Cytogenetic Biomonitoring in Buccal Mucosa Cells from Seaport Dockers

KATYANE NEGREIROS CAMPOS, MARIA ESTHER SUAREZ ALPIRE and DANIEL ARAKI RIBEIRO

Department of Biosciences, Institute of Health and Society, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil

Abstract. Background/Aim: Dockers of seaport working as stevedores are self-employed workers who carry out arduous and dangerous activities. To date, few studies have investigated the human health risks in these professionals. The aim of this study was to evaluate cytogenetic damage in oral cells of dockers of seaports working as stevedores by micronucleus assay in buccal cells. Patients and Methods: For this study, a total of 26 seaport dockers working as stevedores aged 51.2±8.4 years (all men) were included in this study. All volunteers had worked for at least 3 years. The control group consisted of 25 participants aged 55.2±9.9 years (all men), who did not work in the Port of Santos city. Results: The results showed statistically significant differences (p<0.05) of micronucleated cells in buccal mucosa cells of seaport dockers. Pyknosis, karyolysis and karyorrhexis did not show statistically significant differences (p>0.05) between groups. Conclusion: The results of the present study suggest that seaport dockers present mutagenicity in oral cells.

Seaport dockers working as stevedores are self-employed workers who carry out arduous and dangerous activities (1). These professionals work on the deck and in the holds of ships, from loading and unloading cargo, tidying up in the hold and repair. Currently, stevedoring work is no longer predominantly manual work, involving itself in the organization of containers on decks and in holds of ships. In addition to manual and heavy loading and unloading operations on ships that dock at the port, these individuals are continually exposed to unfavorable environmental conditions, which expose them to different occupational hazards (2).

As highlighted by Soares et al. (3), the port work presents a series of risks to the worker. In line with the classification of the Brazilian Ministry of Health (4), the following risks are: i) physical (noise, machine vibrations, bad weather, extreme temperatures); ii) chemical (airborne and chemicals); iii) ergonomic/psychosocial (manual lifting of loads, work tools, lack of guidance and supervision regarding health, low number of team members, gain in productivity and work pace, discomfort in the use of personal protective equipment); iv) mechanical risk/accident (physical conditions at the port terminal, falling suspended objects, working at heights, machinery traffic, shifting over loads, electrical installations, stacking cargo, access stairs to vessels).

Stevedores are exposed to several chemical agents due to the frequent strong odors, and dust arising from the movement of cargo. Furthermore, there is a lack of information on the correct use of protective equipment. Also, it is noteworthy to mention that there is poor ventilation and confinement of workers when performing tasks inside the seaport wagons.

One possible way to ensure that the outcomes in a population exposed to adverse conditions is biomonitoring studies, using relevant biological parameters, such as the micronucleus assay, which can identify DNA damage from occupational exposure. The information can be used as an important tool for predicting long-term health problems, such as chronic degenerative diseases including cancer (5, 6).

Thus, the aim of this study was to evaluate cytogenetic damage in oral cells from seaport dockers working as stevedores.

Patients and Methods

Participants. For this study, a total of 26 seaport dockers working as stevedores aged 40.7±9.3 years (all men) were included in this study. The volunteers were selected in the Union of Port Workers of the Santos city, Brazil. All volunteers had worked for at least 3 years in the work as stevedores. For the control group, a total of 25
participants aged 41.6±6.2 years (all men), who did not work in the Port of Santos city were included in this setting. All individuals (control and experimental groups) were non-smokers. Furthermore, no volunteers were exposed to dental X-ray in the last month. No oral lesion was visible at clinical evaluation. However, alcohol consumption was not recorded in this setting. Demographic characteristics of the participants of the study are presented in Table I. The study was approved by the Ethics Committee of the Federal University of São Paulo, UNIFESP, number #3.939.407. Informed consent was signed by all individuals included in the study.

Micronucleus test on oral mucosal cells. The micronucleus test was made according to the guidelines described by Andrade et al. (7). For this purpose, exfoliated oral mucosa cells from all volunteers were obtained by scraping the right/left cheek mucosa using a moist wooden spatula. After that, oral cells were transferred to falcon tubes containing saline solution, being centrifuged (800 rpm) for 5 min, fixed in 3:1 methanol/acetic acid, and spread over cleaned glass slides. Finally, all slides were stained with the Feulgen/Fast Green method.

Data analysis. All slides were blindly evaluated using a light microscope at ×1,000 magnification to identify the presence of micronucleated cells and metanuclear alterations of cytotoxicity. Micronuclei were scored according to the criteria described by Belien et al. (8) as a parameter of DNA damage (mutagenicity). For cytotoxicity, the following nuclear alterations were considered: pyknosis, karyolysis, and karyorrhexis. Results were expressed as a percentage of total cells examined. This analysis was established in a previous study conducted by our research group being performed by one experienced observer (8). A total of 2000 cells were evaluated per volunteer.

Statistical methods. The student’s t-test was used to compare age, medicines, use of mouth rinses as well as the frequencies of micronucleus (mutagenicity) and cell death parameters (cytotoxicity) among the samples between the experimental and the control group (9). The statistical analysis was conducted using BioStat software, version 5.0 (Maringa, PR, Brazil). The level of statistical significance was set at 5%.

Table I. Demographic characteristics of the participants of the study.

<table>
<thead>
<tr>
<th>Parameters investigated</th>
<th>Control (n=26)</th>
<th>Dockers of seaport (n=25)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>51.2±8.4</td>
<td>55.2±9.9</td>
<td>p=0.4</td>
</tr>
<tr>
<td>Time of working</td>
<td>-</td>
<td>27.1±11.6</td>
<td>-</td>
</tr>
<tr>
<td>Medicines</td>
<td>3 (metformin)</td>
<td>6 (metformin, Angiotensin-II receptor antagonists)</td>
<td>p=0.1</td>
</tr>
<tr>
<td>Dental X-ray</td>
<td>2</td>
<td>3</td>
<td>p=0.1</td>
</tr>
<tr>
<td>Mouth rinse</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smoking</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Illicit drugs</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chemo- or Radiotherapy</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table II. Mean and SD (% of cells) of cytogenetic parameters (micronucleus, pyknosis, karrhyorexis and karyolysis) from seaport dockers working as stevedores.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Micronucleus</th>
<th>Pyknosis</th>
<th>Karrhyorexis</th>
<th>Karyolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>0.1±0.2</td>
<td>113.2±34.6</td>
<td>13.5±9.2</td>
<td>123.5±80.5</td>
</tr>
<tr>
<td>Dockers of seaport (n=11)</td>
<td>1.0±1.7*</td>
<td>98.5± 88.9</td>
<td>9.8±10.9</td>
<td>85.9±99.4</td>
</tr>
</tbody>
</table>

*p<0.05 when compared to control group.

Results

The results showed statistically significant differences (p<0.05) of micronucleated cells in buccal mucosa cells of seaport dockers working as stevedores (Figure 1). The results are shown in Table II.

When parameters closely related to cellular death were evaluated, no significant findings were detected. First, pyknosis was not altered in oral cells from seaport workers. In the same way, karyolysis and karrhyorexis did not show any significant differences (p>0.05) between groups. These findings are shown in Table II. Figure 2 shows normal cell, pyknosis, karrhyorexis and karyolysis.

Discussion

The aim of this study was to evaluate cytogenetic damage and cell death in buccal mucosa cells from seaport dockers working as stevedores, as predictors of chromosomal injury and cytotoxicity, respectively. The study was conducted using the micronucleus test in buccal cells. To the best of our knowledge, the approach has not been evaluated so far. This ratifies this study and others as well.

In recent decades, the micronucleus test has been successfully applied to assess the occupational risk in various scenarios and paradigms, such as in gas station attendants, farmers exposed to pesticides, hairdressers and children exposed to environmental pollutants, among others (10-14). The results of these studies have brought important data regarding the human health risks, justifying protective measures taken to mitigate them, and thus prevent possible harm to human health. Following this line of reasoning, it is recognized that seaport dockers working as stevedores are occupationally exposed to...
adverse conditions, given their daily activities in the port. For this reason, it would be interesting to know if, and to what extent, this important job for foreign trade is able to induce mutagenesis and/or cytotoxicity in oral cells *in vivo*. Our results pointed out high frequencies of micronucleated cells in buccal mucosa cells of seaport workers when compared to the control group. It is important to mention that micronuclei formation is supported by chromosome breakage or loss due to clastogenic/aneugenic effects. Therefore, the presence of this cytogenetic parameter in oral cells suggests mutagenicity as a result of genomic instability. Taking into consideration that 90% of malignant tumors originate from epithelial tissues (15), these results clearly demonstrate the risk that these professionals are continuously exposed to, such as dust and chemical agents, capable of inducing mutations in the oral mucosa. As a result, the consequences of this situation are alarming and warrant rigorous assessment in these professionals.

It is worth mentioning that Tolbert *et al.* (16) have revealed the presence of metanuclear changes suggesting the presence of cytotoxicity in buccal cells, such as karyorrhexis, pyknosis and karyolysis. This approach is valid and necessary because cytotoxicity interferes with the amount of micronuclei. If cytotoxicity is increased, the micronucleated cells are lost at the expense of cellular death. Particularly, this is clearly seen in the oral cells of chronic smokers (17, 18). This is the reason why all volunteers included in this study were non-smokers in order to avoid potential bias. Even so, it is important to emphasize that it was quite complicated to recruit volunteers in the experimental group due to the high rate of smokers, or even illicit drug users. As previously mentioned, an incidence of 30.5% for tobacco smoking, 16.5% for alcohol consumption and 9.1% for cannabis smoking was seen in seaport dockers (19). In any case, our results demonstrated no significant changes of karyorrhexis and karyolysis in the buccal mucosa from seaport workers. Furthermore, pyknosis did not show significant differences between groups as well. By comparison, some authors assumed that stevedores present abnormal electrocardiograms in ~50% of the volunteers evaluated (20). Nevertheless, it seems that these workers did not present increased cellular death in oral cells.

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Figure 1. *Micronucleated cell (arrow) (A and B)*. *Bar*=4 μm. *Feulgen-Fast-Green stain.*

Figure 2. *Normal cells (A); pyknosis (arrow) (B); karyorrhexis (arrow) and karyolysis (circle) (C)*. *Bar*=4 μm. *Feulgen-Fast-Green stain.*
In conclusion, the results of the present study suggest that seaport workers working as stevedores present mutagenicity in oral cells. Certainly, such data are relevant to protect these professionals against developing various types of chronic degenerative diseases, such as cancer.

Conflicts of Interest

All Authors declare that no conflicts of interest exist.

Authors’ Contributions

Conceptualization: KNC and DAR. Data search: KNC, and MESA. Formal analysis: KNC, MESA, and DAR. Writing - review & editing: KNC, MESA, and DAR.

Acknowledgements

DAR is a recipient of a CNPq grant (Conselho Nacional de Desenvolvimento Científico e Tecnologico, grant number #001).

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