Iraqi Journal of Biotechnology, 2017, Vol. 16, No.1 , 10-15

Evaluation of Micronuclei and Other Nuclear Anomalies in Buccal Cells of some Iraqi Women with Breast Cancer

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Received: November 6, 2016 / **Accepted**: November 22, 2016

Abstract: The present study was designed to evaluate the frequencies of micronuclei and other nuclear anomalies in buccal mucosa cells of Iraqi women with breast cancer. This case –control study included 20 primary diagnosis breast cancer patients. They were recruited at The Oncology Teaching Hospital, Baghdad, Iraq. From January to February 2016. As control 20 apparently healthy women were enrolled in the present study. Each participant was interviewed personally about her date of birth, marital status, habits and health state, occupation using a comprehensive questionnaire. Buccal smears were taken from all participants after asking them to rinse their mouths with tap water. The analysis of micronucleated cell and nuclear anomalies were done under a total magnification of X1000, 2000 cells per subjects (patient and control group) were scored and the results are represented as the number of micronucleated ,binucleated and karyolysis cell per 2000 cells. The scoring of the level of the micronuclei in exfoliated cells of oral mucosa showed a significant increase ($P = 0.0001$) in the breast cancer patients in relation to control. The mean score of micronuclei for the breast cancer patients was (19.20 ± 1.36) it was twofold higher than that of healthy women (control group).The frequency of binucleated cells was higher in breast cancer patients than in control, the difference was statistically significant ($P < 0.05$). The number of Karyolytic cells was also elevated in breast cancer patients, but this increase did not attain a significance. Increased frequency of binucleated and micronucleated cells in the buccal mucosa of breast cancer patients shows the genomic instability may be correlated with breast cancer. The results suggest that The Buccal Micronucleus Cytome (BMCyt) assay serves as a sensitive tool for studying genomic instability in primary cancer patients.

Key words : Cytome assay , binucleated cells, karyolysis cells.

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Introduction:

Numerous investigations have shown that the subsistence of malignant tumors in organism initiates considerable changes in chromatin structure and DNA content of buccal mucosa. This changes can serve as a biomarker for the presence of malignant tumor (1). A number of Ukrainian researchers have detected malignancy related change in the buccal mucosa cell in patients with malignant and benign tumors (2).

The micronuclei test in buccal mucosa cells was proposed by Stich *et al*. in 1982 (3) .Micronuclei (MN) are a

round or an oval chromatin-staining bodies in the cytoplasm, it originates from chromatid fragments, acentric fragment chromosomes, and/or whole chromosomes that are not incorporated in the main daughter nuclei through nuclear division, their frequency is quantified microscopically by using diverse staining techniques (4). Subsequently, many laboratories suggested scoring further genome damage markers such as, binucleated, karyorrhectic, karyolitic and broken eggs cells to assures a valuable analytic tool of assessment cytotoxic and genotoxic effect (5,6,7,8).

The Buccal Micronucleus Cytome (BMCyt) assay enables direct measurement of genotoxic and cytotoxic effects by inclusion of other genome instability markers (nuclear bud, binuclear), percentage of apoptosis and necrotic cells by using a cytome approach(9). Till now, many laboratories from different countries have used this assay in addition the number of articles related to the buccal cell micronuclei assay published annually is gradually increasing. Moreover, the buccal cytom assay can identify an increase in MN frequency and other nucleare anomalies in exfoliated buccal cells after exposure to numerous chemical and physical genotoxic factors. This assay similarly has the potential to detect inherited genomic instability for example Xeroderma pigmentosum, Ataxia telangiectasia and Bloom's syndrome (5, 10,11).

The present study was designed to evaluate the frequencies of micronuclei and other nuclear anomalies in buccal mucosa cells of Iraqi women with breast cancer.

Material and Methods

This case –control study included 20 primary diagnosis breast cancer patients, They were recruited at the Oncology Teaching Hospital, Baghdad, Iraq. from January to February 2016. As control 20 apparently healthy women were enrolled in the present study. Informed consent was taken from all the patients and control subjects. The present work was approved by the hospital based ethical committee of the institute.

Each participant was interviewed personally about her date of birth, marital status, habits and health state, occupation using a comprehensive questionnaire. In the control group, the women were excluded from the work if their health status or lifestyle displayed some factor that was possible to alter the expression or induction of micronuclei and other nuclear anomalies in buccal cells(occupational exposed to physical or chemical genotoxic or cytotoxic agents, hereditary disease, recent viral infection ,alcohol and smoking consumption, and medication) .

Buccal smears were taken from all participants after asking them to rinse their mouths with tap water. Toothbrushes were utilized to obtain cells from the buccal mucosa. Exfoliated cells were smeared onto grease free slides and allowed to air-dry. Subsequently smear was fixed with methanol (15 minutes) and then stained using Giemsa stain (20 minutes). For each subject two slides were prepared (12). The analysis of micronucleated cell and nuclear anomalies were done under a total magnification of X1000 . According to the criteria formerly developed by Tolbert *et al.*(13,14) a total of 2000

singly and distinctly non- fragmented staining cells were scored per subjects (patient and control group) in order to determine the rate of micronucleated, binucleated and karyolytic cells per 2000 cells.

Statistical analysis

The obtained data were arranged, saved in the database of the statistical software SPSS (version 15.00) .Non parametric method (Mann-Whitney U-test,) was utilized to compare the frequency of micronucleated cells and other nuclear anomalies in the studied groups. The P value of 0.05 was taken at level of significance.

Results and Discussion

The results of the present study showed that the mean age of breast cancer patients was 42.2 years (ranging 25-60 years) and the mean age of healthy female was 40.5 years (ranging 25-56 years). All study participants were nonsmokers.

The scoring of the level of the micronuclei in exfoliated cells of oral mucosa shown a significant increase ($P = 0.0001$) in the breast cancer patients in relation to control. The mean score of micronuclei for the breast cancer patients was (19.20 ± 1.36) it was twofold higher than that of healthy women (control group) (Table 1).

Increase in the frequency of cells with micronuclei ($P < 0.0001$), in the breast cancer patients, indicating potential changes in the efficiency of DNA repair and increased genomic instability in exfoliated cells reflect recent genotoxic events that occurred in the dividing basal cell (1-3 weeks) (5).

The result of the present work is in agreement with the results of the preceding research regarding elevation micronuclei level in buccal cells of breast cancer patients, Garcic *et al*. (15) reported increased micronuclei frequency in 21 breast cancer patients from Mexico. Similar results were found in a recent Turkish study carried out on 24 breast cancer patients(16).

It is noteworthy that previous studies have shown that elevated frequency of the micronuclei frequency as well as chromosomal aberration in normal somatic cells of cancer patients' (17,18). Other studies found an increased frequency of micronuclei and other nuclear anomalies in buccal cells of patients with oral cancer, head and neck cancer, Alzheimers disease (19,20,21). In addition to increased micronuclei scoring in breast cancer patients the present work found elevated level of other nuclear anomalies including binucleated and karyolysis cells. Binucleated cells are cells having two very close main nuclei rather than one and consider biomarker of cytokinesis arrest or cytokinetic faults as a result of aneuploidy. Karyolitic cells are cells seem to have no nucleus due to exhausted nucleus DNA, and signify a very late phase in the cell death process, moreover these cells denote responses to cytotoxicity (9).

The frequency of binucleated cells was higher in breast cancer patients than in control the difference was statistically significant ($P < 0.05$). The number of Karyolytic cells was also elevated in breast cancer patients,

but this increased did not attain a significant as shown in (table 1). The possible explanation of the lack of a significant result may be due to the low number of breast cancer patients recruited in the present study.

Table (1): Micronuclei level and other nuclear anomalies in buccal cells of breast cancer patients and healthy women

Parameter /2000cell	Breast cancer patients 20 (mean ± S.E)	Healthy women (control) 20 (mean ± S.E)
Micronucleated cells	19.20 ± 1.36***	7.30± 0.954
Binucleated cells	10.30± 0.960*	7.650± 0.689
Karyolytic cells	20.95± 3.52	13.70± 1.22

*** **P < 0.0001;* p < 0.05 compared with healthy women (control), Mann-Whitney U-test .**

Similar to our finding García *et al.* (15) reported increased frequency of binucleated and Karyolytic cells of stage I breast cancer patients compared with 20 healthy women but without getting statistical significance. KhIifi *et al.*(19) reported increased number of binucleated cell in 45 untreated head and neck cancer patients compared with 57 healthy individuals .In present study, the mean MN frequency in the buccal cells of the healthy control group was 7.30± 0.954; per 2000 cells , which is in consonance with the earlier published reports. A wide series of baseline micronuclei frequencies has been scored (0.05–11.5 MN/1000 cells)(5,22). Several studies indicate that the baseline levels of MN are dependent on the various risk factors including health status, occupational factors combined with socio-demographic factors (23,24,25).

The elevation in micronuclei score in breast cancer patients is attributed to susceptibility of these subjects to genomic instability due to acquired or inherited mutation in genes involved in cell cycle checkpoint , fidelity of DNA replication; DNA repair and \ or chromosome segregation such as BRCA1 , BRCA2 , P53 and ATM genes(26).

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(27).

Conclusions:

Increased frequency of binucleated and micronucleated cells in the buccal mucosa of breast cancer patients reflects genetic damage and /or defect in DNA repair system in normal somatic cell of cancer patients. Further, this result suggests that the MN cytome assay serves as a sensitive tool for assessment of genomic instability in primary cancer patients.

Acknowledgments:

The authors thank all participants who kindly giving us the samples.

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