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Increased DNA Damage, Instability and Cytokinesis Defects in Occupationally Exposed Car Painters

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Abstract. *Background/Aim: Car painting is considered an occupational exposure job with high risk for cancer development, due to the association with harmful chemicals and mutagens. This study aimed to profile car painters occupationally exposed and determine its association with DNA damage and genomic instability. Materials and Methods: We collected a questionnaire and buccal cells of 74 individuals (37 car painters and 37 non-exposed workers) paired by age, alcohol and smoking habits. The number of pyknotic cells, karyolitic cells, karyorrhetic cells, condensed chromatin, binucleated cells, basal cells, differentiated cells (DIFF), micronucleated cells and nuclear buds were evaluated using the Buccal Micronucleus Cytome Assay protocol. Results: A statistically significant increase was observed in all parameters ($p<0.05$) in the exposed group, but DIFF showed a statistically significant decrease ($p<0.001$), compared to the control group. Conclusion: In association with the poor work environment and lack of personal and collective protective equipment, occupational exposure of car painters leads to high DNA damage, genomic instability and alterations in cellular kinetics.*

Human biomonitoring studies have tried to establish an association between the risk for future diseases and occupational exposure, life style and environmental and nutritional contamination (1-11). In this context, car painting represents a potential occupational hazard, especially due to the known use of benzene, isocyanates, toluene and xylene (12, 13). The International Agency for Research on Cancer (IARC) already classified commercial painting as a high-risk group for lung and bladder cancer development (14), whereas the major pathways of exposure involve inhalation of vapors and gases, dermal absorption and/or ingestion (15). While, benzene is already classified by IARC as "carcinogenic to humans", isocyanates, toluene and xylene *per se* are classified as "not classified as to their carcinogenicity in humans", even with the crescent literature associating painters with chemical contamination and diseases (16, 17). Another major issue is that some workers do not even know about these classifications, so they tend to ignore personal and collective protective equipment, what could increase the risk for future diseases.

Given the association between car painting and the potential and proven harmful mutagens and carcinogens, this study aimed to profile individuals working as car painters for at least 8 years. We selected the Buccal Micronucleus Cytome assay (BMCyt) (1), as a reliable and cost-effective test to evaluate cellular biomarkers of early toxicity effects. BMCyt was selected since the different cellular types can be easily observed in buccal samples and may be categorized according to DNA damage level, genomic (in) stability and cellular kinetics, providing a full spectrum for a genetic screening using a minimally invasive test.

Here, we matched by age, alcohol and smoking habits 37 car painter workers (exposed group) for at least 8 years with 37 non-exposed individuals. Due to availability, only men were included in this study. Our groups included only individuals with no familial history of cancer or any chronic diseases. The BMCyt test was performed and statistically significant differences were observed in the exposed group in all cellular parameters evaluated ($p<0.05$), indicating high DNA damage, genome instability and alterations in cellular kinetics, arguing for occupational exposition. Furthermore, most of car painters declared no use of personal protective equipment, what likely corroborate the results. All in all, our study demonstrated the feasibility of performing the BMCyt test to identify early biomarkers for occupational exposition in car painters and

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Key Words: Buccal micronucleus cytome assay, cellular kinetics, DNA instability, cancer risk, occupational exposure mutagenesis.

Table I. *Demographic characteristics of the studied population.*

			Smoking		Alcohol consumption		
	N	Age (years)	Smokers	Non-smokers	Non consumer	Moderate consumers	High Consumers
EXP	37	43.2	15	22	6	25	6
CON	37	42.5	15	22	6	25	6

EXP: Exposed group; CON: control group; Age in years calculated as average; NC: non-consumer; MC: moderate consumer; HC: high consumer.

represents an important stepforward to suggest genetic screenings to determine future disease risk, including cancer.

Materials and Methods

Acquisition of sample data. All individuals signed an Informed Consent approved by an Ethics Committee (CAAE: 65453117.4.0000.0107) and completed a questionnaire. The car painters group consisted of 37 workers (exposed group), paired by gender, age, smoking and alcoholic habits with 37 non-exposed individuals (control group). Individuals from our study were born in the same state in Brazil. The exposed group consisted of individuals working as car painters for at least 8 years. Non-exposed individuals included individuals working in no known occupational exposure jobs and without known chemical exposure at home. All individuals filled out a questionnaire to provide identification, family history, smoking habits, alcohol consumption and medical history. Two major groups were created and we were carefull to include only individuals without family history of cancer or any chronic disease. Cigarette smokers were defined as those who had smoked at least 100 cigarettes during their lifetime, or were at the time of recruitment smoking occasionally or every day; ex-smokers were classified as those who had stopped smoking for at least one year prior to collection (18). To evaluate alcohol consumption, individuals were classified as in Silveira *et al*. (8): non-consumer, defined as any alcohol consumption or social consumption; moderate consumer, defined as consuming up to 1 cup (about 100 ml) of alcohol per day, or more than a glass on weekends; high consumer, defined as the consumption of more than 1 l of light alcoholic beverage (beer, wine or cider), or 2 glasses of spirits (rum, vodka, or whiskey) per day, for at least 6 years. Table I shows the demographic data of the studied population.

Workplace conditions. Car painters usually works indoor, for at least 40 h per week. Most of the workers (90%) declared no use of personal protective equipment and that their work environment was not equipped with basic collective protective equipments as working cabins and air extractors.

Buccal micronucleus cytome assay (BMCyt). Both groups (exposed and control) were submitted to the same procedure for sample collection and preparation. Collection of the oral mucosa and the preparation of slides were performed according to the protocol described by Thomas *et al*. (1), with slight modifications already published by Silveira *et al*. (8) and Filho *et al*. (4). Briefly, buccal mucosa cells were collected by scraping the inner cheek using a sterille swab and placed in 3 ml of saline solution. Samples were centrifuged at 1000 rpm for 10 min and fixed using glacial acetic acid and methanol (1:3, v/v) solution, before centrifugation for additional 10 min, followed by refrigeration for 24 h in glacial acetic acid and methanol solution. Cells suspended in the fixative solution were dropped onto cold and clean slides and allowed to dry at room temperature. After 24 h, 5N HCl was added to the slides for 30 min. Distilled water was used for the washes. When totally dry, the slides were stained with Schiff´s reagent for 90 min and counterstained with 0.5% FastGreen for 3 min. One thousand cells were analyzed using an optical microscope and the number of pyknotic cells (PYC), karyolitic cells (KYL), karyorrhetic cells (KHC), condensed chromatin (CC), binucleated cells (BN), basal cells (BC), and differentiated cells (DIFF) were counted. Additional 2.000 cells, in two slides, were analyzed to count micronucleated cells (MNi) and nuclear buds. The images were captured on the 40X objective, in an Olympus Bx60® photomicroscope coupled to an Olympus DP71 camera, with the aid of the DP Controler 3.2.1 program 276.

Table II. *Mean and standard error of the results comparing the exposed group to the control group using 1000 cells# or 2000 cells##.*

Biomarkers	Exposed group (n=37)	Control group (n=37)	*p*-Value
Basal cells#	92.13±30.00*	66.35±25.32	≤0.001
Differentiated cells#	832.84±40.42*	895.18±42.89	≤0.001
Condensed Chromatin#	27.39±13.08*	11.40±4.95	≤0.001
Binucleated cells#	13.52±5.36*	7.81±3.85	≤0.001
Karyolitic#	13.86±5.69*	5.51±3.49	≤0.001
Pyknotic#	10.50±5.48*	5.37±2.25	≤0.001
Karyorrhetic#	7.44±4.57*	4.89±2.92	≤0.05
Micronuclei##	12.57±3.43*	3.70±2.14	≤0.001
Nuclear Buds##	13.13±5.00*	4.91±2.95	≤0.001

t-test used for statistics. *$p<0.05$.

Statistical analysis. Statistical analysis of the data was performed using a paired *t*-test, comparing the exposed group to the control group and considering a confidence interval at 95%.

Results

According to the demographic characteristics of the population no statistically significant differences were observed between the exposed and control groups (Table I), suggesting that paired statistical tests can be used. Furthermore, no significant

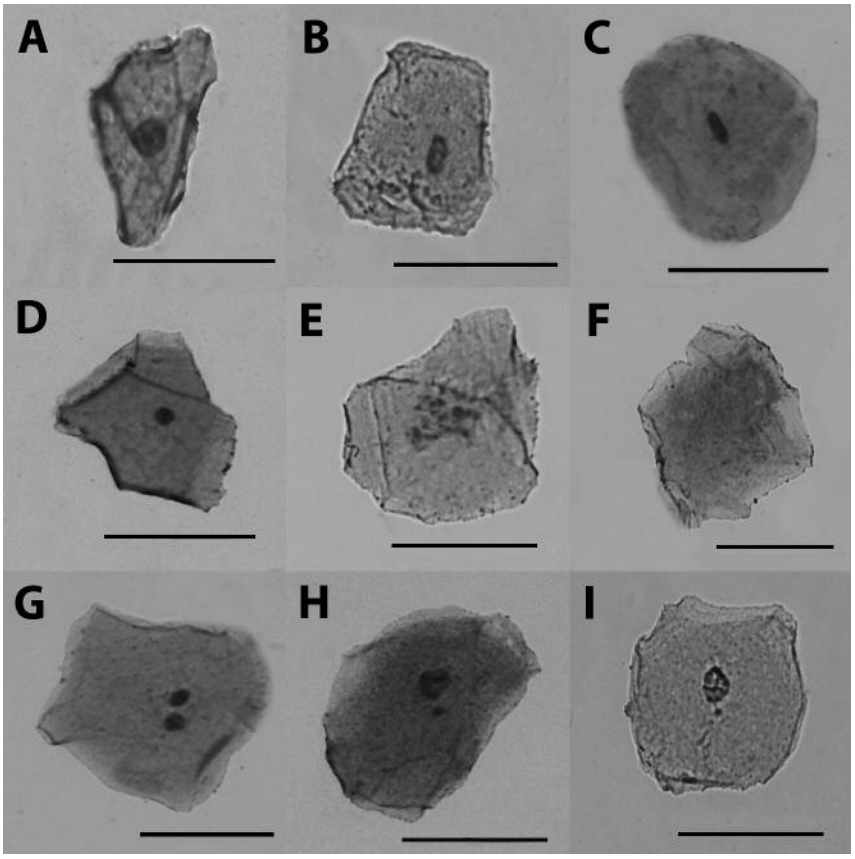

Figure 1. *Main cell types observed. (A) Basal cell. (B) Differentiated cell. (C) Condensed chromatin cell. (D) Pyknotic cell. (E) Karyorrhetic cell. (F) Karyolitic cell. (G) Binucleated cells. (H) Micronucleated cell. (I) Cell with nuclear bud.*

association of the BMCyt results with age, gender and alcohol consumption as confounding variables in the analysis were observed, arguing that our results can be extrapolated due to occupational exposure.

After evaluation of the structural profiling of buccal cells, an increase in all parameters, but DIFF, was observed in the exposed group ($p<0.05$; Table II). DIFF cells were decreased ($p<0.001$) in the exposed group compared to the control group (Table II). In summary, all parameters were statistically different in the exposed group compared to the control group, suggesting an extensive DNA damage and cellular changes due to occupational exposure as a car painter. Figure 1 presents some abnormalities evaluated in our study.

Discussion

The goal of our study was to profile car painters using the BMCyt test and evaluate cellular biomarkers for DNA damage, genomic (in)stability and cellular kinetics. The first two parameters analysed were BC and DIFF since both reflect the proliferative activity of buccal cells (1) and their alteration could argue for possible alterations in other parameters. Indeed, an increase in BC and a decrease in DIFF were the first evidence of cell cycle abnormalities observed in the exposed group ($p<0.001$; Table II) compared to the non-exposed group. Additionally, BN frequencies were significantly increased in the exposed group, another evidence of defects in cytokinesis (1). Silva *et al*. (19) have pointed out that increased frequencies of BN cells favor the appearance of DNA damage.

As follows, according to cell death (necrosis and apoptosis) parameters, the number of PYK, CC, KYL and KHC was significantly increased in the exposed group ($p<0.05$; Table II) compared to the control group. These parameters can be regarded as a response to cytotoxic compounds (20). Apoptosis induction is associated to significant DNA damage (21) and increased rates of cellular proliferation and apoptosis are observed in early stages of tumorigenesis (22). As expected, we observed a statistically significant increase in the frequencies of MNi and NBuds ($p<0.001$; Table II), two parameters of DNA damage and genome instability. NBuds represent nuclear mutations (20)

and a statistically increase in Nbuds and/or other nuclear abnormalities in oral cavity cells are associated with genotoxic and mutagenic exposure (23). Overall, our results pointed out to an extensive DNA damage and genome instability and alterations in cellular kinetics in the buccal cells of car painters occupationally exposed.

Particularly, Moro *et al*. (24) observed an increase in oxidative damage in car painters. Recently, Hoyos-Giraldo *et al*. (16) observed an association between years of occupational exposure as a painter and organic solvents levels in urothelial cells. As a matter of fact, some studies have already shown a positive association between car painting and DNA damage (25, 26), but as far as we know, our study is the first to evaluate the occupational exposure of car painters using a multimarker test as BMCyt, unravelling not only the DNA damage status, but also potential cellular phenotypes associated with genome instability and alterations in the cellular kinetics as early biomarkers for future disease and cancer risk.

Interestingly, Chang *et al*. (27) have observed that painters wearing protective masks were significantly less exposed to inhalation of xylene and ethyl benzene, but not to dermal absorption. In our study, 90% of the workers declared no use of any personal protective equipment. Furthermore, they declared that their work environment had no basic collective protective equipments or proper ventilation, which increases not only their exposure but also indirectly the exposure of others in the same environment, independently of their job. Thus, we can predict that our exposed group was exposed to different substances during a workday, even if they were not directly manipulating specific chemical mixtures.

Finally, it is disturbing to know that literature is full of reports associating painting with cancer, especially considering that >90% of all human cancers are of epithelial origin (16, 17, 28). Recently, Jung *et al*. (28) observed that Canadian painters occupationally exposed had high risk of lung cancer, corroborating the IARC classification.

Overall, we observed statistically significant differences in all parameters in our exposed group ($p<0.05$), summarized in accelerated cell proliferation, defects in cytokinesis, and increase in DNA damage and genome instability. Our study evaluated only cytologic markers, but all parameters could be used as early biomarkers for future disease risk assesement. Furthermore, the use of individual protective equipments is essential to prevent the increased risk, but most of the individuals in our study declared no use. Given the clear association with cancer, relative information should be provided for these individuals, and public policies should be updated, ranging from regular inspections, limited time of exposure and ideal work conditions, in order to guarantee a better quality of life and reduced risk.

Conflicts of Interest

The Authors declare that there is no conflict of interest regarding this study.

Authors' Contributions

APRF collected the sample, performed most of the methods and contributed on data analysis; MADS contributed to conceive the presented hypothesis, data analysis and wrote the manuscript; NRD collected the sample and contributed on methods and data analysis; LPGD conceived the presented hypothesis, supervised APRF and NRD on all procedures and wrote/corrected the manuscript. All Authors discussed the results and contributed to the final manuscript.

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