Chemotherapy effects on acute alterations in the nuclei of buccal mucosa cells at patients with breast cancer

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Abstract

Aim: Micronuclei analysis (MN) in exfoliative human cells presents an opportunity to study genomic changes in the target organs affected by cancer. The aim of this study was to analyze the extent of changes in the nuclei of epithelial exfoliated cells of oral buccal mucosa of patients with breast cancer treated with chemotherapy.
Methods: This study lasted for four months and, during this time period, 22 patients became part of the study. The patients were diagnosed with breast cancer (22.7% male) with an average age of 48.7±11.0 years. Taking the epithelial cells from oral cavity of those 22 patients, we analyzed the degree of nuclei alteration in exfoliated cells of buccal mucosa. The statistical parameters were tested with the t-test.
Results: The mean of degenerative changes in exfoliated epithelial cells of the buccal mucosa in patients with breast carcinoma increased significantly after chemotherapy (17.7±27.2 vs. 29.8±33.6, $P<0.0001$). The data indicates significant increase for all types of nuclei alterations. The frequency of various acute nuclei alterations and the total number of such alterations was similar in both genders.
Conclusion: The results have shown that chemotherapy induced cell cytotoxicity, but it did not induce chromosomal changes and micronuclei formation.

Keywords: acute nuclei alteration, breast cancer, chemotherapy.

Introduction

The origin of Micronucleus comes from the fragments of chromosomes and/or the whole chromosomes, which are not included in the nucleus cells during the process of the nucleus separation. Micronucleus analysis (MN) in exfoliated human cells shows a good opportunity for studying directly genome alterations at the target organs affected from the tumor (1).

Some researches have shown a significant correlation among the level of chromosomal aberrations in lymphatic cells and MN in exfoliative cells of buccal mucosa in subjects exposed to environmental mutagens (2).

Genomic damage can be caused by the action of external environmental genetoxis (e.g. radiation and chemical substances), the lack of micro-nutrients (e.g foliate), life habits (e.g. alcohol, smoking, medicine, and stress) and genetic factors (inherited defects in metabolism and DNA repair).

Based on this, it is supposed that the epithelial cells of oral mucosa are a favorite place for early genotoxic changes caused by carcinogenic agents that enter into the body by inhalation and ingestion (3).

Methods

Patients' recruitment

This study included 22 consecutive patients. They were hospitalized at the Institute of Oncology, UCCK (University Clinical Centre of Kosovo), in Prishtina. Patients' recruitment begun on 15th October 2012 and it ended on 15th January 2013. We prepared inclusion and exclusion criteria. The inclusion criteria were as follows: the patients had to be diagnosed with breast cancer and the patients had not yet begun chemotherapy treatment.

All the patients were informed about the reasons of this research, and they all have willingly accepted to be part of the study. We have received permission to start this research from Institute of Oncology, UCCK and from the General Director of UCCK, in Pristina, Kosovo.

Epithelial cells from oral cavity were taken from the same patient two times. The first time the epithelial cells were taken from the patient was before starting the chemotherapy, on the day they had their first appointment for chemotherapy treatment. The second time the epithelial cells were taken from the same patient's mouth was on the 21st day after the first day of treatment with chemotherapy.

Also, all patients had to complete a questionnaire about their life habits. The questionnaire was prepared specifically for this research.

Technique

The research work was conducted at Department of Molecular Pharmacology, Faculty of Biology, University of Prishtina, Prishtina, Kosovo.

With the CytoBrush (Koslabor, Prishina, Kosovo) we have taken the epithelial cells from buccal region of oral cavity. In the test tubes we have added 5 ml of physiological digestion. Next, the material obtained was placed in the device for centrifugation for 10 minutes in 1200 rpm.

After the centrifugation we removed the supernatant from the tube, leaving 1 ml of digestion together with the rotter. The rotter and the digestion were easily mixed together with a clean Pasteur Pipette. After that, we took some of that mixed amount, and that amount was spread on the surface of three microscopic slides. The materials were left to dry in room temperature for 2 to 3 hours.

After the material dried we added one drop of 96% alcohol to each slide with Pasteur Pipette, which was left to dry too. The microscopic slides after that were placed in a diluted Gimze 1:5, and the slides stayed there for 60 minutes. After dyeing the preparates, we rinsed them with distilled water. Subsequently, the slides were left to dry and finally they were analyzed in optical microscope.

Statistical analysis

All the results were collected and all the data were analyzed. We constructed the tables, calculated the means, standard deviations, and statistical testing for the level of significance set at 5%. The results are presented in three tables. The program used for analyzing the results was Sigma STAT 3.1.

Results

From the total number of patients with breast cancer, 22.7% were male. The average age of analyzed patients with breast cancer was 48.7±11.0 years old.

About 82% of the total number of the patients were in the age group 40-59 years.

When asked about their habits, 45.5% answered that they were tobacco users, and 4.5% were alcohol users (Table 1).

Table 1. Demographic data for breast cancer patients treated with chemotherapy (N=22)

Characteristics	Number	Percentage
Sex		
F	17	77.3
M	5	22.7
Age		
40-59 years	18	81.8
60-79 years	4	18.2
Age, mean (SD)	48.7	11.0
Smoking		
No	12	54.5
Yes	10	45.5
Alcohol abusers		
No	21	95.5
Yes	1	4.5

The average number of degenerative changes in epithelial exfoliate cells of buccal mucosa at patients with breast cancer had a significant increase after the chemotherapy treatment. The value of degenerative changes before treatment was 17.7±27.2, while on the 21st day after treatment this value was higher (29.8±33.6, P<0.0001). We found a significant increase for all types of nuclear changes (Table 2).

Table 2. Average degeneration of epiteliate exfoliated cells of buccal mucosa in breast cancer patients (N=22)

Type of degeneration	Baseline		After therapy		Mean	P
	Mean	SD	Mean	SD	difference	
Micronukleus (MN)	0.1	0.0	0.0	0.0	-0.1	NT
Nuclei picnotic (Pik)	7.5	9.7	15.5	12.4	8.0	0.00001*
Karyorhectic cells (KR)	5.1	6.5	19.7	13.5	14.6	0.0001*
Karyolysis (KL)	51.8	33.9	68.5	44.2	16.7	0.0003*
Binuclear cells (BN)	6.1	3.8	12.7	7.5	6.7	0.00002*
Total change	17.7	27.2	29.8	33.6	12.1	0.0001*

* significant difference.

The frequency of various acute nuclei alterations and total number of such alterations was similar in both genders, and there was no significant difference with t-test while comparing these values according to the gender (Table 3).

Discussion

Micronucleuses are fragments of chromosomes or whole chromosomes, which can not reach the poles in axis of separation during mitosis and in the telephase they remain encapsulated as separate

Table 3. Average difference in degenerative changes in epitaliale exfoliate cells in buccal mucosa according to gender

Type of degeneration	Female	Male		
	Mean difference	Mean difference	T-test	P
Micronukleus (MN)	0.12	0.00	--	NT
Nuclei picnotic (Pik)	8.00	11.20	1.0	0.32
Karyorhectic cells (KR)	13.59	19.00	0.67	0.51
Karyolisis (KL)	16.29	22.00	0.6	0.55
Binuclear cells (BN)	8.24	4.00	1.6	0.12
Total changes degenerative	9.20	11.24	0.7	0.49

nucleuses. Micronucleus testing helps detection of lost chromosomes or malfunction in mitotic axis, caused from aneugenic mechanism (4).

The epithelium of oral cavity is permanently regenerated through constant production of new cells in a base layer with mitosis and the migration of the cells to the surface for replacing the old ones. The base layer contains source cells that can show genetic damage (detachment or lost chromosomes) as MN during the separation of the nuclei. The new cells that are produced may or may not contain MN, eventually differentiated on the thorny cell layer. Some of these cells may degenerate into condensed chromatin cells, fragmented nucleus (karyorrhexic cells), picnotic nuclei, or they may lose the whole material of nuclei (karyolysis) (5). In rare cases, some of the cells can be blocked in binuclear phase or can express nuclei in stitch form, known as "broken eggs" in the cells of buccal mucosa, as biomarkers of gene proliferation. These biomarkers can be observed in lymphocytes, and also in buccal cells too, which shows a wider damage of genome, than only MN, in the cytotoxics and cytostatic effects context (6). Casartelli and collaborators (7) have analyzed the frequency of MN in exfoliated cells of normal buccal mucosa, in precancerous lesions and in squamous cells carcinoma. They realized that gradually increased number of MN from normal mucosa towards precancerous lesions to carcinoma shows a relation of this biomarker in neoplastic progress. The biomonitoring publications that use evaluation of MN in buccal mucosa analyzed the effects of many factors, including the influence of environment exposure, working place, radiotherapy, chemoprevention, the effect of life habits, the effects of tumors and other diseases (8).

Binuclear cells have two nuclei. This kind of cell is frequently noticed at cancer cells and can appear from many factors. If during the separation of the cell the hull of cell's separation will start to regret, the cells will be joined again, causing non-separation of the chromosomes (9). The binuclear cells can appear also during the failure of cytokinesis, so that the hull of cells's separation cannot be formed, causing both nuclei to remain in the same cell. Picnosa or karyopicnosa presents irreversible condensation of cromatina in cell nuclei that undergo necrosis. This is followed by caryorrhexis or with the fragmentation of the nuclei (10). Caryorrhexis is destructive fragmentation of dying cells nuclei, in which chromatin is spreading in irregular way along cytoplasma. This usually is followed by karyolysis (11). Karyolysis presents the complete digestion of chromatin due to the activity of DNA enzymes in the dying cell. In apostosis, after caryorrhexis nuclei is usually digested in apoptotic bodies (12).

The cell exposure to cytotoxic substances may result with different ending for those cells. The cells can undergo necrosis, which can cause the loss of cells membrane integrity and the immediate death as a result of cell lyses. The cells growth and their active separation can stop (constant cellular decreasing), or the genetic program which controls the death of cells can be activated (apoptosis). The

cells that undergo rapid necrosis do not have enough time to activate the mechanism of apoptosis – this is the reason they do not show apostosis markers (13).
Breast cancer is the most common cancer diagnosed in most women all over the world, with 1.38 million newly diagnosed cases in 2008 (about 23% of the total only in women, and about 11% of the total number, in both genders). The level of incidence of breast cancer is higher in Western Europe and lower in East and Mid-Africa (14).
On the other side, due to the application of cytotoxic medicine, the number of binuclear cells is getting lower, whereas the number of karylictic cells is increasing. According to the authors, those parameters can be used as the markers of the cytotoxity in future studies for different medicines (15).
Fitim Alidema and his collaborators in their studies related to acute alterations in nuclei of buccal cells in patients with cancer treated by chemotherapy observed a significant increase of frequency of karyorrhexis, karyolysis and picnosis compared to the control group. Also, the karyorrhexis was significantly more frequent after the treatment with chemotherapy, compared with the period before the treatment (6.4±3.0 vs. 3.9±4.8, P<0.006). According to the results obtained, the authors realized that the acute therapy does not induce chromosomal damage, but it can cause a cytotoxic action (16).
In our research, the average number of picnotic changes (P<0.00001), karyorrhexis (P<0.0001), karyolysis (P<0.0003), and the average number of binuclear cells (P<0.00002) is significantly increased. Also, a significant increase is observed in all changes in nuclei of exfoliate cells of buccal mucosa among patients with breast cancer who are treated with chemotherapy (P<0.0001). Gender is not a factor which has influenced significantly the frequency of nuclei alterations after the chemotherapy of breast cancer.

Conclusion

The acute changes in nuclei of the buccal cells can be used as important markers for estimating the cytotoxity of chemotherapy among patients with breast cancer.
The results have shown that all the applied protocols for treatment of breast cancer have induced the cytotoxity of cells, but they did not induce the chromosomal changes and the micronucleus formation.

Conflicts of interests: None declared.

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