Micronucleus Score in the Buccal Mucosa of Women with Breast Cancer and the Relationship to Chemotherapy

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ABSTRACT—
Background: Breast cancer is the most common type of cancer among women so many studies have be carried out about breast cancer chemotherapy. It is possible that breast cancer patients possess elevated chromosomal brekage/loss and MN formation in epithelial cells of the buccal smear and also the MN numbers may be increased in epithelial cells after chemotherapy in breast cancer patients. The objective of this study was to evaluate the MN scores in buccal smear compared to healthy women and women with breast cancer before and after chemotherapy.

Materials and methods: This study included 32 healthy women and 24 breast cancer patients. The buccal smears of breast cancer patients were taken three times: before chemotherapy, and after two and four cycles of chemotherapy. Buccal smears of the control group and patients were taken, and 1000 epithelial cells were counted per patient, with the criteria of The Human Micronucleus Project for the assay of micronucleated cells.

Results: The MN scores of breast cancer patients before chemotherapy, after 2 cycles chemotherapy and after 4 cycles chemotherapy were statistically significantly higher than the MN scores of the healthy control group ($p<0.001$).

Conclusion: The MN scores were observed higher in breast cancer patients than in healthy individuals and also it increased with the application of chemotherapy. These results and our findings propose that the MN assay of buccal mucosa samples can be used to determine the genotoxic effect of chemotherapy.

Keywords— *Breast Cancer; Micronucleus; Buccal Mucosa; Chemotherapy.*

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1. INTRODUCTION

Breast cancer is the most common type of cancer among women so many studies have be carried out about breast cancer chemotherapy. It is possible that breast cancer patients possess elevated chromosomal brakeage/loss and Micronucleus (MN) formation in epithelial cells of the buccal smear [1] and also the MN numbers may be increased in epithelial cells after chemotherapy in breast cancer patients.

Micronucleus (MN) is extranuclear body surrounded with an isolated membrane in cytoplasm that is formed at anaphase during cells cycle from lagging whole chromosome or chromosome fragments [2]. Therefore, MN can be measured chromosomal brakeage/loss and also indicate chromosome damage using standard metaphase chromosome analysis or MN assay [3]. A major benefit of the MN assay is more simple method and statistical power including scoring greater number of cells than classical metaphase analysis. The MN assay can be used as a method to monitor chromosomal damage of buccal mucosa cells [3]. In addition, the MN assay has been utilized as a biomarker for in vitro and in vivo studies, such as occupational exposure, genotoxicology and cancer [2]. In breast cancer cases may increase the number of cells with MN [1]. The number of MN can be easily evaluated from rapidly dividing epithelial cells of the buccal mucosa treated with genotoxic agents [4].

The application of chemotherapy can be the reason for chromosome loss and chromosome breakage and increase of MN numbers. Chemotherapy can affect the MN numbers in cancer patients [4]. In addition, the genotoxic effect of chemotherapy is associated with MN numbers of buccal mucosa [4]. The other reasons for the induction of MN containing cells are exposure to organic solvents, polycyclic aromatic hydrocarbons, and diesel derivatives, carcinogenic compounds in tobacco, betel nuts, alcohol, solvents, lead-containing paints, and arsenic contaminated drinking water [5-7].

The objectives of this study was compare the MN scores in buccal mucosa of healthy women, women with breast cancer before and after chemotherapy by using MN assay, and also compare the results to reveal the statistical difference among them to determine the genotoxic effects of chemotherapy.

2. MATERIALS AND METHODS

This study included 32 healthy women (control group) and 24 breast cancer patients, in whom the disease was diagnosed by an incisional biopsy and pathological examination. In the control group, persons with a history of radiation therapy, cancer, recent viral infections, or used alcohol or had been on medication were excluded from the study.

The buccal smears of breast cancer patients were taken three times: before chemotherapy, and after two and four cycles of anthracycline-containing chemotherapy (AC chemotherapy protocol; Adriamycin 60 mg/m² and cyclophosphamide 600 mg/m², repeated every three weeks). Samples were prepared and stained according to technique proposed previously [8]. Buccal smears of the control group and patients were taken after rinsing their mouth with tap water. A wooden spatula was used to obtain the buccal smear. The scraped exfoliated cells from buccal mucosa were placed on a clean glass slide and smears were prepared. The smears on the glass slide were fixed with 80% methanol. After fixation of the buccal smears, a basic dye, pararosaniline, was used to stain the cell nucleus and MN. Fast green contrast stain was used to determine the cell cytoplasm border. After the smears dried, 1000 epithelial cells were counted per patient from each slide, with the criteria of The Human Micronucleus Project (HUMN) for the assay of the micronucleated cells [3, 9]. Epithelial cells were counted by using fluorescence microscope with two independent observers. The MN scores were defined per 1000 cells. Singly and separately staining cells were preferred for the scoring of the cells. Normal buccal mucosa epithelial cells and MN containing buccal mucosa epithelial cells are shown in Figures 1A and 1B.

Statistical analyses were conducted with SPSS for Windows statistical package, version 11.0, and values of $p \leq 0.05$ were considered statistically significant. The appropriate distribution of numerical parameters was tested with the Kolmogorov-Smirnov test. Spearman's and Pearson's correlation analyses were used to evaluate the correlation between parameters. The independent samples t-test was used to test the differences between the two independent groups. Wilcoxon signed-rank test was used to test the differences between the two dependent groups. The data of the obtained MN were pooled together into their different groups and were expressed as mean frequency per 1000 cells along with the standard error of count means (mean±standard deviation).

This study received approval from the ethics committee and followed the principles of the Declaration of Helsinki.

3. RESULTS

Demographic characteristics of the patients and the control group are shown in Table 1A. The mean score of MN for the control group was 4.38±2.71 (1-10). For breast cancer patients, the mean score of MN in the buccal smear before chemotherapy, and after two and four cycles of anthracycline-containing chemotherapy were 14.8±11.94 (4-39), 26.15±12.5 (11-55), and 20.45±11.9 (6-47), respectively (Table 1B, Figure 1C). In the healthy control group, there was no relationship between age and MN score (p: 0.96). In the breast cancer patient group there was no significant correlation between MN score and age (p: 0.842), estrogen receptor status (p: 0.608), progesterone receptor status (p: 0.404), and c-erbB2 status (p: 0.298).

The MN scores of breast cancer patients before chemotherapy, after two cycles chemotherapy and after four cycles chemotherapy were statistically significantly higher than the MN scores of he healthy control group ($p<0.001$). In the breast cancer patient group, the difference between MN scores before chemotherapy and after two cycles of chemotherapy was statistically significant (p: 0.019). However, there was no statistically meaningful difference between MN scores before chemotherapy and after four cycles of chemotherapy (p: 0.068), and between the MN scores after two and four cycles of chemotherapy (p: 0.842) (Table 1B).

4. DISCUSSION

MN studies have revealed an association between MN frequency and cancer [1, 10-11], In some of these studies, MN frequencies of untreated cancer patients compared with MN frequencies of healthy subjects, and significantly increased MN frequencies have been found in untreated cancer patients [10-13]. MN frequency values were analyzed in extra-cancerous tissues, such as lymphocytes, buccal mucosa cells, and exfoliated bladder cells [12].

This study investigated the MN scores of healthy women, breast cancer petients before and after chemotherapy, and the effects of chemotherapy on MN frequencies. Like other studies performed on cancer patients (stomach, lung, and colon) [11], we determined a significant increase in the MN scores of breast cancer patients compared to healthy subjects.

In our study, the mean MN scores in the buccal smear of the healthy control group and breast cancer patients before chemotherapy were 4.38±2.71 and 14.8±11.94 per 1000 cells, respectively. Bansal et al. established the mean numbers of MN in healthy control groups' buccal mucosa cells as 4.17±2.99 per 1000 cells [5]. In another study from Turkey, the mean MN score in buccal mucosa cells of a healthy control group was 0.84±0.22 per 1500-3500 cells [14]. In a study conducted by Dey et al., the mean MN scores in buccal smears of benign and breast carcinoma groups were 0.5014±0.45768 and 2.1938±1.08656 per 1000 cells, respectively, and there was a statistically significant difference between two groups ($P < 0.001$) [1]. These different results may be due to age, different ethnicity, or unknown exposure to toxic agents.

The higher MN levels found in the patient group could be explained by the endogenous and exogenous factors that play role in cancer formation. Hormonal effects can be classified as an endogenous factor in MN formation. In a study carried out on infertile couples revealed increased MN frequencies [15]. Another study carried out on hirsutismus patients reported a high MN frequency compared to healthy subjects [16]. Both infertility and hirsutismus hormonal effects are the causes of the diseases, and hirsutismus patients are prone to develop cancer at any point in their lives [16].

The genotoxic effects of exogens have been studied using MN assay for occupational exposure. In a study carried out on nurses that administer drugs to patients revealed a higher MN frequency compared to the control group [17]. These findings and our results indicate the roles of endogen and exogen factors in the formation and development of cancer, and also the usefulness of MN assay in the genotoxic effects of these factors.

After determining the basal MN levels of the patient group, MN frequencies after chemotherapy were determined and compared to the basal MN level. The MN score after the second cycle was found higher than the basal level, and there was a statistically significant difference between both values ($p<0.001$). Interestingly, the MN score obtained after the fourth cycle of chemotherapy was lower than the second cycle. This could be explained by the different acting mechanisms of anthracyclines, such as topoisomerase inhibition, free radical production.

Antineoplastic drugs act on DNA and on other cellular mechanisms to induce the cells to apoptosis [18]. Anthracyclines are a group of antineoplastic drugs that lead to DNA double-strand breaks by intercalating DNA [19] . Additionally, they enhance the catalysis of oxidation-reduction reactions and promote the generation of oxygen free radicals [19] , which may be involved in genotoxicity. The effects of anthracyclines on the body can be monitored by MN assay.

In a study performed with chemotherapy regimens demonstrated increased MN frequency after the application of ifosfamide and epirubicin-containing chemotherapy regimens [4]. In the same study, there was no increase in MN scores after the use of cisplatin plus 5-Flourouracil and carboplatin plus 5-Flourouracil-containing chemotherapy protocols. This study also showed the MN frequency changes by epirubicin dosage. In this study, mean MN scores were determined to be 2.9 after 50 mg of epirubicin administration and 3.5 after 80 mg of epirubicin administration [4].

In conclusion, the MN score of the buccal mucosa is higher in breast cancer patients than healthy persons and also it increased with the application of chemotherapy. Micronucleus assay can be used for the evaluation of cancer treatment strategies and effects of the exposure to genotoxic agents. The importance of using the MN score in exfoliated epithelial cells from the buccal mucosa is understood in the fact that 90% of cancers originate from epithelial cells, and the tissues that are the source of exfoliated epithelial cells are the main targets of the genotoxic agents [10, 20].

Finally, these results and our findings propose that MN assay of buccal mucosa samples can be used to determine the genotoxic effect of chemotherapy.

Table 1A: The demographic characteristics of breast cancer patients and control group.

	Breast cancer patient group (n=24)	Healthy control group (n=32)
Age (year)	46,21±10.43 (27-67)	41.5±16.47 (22-78)
Estrogen receptor	Positive: 17/22 (77%) Negative: 5/22 (23%)	
Progesterone receptor	Positive: 14/22 (64%) Negative: 8/22 (36%)	
c-erb B2	Positive: 5/22 (23%) Negative: 17/22 (77%)	
Smoking	Yes: 2/24 (8%) No: 22/24 (92%)	
Disease stage	Non-metastatic: 21/24 (87.5%) Metastatic stage: 3/24 (12.5%)	

Table 1B: The MN score in buccal smear for breast cancer patients and control group.

	Micronucleus Score of Breast cancer patient group *(mean±standard deviation)*	**Micronucleus Score of Healthy control group** *(mean±standard deviation)*	p	r
A: Beginning	14.8±11.94	4.38±2.71	p< *0.001*	0.588
B: After 2 cycles of anthracyclin containing chemotherapy	26.15±12.5			
C: After 4 cycles of anthracyclin containing chemotherapy	20.45±11.9			

Footnote: A vs **B**; p: *0.019* **A** vs **C**; p: 0.068 **B** vs **C**; p: 0.842

Figure 1A: Normal buccal mucosa epithelial cell.

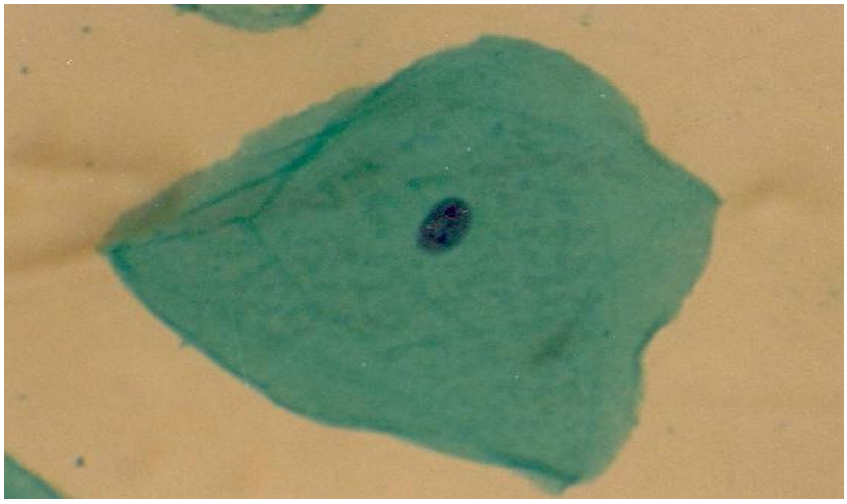


Figure 1B: Micronucleus containing buccal mucosa epithelial cell.

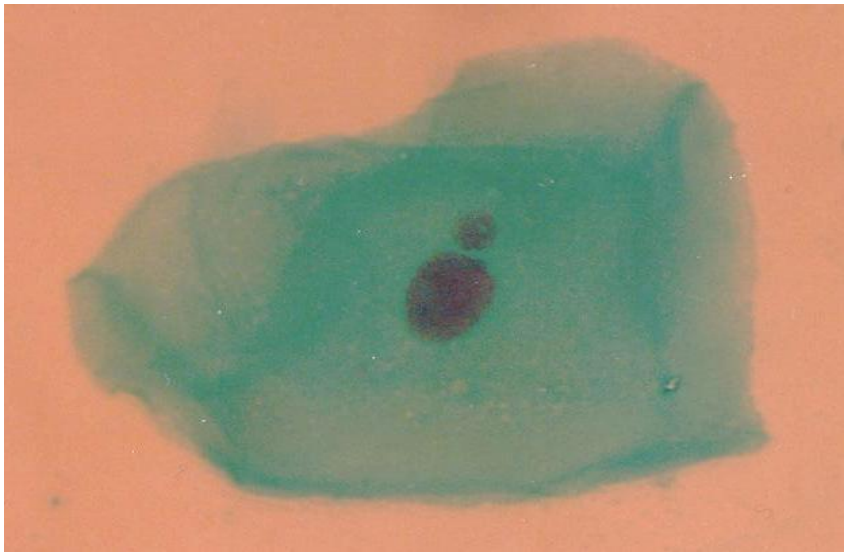
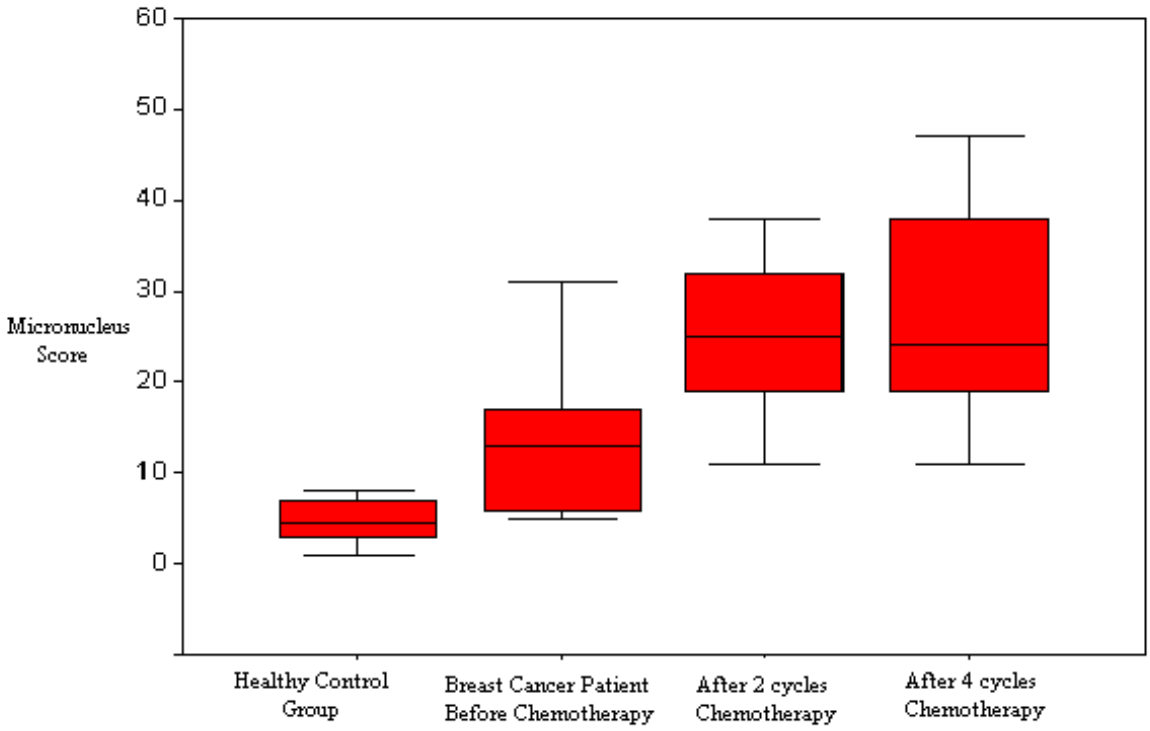


Figure 1C: Micronucleus scores.



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