Cytogenetic Biomonitoring in Buccal Mucosa Cells from Women Submitted to Chemotherapy After Mastectomy for Breast Cancer

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Abstract. *In addition to surgery, one of the most widely applied treatments for breast cancer is chemotherapy. Chemotherapy is currently considered efficient in curing this disease; however, the therapy may induce damage to the patient's genetic material. Thus, the aim of this study was to evaluate putative cytotoxic and mutagenic effects induced by chemotherapy in women diagnosed with breast cancer. For this purpose, a cross-sectional study was carried out in 42 women, aged 18 to 70 years, allocated according to the diagnosis and stage of breast cancer treatment: control group (healthy) (n=15), chemotherapy group (n=11) and post-chemotherapy group (n=16). Cytotoxicity and mutagenicity were analyzed by the micronucleus test in buccal mucosa cells. A higher frequency (p<0.05) of micronucleated cells was detected in the chemotherapy and post-chemotherapy groups when compared to the control. A higher frequency (p<0.05) of karyorrhexis and pyknosis in the chemotherapy group was also noted. Taken together, our results indicate that chemotherapy induces mutagenicity and cytotoxicity in buccal mucosa cells of women diagnosed with breast cancer, being persistent after finishing their treatment.*

Breast cancer is the leading cause of death among women worldwide, both in developed or in developing countries. In Brazil, it has been estimated that there will be 52,000 new cases in 2016 (1). Recent epidemiological studies indicate that lifestyle as well as environmental factors play a crucial role in the etiology of the disease (2). Risk factors for breast cancer include early age of menarche, late menopause, contraceptive use, hormonal replacement therapy, obesity, environmental pollutants, smoking and alcohol use (3). It is accepted that the initiation of breast cancer is characterized by alterations of expression of cell cycle-regulatory proteins caused by mutagenic agents and carcinogens.

The micronucleus test is well recognized as a reliable biomarker of chromosomal damage, being widely applied in genetic toxicology for human biomonitoring studies (4). Nowadays, the micronucleus test is one of the gold-standard assays able to evaluate with accuracy either *in vitro* or *in vivo* for regulatory purposes and for risk assessment of chemicals (5, 6). The test is very successful in monitoring human populations exposed to putative genotoxic compounds (7). Recent prospective studies have demonstrated higher frequencies of micronuclei in eukaryotic cells in association with increased risk of cancer. This provides scientific evidence that this biomarker could be predictive of cancer risk after therapy for cancer such as radio- or chemotherapy (8, 9). Micronuclei are defined as acentric fragments or whole chromosomes that are not included in the main nuclei of the daughter cells. Micronuclei are induced by substances that cause chromosomal breakage (clastogens), or disruption of the spindle apparatus (aneugens) (10). According to Tolbert *et al.*, the specificity of the test to detect chromosomal damage is improved by analyzing other degenerative nuclear alterations indicative of cell death (11). Among them, pyknosis, karyolysis and karyorrhexis are suitable for this purpose.

Nowadays, the treatment of breast cancer includes surgery, radiotherapy and chemotherapy. Anti-neoplastic drugs are used in the treatment of cancer worldwide. Nevertheless, mutagenicity of such anti-neoplastic drugs has been detected in short-term assays reflected in several biological endpoints. In fact, anti-neoplastic drugs have demonstrated genotoxicity using *in vitro* test systems (12, 13). *In vivo*, mutagenic outcomes have been found in patients with cancer treated with anti-neoplastic drugs (14, 15).

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Many published data have shown the application of the micronucleus assay in untreated women with breast cancer (16, 17). These demonstrated an increase of micronucleus frequency in patients compared with control groups, with a large variability among the tumors and studies (18-21). However, studies investigating the outcomes induced by chemotherapy in patients after mastectomy for breast cancer are fairly limited in the literature.

As a result and because of the lack of scientific evidence, the aim of this study was to investigate mutagenicity and cytotoxicity in buccal mucosa cells induced by chemotherapy in patients after mastectomy for breast cancer.

Materials and Methods

Participants. The subjects of this study consisted of 42 women provided from Neo Mama Institute (Santos City, SP, Brazil), aged 18 to 70 years, allocated according to the diagnosis and stage of breast cancer treatment: control group (healthy) (n=15), chemotherapy group (n=11) and post-chemotherapy group (n=16). All volunteers included in chemotherapy were submitted to mastectomy and the post-chemotherapy group had finished the therapy up to 2 years earlier. None of the participants were smokers. The study was approved by the Human Ethics Committee of the Federal University of Sao Paulo (no. 887029). Informed consent was obtained from all participants. All patients with breast cancer were diagnosed with invasive ductal carcinoma II or III (22).

Micronucleus test in oral mucosa cells. After rinsing their mouth with tap water, cells were obtained by scraping the right/left cheek mucosa of each participant with a moist wooden spatula. The cells were transferred to a tube containing saline solution, centrifuged at 180 × *g* for 5 min, fixed in 3:1 methanol/acetic acid, and placed on pre-cleaned slides. Later, the air-dried slides were stained using the Feulgen/Fast-Green method, and examined under a light microscope at ×1000 magnification to determine the frequency of micronucleated cells. Two thousand cells were scored from each participant.

Data analysis. Micronuclei were scored according to the criteria described by Belien *et al*. (10) 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. The analysis was evaluated independently by two biomedical doctors in a blinded fashion.

Statistical methods. The Mann–Whitney non-parametric test was used to compare the frequencies of cytotoxicity among the samples between experimental and control groups. Micronuclei were evaluated as established by Pereira *et al*. (23). The statistical analysis was conducted using BioStat software, version 5.0 (Maringa, PR, Brazil). The level of statistical significance was set at 5%.

Results

Table I shows the frequencies of micronucleated cells in buccal mucosa cells of women submitted to chemotherapy for breast cancer. A higher frequency ($p<0.05$) of micronuclei was obtained for the chemotherapy group compared with the matched controls. Furthermore, significantly statistical differences ($p<0.05$) were detected between post-chemotherapy and control groups.

When cytotoxicity parameters were evaluated, interesting findings were made. Chemotherapy induced cytotoxicity in buccal mucosa cells from women diagnosed with breast cancer since significant statistically differences ($p<0.05$) were found for karyorrhexis, pyknosis. Karyolysis was not remarkable different between groups ($p>0.05$). After chemotherapy, no significant statistically differences ($p>0.05$) were found between groups. Such findings are shown in Table II.

Finally, exposure to known genotoxins was not investigated since all participants were non-smokers. Daily alcohol consumption was not considered in this study because recall bias phenomenon had occurred.

Discussion

The aim of this study was to evaluate chromosome damage and cellular death induced by chemotherapy comparatively in women with breast cancer submitted to chemotherapy as indicators of mutagenicity and cytotoxicity, respectively. The investigation was conducted using the micronucleus test in oral exfoliated cells. To the best of our knowledge, this approach has not been taken before.

The micronucleus assay is widely recognized by scientific literature as an easy, cheap and reliable technique for cytogenetic biomonitoring (10). Micronuclei contain genetic material that is lost from whole DNA during mitosis, as a result of clastogenic or aneugenic events (10). Biological events that lead to the formation of micronuclei take place in the basal layer of the epithelial tissue, where cells undergo mitosis. Programmed turnover of epithelial tissues brings these cells to the surface where they exfoliate and, therefore, it is possible to detect them (10).

Genomic damage plays a pivotal role during carcinogenesis. It has been well established that genomic damage is produced by environmental exposure to mutagens and carcinogens, as well as to genetic factors such as defects in xenobiotic metabolism and DNA-repair deficiency (24). Micronuclei frequencies predict genomic instability (25). The detection of an elevated frequency of micronuclei in a given population indicates an increased risk of cancer (26). However, cell types that repair DNA damage efficiently are likely to have lower levels of residual damage than cells less proficient in DNA repair (27). Buccal cells have been shown to have limited DNA-repair capacity relative to peripheral blood lymphocytes, and therefore may more accurately reflect genomic instability events in epithelial tissues (10).

Nowadays, chemotherapy plays a key role in the treatment of many tumor types, improving the prognosis of patients

Table I. *Frequency of micronucleated cells in buccal mucosa cells of women submitted to chemotherapy for breast cancer.*

Group	Frequency of micronuclei
Control (n=15)	0.07±0.01
Chemotherapy (n=11)	0.36±0.1*
Post-chemotherapy (n=16)	0.38±0.1*

Data are the mean±S.D. *$p<0.05$ Compared to the control group.

Table II. *Cytotoxicity parameters (karrhyorexis, pyknosis and karyolysis) in buccal mucosa cells of women submitted to chemotherapy for breast cancer.*

Group	Pyknosis	Karyorrhexis	Karyolysis
Control (n=15)	16.40±7.78	0.0±0.0	33.13±45.96
Chemotherapy (n=11)	40.45±16.26*	1.09±0.71*	31.09±6.36
Post-chemotherapy (n=16)	48.31±56.57	0.25±1.41	24.91±7.78

Data are the mean±S.D. *$p<0.05$ Compared to control group.

with cancer (3). However, it is well known that some cytostatic drugs can induce DNA damage in both somatic and germ cells, either increasing the incidence of a second malignancy (2) or the incidence of primary tumor in the offspring, when administered in the reproductive stage (28). The age of an individual, their genetic predisposition, as well as the nature of the treatment, have been assumed to be causative factors for development of a second tumor (29). Therefore, the elucidation of the mutagenicity and cytotoxicity induced by chemotherapy is relevant in order to determine the degree of cancer risk, as well as to mitigate further neoplasms induced by therapy. It is important to stress that the goal of this study was mainly to evaluate chromosomal damage induced by chemotherapy rather than measuring genomic instability in patients with cancer.

Our results demonstrated an increase of micronuclei in buccal cells from women undergoing chemotherapy. The same occurred after chemotherapy, *i.e.* the frequency of micronucleated cells was increased when compared to controls. By comparison, some authors have demonstrated genotoxicity in patients with breast cancer who were submitted to radiotherapy, specific patterns of DNA damage being observed in the majority of patients after prolonged exposure to ionizing radiation as a result of adaptive response (30). Radio- and chemotherapy significantly increased the frequency of micronuclei in patients' lymphocytes, but without significant differences in the frequency of therapy-induced micronuclei between groups (31). Others demonstrated no difference in the DNA-repair capacity of lymphocytes from patients with breast cancer and healthy women after etoposide-induced DNA damage (32). Taken as a whole, our data emphasize the well-known influence of chemotherapy on oral mucosal cells. In particular; we assumed that cytogenetic damage in buccal mucosal cells persists after finishing chemotherapy.

To monitor cytotoxic effects, the frequencies of karyorrhexis, karyolysis and pyknosis were included in our experimental design. Our results showed that chemotherapy increased the frequencies of pyknosis and karyorrhexis. Post-chemotherapy, cytotoxicity had not been induced as depicted by the lack of statistically significant differences ($p>0.05$) between groups. Some authors have argued that chemotherapy is able to induce cellular death *in vitro* and *in vivo* (33). However, the cytotoxic insult does not accumulate after finishing the therapy. This issue requires further investigation.

In conclusion, the results of the present study suggest that chemotherapy induces cytogenetic damage in women diagnosed with breast cancer. Cytogenetic damage found herein reflects a certain degree of genomic instability, reinforcing the need for evaluating the side-effects induced by chemotherapy on health, as well as contributing to the micronucleus database for understanding and improving this methodology. Overall, these patients should be examined regularly in order to detect and prevent second tumors developing.

Conflicts of Interest

None declared.

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