

ORIGINAL ARTICLE

Micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of Mexican women with breast cancer

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Summary

Purpose: Breast cancer (BC) is the most frequently diagnosed form of cancer and the leading cause of cancer-related deaths among females in the world. Results of several studies showed that the genome of primary cancer patients (naïve for any treatment) is unstable. The purpose of the present study was to evaluate the genomic instability in BC patients by means of buccal cells micronucleus (MN) cytome assay

Methods: The frequencies of nuclear anomalies including MN, binucleates (BN), broken eggs (BE), condensed chromatin (CC), karyorrhexis (KR) and karyolysis (KL) were evaluated in exfoliated buccal mucosa cells of Mexican women with primary BC and healthy women. Buccal cells were collected from 21 BC patients (9 with stage I and 12 with stage

II) and from 20 healthy females used as control group.

Results: The results of the evaluation of cells showed that the frequencies of MN, BN, BE, KR and KL were significantly increased in the pooled group of BC patients compared with the control group. However, no one parameter of buccal MN-cytome assay in patients with stage I BC was significant compared with controls and BC patients with stage II.

Conclusion: Application of the buccal MN-cytome assay for the study of genomic instability in primary BC patients showed that both genotoxic and cytotoxic effects can be evaluated in such patients

Key words: breast cancer, buccal micronucleus cytome assay, cytotoxicity, genotoxicity

Introduction

BC is the most frequently diagnosed form of cancer and the leading cause of cancer-related deaths among females in the world, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400 subjects) of the total cancer deaths in 2008 [1]. In Mexico, the incidence and mortality of BC have risen in the last years. Low mammography coverage, poor quality control, insufficient physical and human resources for clinical care and limited access to diagnosis and treat-

ment are factors that contribute to this growing public health problem in this country [2].

Cancer is a genetic disease associated with an accumulation of chromosomal aberrations, which cause cell overgrowth through overexpression/activation of an oncogene, or by deletion of a tumor suppressor gene [3].

Results of several studies showed that the frequencies of micronuclei (MNI) in the lymphocytes of BC patients are significantly increased compared with healthy women [4-7].

A minimally invasive and potentially useful

method for monitoring genetic damage in humans is the MN assay in exfoliated buccal mucosa cells [8,9]. In addition to MNi, other nuclear anomalies, reflection of both genotoxic and cytotoxic effects, can also be monitored with this assay [8].

It has been claimed that this procedure may be a reliable method for the detection of human cancer risk as most tumors are of epithelial origin [10]. Except MNi, also other nuclear anomalies such as BN, BE phenomenon, CC, KR, pyknotic nuclei (P) and karyolysis (KL) can be scored in buccal cells [8,11]. MNi and BE phenomenon are considered as genotoxic events, BN as a spindle disturbance (aneugenic effects), and CC, KR, KL and pyknosis as acute cytotoxic effects [8,11].

Bonassi et al. [12] analyzed all the data concerning MN assay in buccal cells of cancer patients and postulated that a diagnosis of cancer significantly increased MN and other endpoints frequencies. Especially high correlation was found for oro-pharyngeal cancers, respiratory system cancers, and for all the other cancers pooled together.

Nersesyan et al. [13,14] have shown significantly increased number of MNi in exfoliated buccal cells of primary BC patients (N=27) compared with control subjects in Armenian women, but in women with benign breast tumors no such effect was found. Two Indian research groups also reported about increased number of MNi in oral mucosa cells of BC patients compared with healthy controls [15] and patients with benign breast lesions [16].

Hence, several groups of investigators reported increased frequencies of MNi in buccal mucosa cells of BC patients. However, there are no data concerning nuclear anomalies in buccal cells of BC patients.

The aim of the present study was to evaluate the frequencies of MNi and other nuclear anomalies in exfoliated buccal mucosa cells of Mexican women with primary BC compared with healthy females.

Methods

Subjects

A case-control study was performed in 21 never-treated primary BC women (all patients had stages I and II disease), and in 20 healthy women-volunteers used as controls from the Centro Estatal de Cancerología de Nayarit, of the Servicios de Salud de Nayarit, Tepic, México. Informed consent was obtained from all participants and the study was performed after

approval of the local Ethics Committee.

Both groups had similar age and socioeconomic backgrounds. All individuals who agreed to participate were interviewed to obtain personal information and were asked to complete a questionnaire concerning smoking habits, alcohol and coffee consumption, health status, age, alimentary habits, lactation, consumption of drugs, contraceptive or antioxidants, viral infections in the last 3 months, vaccination and hereditary diseases. Participants were excluded from the study if their lifestyle or health status showed any factor that was likely to affect the induction or expression of MNi.

Sample collection

Exfoliated buccal mucosa cells were collected from each subject. All participants were asked to rinse their mouths with water. A polished slide was used to collect cells from the buccal mucosa of the inner lining of both cheeks in each subject. Exfoliated cells were smeared on two slides. Smears were air-dried, fixed in 80% ethanol for 48 hrs and then stained with acridine orange (Sigma-Aldrich, Mexico) 0.02 mg/ml of phosphate buffer (pH 7.4) [17].

Slides evaluation

Cells were scored at 100x magnification using oil immersion with a Carl Zeis Axio Scope.A1 microscope (Gottingen, Germany). MNi and other nuclear anomalies (BN, BE, CC, KR, P, and KL) were scored according to the criteria described by Thomas et al. and Bolognesi et al. [8,11]. The number of cells with MNi and nuclear anomalies other than MNi, namely BN, BE, CC, KR, P, and KL, were evaluated in 2000 cells. Cells with CC are nuclei with areas of aggregated chromatin, which leads to differences in the staining intensity, and KR cells are characterized by more extensive nuclear chromatin aggregation as compared to CC cells. A small shrunken nucleus and a high density of chromatin characterize P cells. KL cells are cells that are classified by nuclei, which are completely depleted of DNA (ghost-like cells) [8,11].

Statistics

Results were evaluated using the Statistical Program for Social Sciences (version 16.0; SPSS Inc., Chicago Illinois, USA). A non-parametric method (Mann-Whitney U-test) was used to compare the frequencies of nuclear anomalies in the pooled group of BC patients and the controls. A non-parametric Kruskal-Wallis test with Dunn's post test comparison was applied to compare nuclear anomalies in BC patients with different disease stages and the controls. A p-value <0.05 was considered statistically significant.

Results

The demographic data of the study partici-

Table 1. Demographic data of study participants

Parameters	Breast cancer patients (stage I, N=9)	Breast cancer patients (stage II, N=12)	Healthy women (N=20)
Age (years)	53.1±12.7	52.25±16.52	50.2±9.3
Body mass index (kg/m ²)	24.1±2.0	25.1± 2.4	23.4±1.3
Women with normal menstrual function, N (%)	5 (55.6)	7 (58.3)	10 (50.0)
Start of menarche (years)	12.9±1.5	12.2±1.3	12.9±1.6
Age at first pregnancy (years)	21.2±4.2	20.8±3.2	22.2±3.9
Number of abortions	0.33±0.71	0.25±0.45	0.25±0.55
Pregnancy at term, N (%)	9 (100.0)	11 (91.7)	17 (85.0)

Results are expressed as mean±standard deviation, unless otherwise noted

Table 2. Nuclear anomalies in buccal mucosa cells of breast cancer patients and healthy women

Parameters (‰)	Breast cancer patients N=21	Healthy women N=20
Micronucleated cells	1.43 ± 0.65**	0.78 ± 0.34
Total number of MNi	1.79 ± 0.77**	0.85 ± 0.40
Binucleates	6.26 ± 2.51**	3.75 ± 1.39
Broken eggs	1.64 ± 1.03**	0.55 ± 0.39
Karyolysis	5.14 ± 3.22*	2.83 ± 2.05
Karyorrhexis+condensed chromatin	7.26 ± 2.27**	4.10 ± 1.92

*p<0.01 and **p<0.001 compared with control (healthy women); Mann-Whitney U-test
Results are expressed as mean±standard deviation. MNi: micronuclei

pants are presented in Table 1. No parameter was significantly different in each group. All women were from families with poor social-economical status. None of them was smoker or consumer of alcohol and coffee.

We found that CC could not be clearly distinguished from KR. It was also not possible to clearly identify pyknotic cells. Hence, cells with CC and KR were considered together, and pyknotic cells were not considered to avoid misevaluation.

The results of analysis of buccal cells of pooled BC patients and the controls are presented in Table 2. It was clear that the frequencies of all nuclear anomalies in buccal cells of BC patients were significantly increased compared with the controls. (for karyolytic cells only, p<0.01; in all others cases, p<0.001). The anomalies connected with genotoxicity, i.e. cells with MNi, total number of MNi and BE were increased by 83.3, 110.5 and 198.2%, respectively. Other studied anomalies were also increased by 66.9 – 81.6% in BC patients.

We also investigated possible differences of nuclear anomalies between BC patients with stage I and II and healthy females. The results are presented in Table 3. As it can be seen, none of the cytome assay parameters was increased in buccal

cells of stage II BC patients compared with stage I patients, as well as in BC patients with stage I compared with the controls. It can be noted (Table 3) that all parameters (reflecting both genotoxicity and cytotoxicity) in cells of stage I BC patients were higher compared to controls, but without statistical significance.

At the same time all the parameters of the cytome assay in buccal cells of stage II BC patients except BN were significantly increased compared with corresponding parameters of the controls (p<0.01). The anomalies reflecting genotoxicity, namely cells with MNi, total number of MNi and BE were increased in cells of stage II BC patients compared with the controls by 131, 163 and 259%, respectively. The parameters reflecting cytotoxicity, such as KL and CC+KR, were increased by 120.8 and 100.2%, respectively. At the same time, only the frequencies of one anomaly, namely KL, were significantly different in cells of stage II BC patients compared with stage I BC patients (p<0.001).

Discussion

The results presented herein confirmed the data of other research groups concerning in-

Table 3. Nuclear anomalies in buccal mucosa cells of stage I and II breast cancer patients and healthy women

Parameters (%)	Breast cancer patients (stage I, N=9)	Breast cancer patients (stage II, N=12)	Healthy women (N=20)
Micronucleated cells	0.94 ± 0.58	1.79 ± 0.45**	0.78 ± 0.34
Total number of MNi	1.22 ± 0.71	2.21 ± 0.50**	0.85 ± 0.40
Binucleates	4.94 ± 1.69	7.25 ± 2.64	3.75 ± 1.39
Broken eggs	1.28 ± 1.06	1.92 ± 0.95**	0.55 ± 0.39
Karyolysis	3.67 ± 1.60	6.25 ± 3.72**	2.83 ± 2.05
Karyorrhexis + condensed chromatin	6.01 ± 1.97	8.21 ± 2.06*	4.10 ± 1.92

*p<0.01 and **p<0.001 compared with controls (healthy women); Kruskal-Wallis test with Dunn's post-test comparison. Results are expressed as mean±standard deviation

creased number of MNi in buccal cells of BC patients [13-16]. Increased frequencies of MNi in buccal cells of BC patients are in concordance with data obtained in studies with lymphocytes in MN- and chromosomal aberrations assay. A Turkish group reported increased number of chromosomal aberrations in BC patients due to fragile site in chromosomes which are specific points where gaps, breaks, or rearrangements are observed due to exposure of cells to aphidicolin and caffeine [7]. It is also noteworthy that 72% of fragile site are coincidental with oncogene loci on human chromosomes [7].

The rates of other nuclear anomalies other than MNi were studied in BC patients for the first time, but in this work we did not include pyknotic cells due to the difficulty in their identification [18].

Ban et al. [19] found increased MNi frequencies in lymphocytes of 136 BC patients (stage not specified) compared with 48 healthy women. Aristei et al. [6] 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 rates of sister chromatid exchanges (SCE) were also significantly increased. Varga et al. [20] also found a highly significant difference between frequencies of MNi in BC patients from Germany (N=91) and healthy women (N=96). Milosević-Djordjević et al. [21] reported that not only in BC patients but also in patients with other tumor localizations MNi levels were increased irrespective of site.

In the study of Celik et al. [5] highly significant differences were found between BC patients with stages I, II and III and the control subjects in the numbers of lymphocytes with MNi and SCE. However, they did not find significant differences in MNi levels in BC patients with different disease stages. These authors found a significant increase (p<0.001) in the frequencies of

lymphocytes with MNi in first-degree relatives compared with controls.

Similar results were found in an Indian study with evaluation of buccal cells, i.e. the number of MNi in patients was higher than in first-degree relatives, and in turn, MNi frequencies in buccal cells of first-degree relatives of BC patients were significantly higher compared with controls [15].

We also found elevated frequencies of MNi and other nuclear anomalies (reflecting both genotoxicity and cytotoxicity) in buccal cells of the pooled group of BC patients (Tables 2 and 3).

As it can be noted, all of the parameters in the cells of stage I BC patients were higher compared with the controls, but without reaching statistical significance. Possible reason for this may be the low number of BC patients studied.

A reason of increased genetic instability in BC patients are the 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 [22]. Defective DNA repair leads to genetic instability which appears in the elevation of MNi in somatic (epithelial) cells.

It is worth noticing that the study of nuclear anomalies other than MNi in primary BC patients was carried out for the first time. To the best of our knowledge, there are only two publications concerning nuclear anomalies in buccal cells of primary cancer patients. In one of them a heterogeneous group of 37 patients with cancer in different organs (prostate, ovary, breast, bladder, cervix) was studied. No significant changes in the number of MNi were observed but BN and KL were significantly increased in this pooled group [17]. In the second one Parsadanyan et al. [23] reported increased levels of BN, CC and KL in buccal cells of primary cervix cancer patients (N=20).

Conclusions

Hence, we confirmed the results of studies of several research groups that BC patients have increased level of MNi. Increase of other parameters of the buccal MN-cytome assay was shown for the first time. The results also show that the MN cytome assay may be useful in studying genetic instability in cancer patients.

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