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Micronuclei and Other Nuclear Anomalies in Exfoliated Buccal Mucosa Cells in Breast Cancer

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Abstract
Objectives: This study designed to assess the genomic instability between healthy women and women with breast cancer by means of buccal cells micronucleus (MN) cytome assay.
Methods: The current study comprised 25 healthy women and 30 breast cancer patients. The exfoliated cells of buccal mucosa were taken after each participants rinse their mouths with tap water. The micronucleated cell and nuclear anomalies were analyzed under a total magnification of X1000, 2000 cells per subjects (patient and control group) were scored and the frequencies of nuclear anomalies including MN, binucleates (BN), Pycnotic cell, karyolysis (KL) and karyorrhexis (KR) were evaluated in exfoliated buccal mucosa cells of women with primary BC and healthy women.
Results: The frequencies of micronuclei and all nuclear anomalies in buccal cells of BC patients were significantly increased compared with the controls. (For Binucleates cells only, $P < 0.001$; in all other cases, $P < 0.0001$). The mean scores of micronuclei and all nuclear anomalies for the breast cancer patients were (10.66 ± 0.3845, 6.20 ± 0.26, 8.40 ± 0.22, 18.40 ± 0.34, 19.13 ± 0.40) were significantly higher than that of healthy women).
Conclusion: Elevated frequency of micronucleated cells and all nuclear anomalies in the buccal mucosa of breast cancer patients reveal the genomic instability. These findings propose that the buccal MN-cytome assay can be used to measure both genotoxic and cytotoxic effects in primary cancer patients.
Keywords: Breast cancer, buccal micronucleus cytome assay, genotoxicity, cytotoxicity

Introduction

Breast cancer is one of the most mutual cancers in women worldwide, Over 1.5 million women (25% of all women with cancer) are diagnosed with breast cancer every year throughout the world.[1,2] Breast cancer is a metastatic cancer and can usually relocation to distant organs such as the bone, lung, liver and brain, which mainly accounts for its incurability. Early diagnosis of the disease can lead to a good prognosis and a high survival rate.[3] There're numerous peril factors such as sex, family history, estrogen, aging, gene mutations and insalubrious lifestyle, which can increase the opportunity of evolving breast cancer.[4] Breast cancer commonly occurs in women and the number of states is 100 times greater in women than that in men.[5]

Formation of the MN in dividing cells is the product of chromosome fracture due to unrepaired or chromosome malsegregation or misrepaired DNA lesions as a result of mitotic malfunction. These incidents may be produced through oxidative stress, contact to aneugens, clastogens or, genetic defects in cell cycle checkpoint and/or DNA mending genes, and deficiencies in nutrients required as cofactors in chromosome segregation machinery and DNA metabolism.[6-10] All these incidents contribute in the generation of MN through changed gene expression or aneuploidy, chromosomal rearrangements, impacts associated with the instability chromosome oftentimes observed in cancer.[7,11]

The chromosome aberration assay notices only the genome damage, whereas micronucleus assay also detects malfunction of mitotic spindle or chromosome loss initiated by aneugenic mechanisms.[12] There is a hypothesis that micronuclei and chromosomal aberrations could have a prophetic value for cancer and hence substitute chromosomal aberrations as cancer peril biomarkers or provide further evidence on the mechanism action of aneugenic agents.[13]

A minimally invasive and possibly useful method for monitoring genetic damage in humans is the MN assay in exfoliated buccal mucosa cells.[14,15] Furthermore to micronuclei (MNi), other nuclear anomalies, repercussion of both genotoxic and cytotoxic effects, can also be monitored with this assay.[14] It has been alleged this procedure may be a dependable method for the revealing of human cancer risk as most tumors are of epithelial origin.[16] Excluding MNi, also other nuclear anomalies such as BN, phenomenon, condensed chromatin (CC), pyknotic nuclei (P), karyorrhexis (KR), and karyolysis (KL) can be scored in buccal cells.[14,17] MNi and, broken eggs (BE) phenomenon are considered as genotoxic events, BN as a spindle disturbance (aneugenic effects), and condensed chromatin CC, KR, KL and pyknosis as acute cytotoxic effects.[8,11] Bonassi et al.[18] investigated all the data concerning MN assay in buccal cells of cancer patients and assumed that a diagnosis of cancer significantly increased MN and other endpoints frequencies. Especially high association was found for respiratory system cancers, oro-pharyngeal cancers, and for all the other cancers pooled together.

The aim of the present study was to evaluate the frequencies of micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of kurdistan women

Materials and Methods

The study group consisted of 30 females with diagnosed breast cancer (Cancerous group) from Nanakaly Hospital at the Erbil city, Kurdistan region in the age ranged between 33 years to 70 years for both healthy and breast cancer patients. All the patients were scored in the study before getting the first course

Table 1. **Micronuclei level and other nuclear anomalies in buccal mucosa cells of breast cancer patients and healthy women**

Parameter /2000 cell	Breast cancer patients (mean ± S.E)	Healthy women (mean ± S.E)
Micronucleated cells	10.66 ± 0.3845***	5.47 ± 0.33
Binucleates	6.20 ± 0.26**	3.88 ± 0.23
Pycnotic cell	8.40 ± 0.22***	4.47 ± 0.28
Karyolysis	18.40 ± 0.34***	5.52 ± 0.37
Karyorrhexis	19.13 ± 0.40***	6.41 ± 0.297

**$P < 0.001$ and *** $P < 0.0001$compared with healthy women (control), *t*-test groups.

of chemotherapy. The control group consisted of 25 healthy subjects (Non-cancerous group).

All individuals were interviewed to obtain personal information and were asked to complete a questionnaire concerning smoking habits, alcohol and coffee consumption, health status, age, consumption of drugs, contraceptive or antioxidants, and hereditary disease.

The exfoliated cells of the buccal mucosa were obtained by scraping the buccal mucosa with a wooden spatula after each participants rinse their mouths with tap water. For each individual, two slides were prepared by smearing the cells directly onto the center of clean glass slides. After smearing the sample to a glass slide and drying it in the air, fixation was achieved with methanol (15 minutes). Afterwards, the glass slide was dried again then stained with Giemsa stain (20 minutes). The glass slide was rinsed with tap water and dried in the air.[19] The micronucleated cell and nuclear anomalies were analyzed under a total magnification of X1000. MNi and other nuclear anomalies (BN, P, KL, and KR) were scored according to the criteria designated by Thomas et al. and Bolognesi et al.[20,21] The number of cells with MNi and nuclear anomalies other than MNi, namely BN, P, KL, and KR were evaluated in 2000 cells.

Statistical Analysis

All the data are expressed as the mean ± standard error of the mean. The data were analyzed statistically using the student's *t*-tests for independent samples. *P*-values less than 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS statistical software program (version 15.00).

Results

The results of the present study showed that the frequencies of micronuclei and other nuclear anomalies in buccal cells of BC patients were significantly increased compared with the controls. (for Binucleates cells only, $P < 0.001$; in all other cases, $P < 0.0001$ the anomalies related with genotoxicity. The mean score of micronuclei for the breast cancer patients was (10.66 ± 0.3845) it was higher than that of healthy women (Table 1).

Discussion

Increase in the frequency of cells with micronuclei ($P < 0.0001$), in the breast cancer patients, representing possible changes in the efficiency of DNA repair and increased genomic instability in exfoliated cells reflect recent genotoxic events that arose in the dividing basal cell.[22] The result of the present work is in covenant with the results of the previous research regarding elevation micronuclei level in buccal cells of breast cancer patients, Kalender et al.[23] reported increased micronuclei frequency in 24 breast cancer patients.

Ban et al.[24] found increased MNi frequencies in lymphocytes of 136 BC patients compared with 48 healthy women. Aristei et al.[25] also found increased number of lymphocytes with MNi in 20 BC patients with stage I and II compared with 12 healthy women. In patients the mean of sister chromatid exchanges (SCE) were also significantly increased. Varga et al.[26] also found a highly significant between frequencies of MNi in BC patients from Germany ($N = 91$) and healthy women ($N = 96$). Milosević-Djordjević et al.[27] reported that not only in BC patients but also in patients with other tumor localizations MNi levels were increased regardless of site.

Furthermore to increased micronuclei scoring in breast cancer patients the current work found elevated level of other nuclear anomalies including binucleated, broken eggs (BE), condensed chromatin (CC) karyolysis cells and Karyorrhexis (reflecting both genotoxicity and cytotoxicity), Comparable results have also been demonstrated in Mexico study carried out on 21 breast cancer patients.[28]

Our results elucidate that the increased MN and other nuclear anomalies frequencies in exfoliated cells of the buccal mucosa of patients with BC may reflect genomic instability or by mutated BC susceptibility genes BRCA1 and BRCA2. BRCA proteins have many critical functions, the most notable of which is repair of double-strand DNA breaks.[29] Defective DNA repair leads to genetic instability which appears in the elevation of MNi in somatic (epithelial) cells.

Hence, the result of the present work confirmed the finding of previous case control studies concerning increased micronuclei frequency and other nuclear anomalies in primary cancer patients.[30,31]

Conclusion

Increased frequency of micronuclei and other abnormalities in the buccal mucosa of breast cancer patients (reflecting genotoxicity and cytotoxicity) reflects genetic damage and /or defect in DNA repair system in normal somatic cell of cancer patients. The results also show that the MN cytome assay may be useful in studying genetic instability in primary cancer patients.

Conflicts of Interest

None.

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