from activities related to urban solid waste management are heavy metals, synthetic organic compounds, methane and volatile organic compounds.

Mutagenic agents, such as chemicals, could cause DNA damage. Such damage may not be repaired and may cause the development of cancer and neurodegenerative diseases (Bolognesi et al., 2015). A widely applied assay for biomonitoring occupational exposure to genotoxic agents is the micronuclei (MN) assay in epithelial cells of the oral mucosa (Thomas et al., 2009; Bolognesi et al., 2015). Its wide use is due to the fact that it provides both an assessment of genetic and cytotoxic damage, including MN, broken-eggs/buds, binucleated cells and various forms of cell death, such as karyorrhexis (Bonassi et al., 2011; Bolognesi et al., 2013). This technique is particularly attractive as buccal cells can be collected in a minimally invasive manner (Bolognesi et al., 2013). In addition, buccal cells are in constant contact with environmental agents, thus being a target site for inhaled or ingested toxic substances (Thomas et al., 2009; Bolognesi et al., 2015).

Although some studies have shown that solid waste collectors and waste recyclers present an increased risk of cancer and neurodegenerative diseases (Pukkala and Pönka, 2001; Jarup et al., 2002; Viel et al., 2008), only but a few have assessed DNA damage rates in this group of professionals (Gonsebatt et al., 1995; Hartmann et al., 1998; Sul et al., 2003; Bakare et al., 2007; Wultsch et al., 2011), and none of these studies were performed in Brazil.

Given the occupational exposure and the potential risks to the workers involved in the collection and recycling of urban solid waste (Klaunig et al., 2009), genetic biomonitoring is important since it enables the identification of risk factors at a time when control measures may still be implemented (Sailaja et al., 2006; Ergene et al., 2007). In addition, studies to assess genotoxicity in urban solid waste collectors are scarce as well as contradictory. Accordingly, the aim of the present study was to evaluate the mutagenic potential of occupational exposure of workers involved in the collection and segregation of solid urban waste from two municipalities in the southern region of Brazil.

2. Material and methods

Individuals, from two municipalities in the Sinos Rver Valleyi, a region in the South of Brazil, who perform activities related to the collection, segregation and recycling of solid waste, formed the population of the study. The exposed group consisted of 44 workers aged 18–65 years and with at least three months of activity in the workplace. The control group consisted of 45 participants of the same age group, who did not perform activities with a recognized exposure to chemical substances, employed in the fields of administration, commerce, and education, from the same region. The study protocol was approved by the Feevale University Research Ethics Committee.

All participants signed an Informed Consent document, answered a questionnaire, which included questions related to age, sex, working time, smoking habits, alcohol consumption, among others.

Shortly after the interview, buccal mucosa cells were collected from inside the two cheeks by using a cytobrush (Thomas et al., 2009). Subsequently, the brushes were inserted into plastic tubes containing saline solution and were fixed with acetic acid and methanol (1:3) after centrifugation. Finally, the cells were dripped onto slides for further Feulgen-Fast Green staining.

A total of 2000 cells of each individual were analyzed to check the frequency of MN and other nuclear alterations. The slides were analyzed using an Olympus BX41 light microscope. The criteria for inclusion of the cell in the analysis were those suggested by Tolbert et al. (1992): (a) intact cytoplasm and relatively flat position on the slide, (b) little or no overlap with adjacent cells, (c) few or no cell moieties, and (d) normal or intact nucleus, smooth and distinct nuclear perimeter. According to Bolognesi et al. (2013), cells with the following characteristics were classified as micronucleated: (a) one major nucleus and one or more MN; (b) oval or round MN; (c) Feulgen-positive MN; (d) MN with the same texture and color as the main nucleus; (e) MN has 1/3 to 1/6 of the nucleus diameter; (f) MN not connected to the main nucleus; (g) MN boundary should be clearly distinguishable from the main nucleus. In addition to the MN analysis, other alterations were also analyzed, according to Bolognesi et al. (2013), such as binucleation, broken-egg/nuclear buds and karyorrhexis. In the present study, cells identified as containing MN and other nuclear anomalies were not confirmed as being positive by examining the cells under fluorescence (as suggested by Thomas et al., 2009).

The studied cytogenetic variables departed significantly from normality and, therefore, the non-parametric Mann–Whitney Test was applied to data. The associations between two variables were analyzed by means of the Spearman correlation. The chi-square test was used to compare frequencies between groups (sex, smoking and alcohol consumption). The level of significance was taken as $p \leq 0.05$. All analyses were conducted using the statistical package for social sciences (SPSS) for Windows, version 18.0. The results were expressed as mean ± standard deviation (SD).

3. Results

The demographic data of the exposed and control groups are presented in Table 1. Age, sex and alcohol intake are similar between the two groups. Smoking frequency was higher in the exposed group ($p = 0.02$). In both groups, smokers have reported smoking less than 1 pack/day and alcohol drinkers reported occasional alcohol consumption. The workers involved in the collection and segregation of urban solid waste have showed an average exposure time of 6.37 ± 6.07 years. Most of workers have reported the use of personal protection, including overalls, gloves, boots and goggles.

The frequencies of MN, buds, binucleation and karyorrhexis are shown in Table 2. The frequencies of MN, binucleation and karyorrhexis were significantly different ($p < 0.05$), and the exposed group presented higher values than the control group.

In the exposed group, the frequencies of MN and binucleated cells presented a significant positive correlation ($p < 0.001$; $r = 0.51$). The other cytogenetic parameters did not correlate with each other or with age and time of exposure. The analysis of the influence of gender, smoking and alcohol intake on the cytogenetic

Table 1
Characteristics of the exposed and control groups.

Characteristic	Exposed	Control	p
Sample size	44	45	
Age (mean in years ± SD)	39.91 ± 10.56	39.40 ± 12.06	0.97
Sex			
Female (%)	22.72	26.67	0.95
Male (%)	77.28	73.33	
Smoking			
Smoker (%)	21.95	4.44	0.02[a]
Non-smoker (%)	78.05	95.56	
Alcoholic beverages ingestion			
Yes (%)	45.45	66.67	0.06
No (%)	54.55	33.33	
Exposure time (mean in years ± SD)	6.37 ± 6.07		

[a] Statistically significant difference ($p < 0.05$) between the two groups.