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MICRONUCLEI LEVEL IN EXFOLIATED BUCCAL MUCOSA CELLS OF PATIENTS WITH BENIGN AND MALIGNANT TUMORS OF FEMALE REPRODUCTIVE ORGANS AND BREAST



Micronucleus (MN) levels in exfoliated buccal mucosa cells of primary breast, cervix and corpus uteri cancer patients, and patients with benign tumors of uterus (myoma) and breast (fibroadenoma) were studied. Significantly increased number of MN in cells of cancer patients was observed compared to both healthy persons and patients with benign tumors. In patients with benign tumors no increase in MN quantity was observed. The evaluation of MN number in buccal mucosa cells shows genomic instability caused by malignant tumor in somatic cells of humans.

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Introduction. It is known that existence of malignant tumor in organism leads to so-called «malignancy associated changes» in buccal epithelium [1]. A group of the Ukrainian investigators have shown substantial changes in DNA content and indices characterized the texture of chromatin in oral mucosal cells of patients with benign and malignant tumors [1–4]. They have revealed that the mentioned changes in buccal mucosa cells can serve for differential diagnosis between benign and malignant processes, and also as a marker of the presence of malignant tumor in organism [1–4]. The mentioned changes in oral mucosal cells were observed in patients with benign and malignant tumors located in thyroid and mammary glands [1–4]. Hence, the study of buccal mucosa cells is of great importance and interest for cancer diagnostics.

Human exfoliated buccal mucosa cells are intensively studied also for presence of micronuclei (MN), DNA containing bodies in the cytoplasm of cells. This test is one of the modern techniques to study the genetic instability in human organism [5]. MN form from chromosomal fragments and/or a whole chromosome that are not included in the main daughter nuclei during nuclear division [5]. Hence, scoring of MN provides a measure of both structural and numerical chromosomal aberrations. MN analysis in exfoliated human cells gives a possibility to study genomic changes both in target and distant organs of organism affected with tumor [5, 6].

As in buccal epithelial cells of patients with benign and malignant tumors substantial changes in DNA content and indices characterizing the texture of chromatin are present, it would be of interest to investigate if MN level in these cells also differs from that in healthy persons. The aim of the present work was to investigate the level of MN in exfoliated cells of primary patients with benign and malignant tumors of female reproductive organs.

Materials and methods. Exfoliated buccal mucosa cells were collected from 12 breast cancer patients, 28 cervix uteri cancer patients and 8 corpus uteri cancer patients. In addition 9 patients with myoma and 8 patients with fibroadenoma of mammary glands were studied. The idea was to compare the difference in nuclear anomalies in buccal cells in the subjects with the same site of affection with tumor either malignant or benign. All patients were under study at Cancer Research Center, Ministry of Health of Armenia, Yerevan

and did not receive any antitumor treatment. All cancer patients were at II-III stages of disease. The mean age of female patients was 46.6 years.

As control we used the cells of 15 women-volunteers of corresponding ages who underwent a complete medical examination at the Center of Obstetrics and Gynecology, Yerevan, Armenia and were considered healthy. Each volunteer was interviewed personally, using a detailed questionnaire about his/her habits and health condition, to control possible confounding factors that potentially play a role in the induction or expression of MN (exposure to physical and chemical mutagens, consumption of alcohol and coffee, smoking habits, food habits, medication, viral infections suffered in the last 3 months, vaccination, hereditary diseases etc.). Participants were excluded from the study if their life style/health showed any factor that was likely to affect the induction or expression of MN. All subjects under study were non-smokers. Preparations of the buccal mucosa cells were carried out according to routine method [6]. All subjects were asked to rinse their mouths with water. Wooden spatula was used to sample cells from the buccal mucosa. Exfoliated cells were smeared onto the slides and allowed to air-dry. Smears were fixed in methanol-glacial acid (3:1). The standard protocol for the Feulgen staining technique with fast green counterstain was used. The scoring of MNC was performed on coded slides. 2000 cells were scored from each individual. Only cells containing intact nuclei, that were not clumped, smeared or overlapped were included in the analysis. Cells with degenerative processes and so-called nuclear anomalies, such as karyorrhexis, karyolysis, pyknosis, binucleates, condensed chromatin, «broken

egg» were not considered. The criteria of scoring the cells with MN were the same as described by Rosin [8]. Totally 84,000 cells were studied. Questionable MN were disregarded. Non-parametric criterion (Mann-Whitney U-test) was used to analyze the frequency of MN.

Results and discussion. The data obtained are presented in Table. Analysis of the data has showed significant increase of MN number in the cells of malignant tumors patients compared to control subjects (2.2–3.2-fold increase; $p < 0.001$). The highest number of MN was registered in exfoliated cells of patients with corpus uteri cancer although it was not differed significantly from the data of patients with other localization of tumors. The mean of pooled data of MN number for all cancer patients was 2.4-fold higher than that of controls (2.24 ± 0.06 and 0.93 ± 0.05 ‰, respectively, $p < 0.001$). MN frequency in buccal cells was not increased in patients with benign tumors of uteri and breast compared with the data of healthy females (1.00 and 1.12 ‰ vs 0.93 ‰ in control). In the contrast to our findings, the Ukrainian investigators studying changes in DNA content and indices characterizing the texture of chromatin in oral mucosal cells have shown substantial changes in cells of patients with benign tumors [1–4].

The MN level in control (healthy) female subjects was 0.93 ‰. It is close to our previous results [7] and the data obtained from healthy subjects in Spain and Italy [8, 9].

Hence, we observed an increased cytogenetic disturbances in buccal mucosa cells of patients with malignant tumors, but not with benign ones. It is noteworthy that in numerous investigations it has been shown that in normal somatic cells of primary

Micronucleus level in exfoliated buccal mucosa cells in primary cancer patients (mean \pm S.E.)

Diagnosis	Mean age, years	Number of subjects	MN level in exfoliated oral cells	Range of cells with MN
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Breast cancer	46.6 \pm 0.7	12	2.08 \pm 0.28 *	1–4
Fibroadenoma of mammary gland	42.5 \pm 0.8	8	1.12 \pm 0.40	0–3
Cancer of cervix uteri	51.0 \pm 0.5	28	2.07 \pm 0.24 *	1–5
Cancer of corpus uteri	45.9 \pm 1.1	8	3.00 \pm 0.60 *	1–6
Myoma	44.6 \pm 1.2	8	1.00 \pm 0.27	0–2
Healthy females (controls)	49.0 \pm 0.4	15	0.93 \pm 0.05	0–1

* Difference with controls (healthy females) at significance level of $p < 0.001$.

cancer patients (lymphocytes), independently on the tumor site, CA as well as MN levels are at least two-fold higher than in corresponding healthy persons (both males and females) [10–13]. The significance of these alterations in peripheral blood cells of cancer patients remains unclear. It has been suggested that the mentioned chromosomal changes may be connected to so called «cancer status», «inflammatory status» associated with cancer, genomic instability in organism of patients, and environmental exposure. It is believed, that tumor-triggering agents may interact with normal («healthy») cells of the body [13].

It is noteworthy that in somatic cells of primary patients with corpus uteri cancer the number of chromosomal aberrations (CA) is higher than in cells of patients with other site of tumors and much higher than that in healthy persons [1]. We have also revealed the same tendency although the number of MN in patients with corpus uteri cancer was not significantly higher than in the other groups of patients.

MN level in exfoliated cells of breast cancer patients was studied recently in India [14]. An increased level of MN in their cells has been shown as compared with healthy subjects although mentioned increase was surprisingly high (see comments to this article — [15]). As for patients with other localization of tumor, MN in their buccal cells has not been studied yet. In lymphocytes of breast, cervix and corpus uteri cancer patients CA level was significantly increased [12]. It is well known that there is a significant correlation between the CA level in lymphocytes and MN level in exfoliated buccal mucosa cells of persons exposed to environmental mutagens/carcinogens [9, 16].

Hence, we revealed the increased MN level in buccal mucosa cells of primary cancer patients reflecting genetic instability in healthy somatic cells of an organism affected with malignant tumor. In our opinion, the study of MN in buccal mucosa cells can serve as an index of possible exposure of subjects to environmental mutagens/carcinogens and/or of the presence of malignant tumor. Further investigations in this area are certainly warranted to confirm our finding because, to our knowledge, this work is one of the first investigations in this field.

The authors wish to thank all volunteers for their enthusiastic participation in the study, Ms. N. Vartazaryan for her excellent technical work and Drs.

E. Bakhshinyan, A. Khangheldyan and H. Davtyan (Cancer Research Centre, Yerevan) for their kind help.

РЕЗЮМЕ. Изучен уровень микроядер в эксфолиативных клетках ротовой полости больных со злокачественными и доброкачественными новообразованиями женских репродуктивных органов и молочной железы. Достоверно повышенный уровень микроядер был обнаружен только в клетках больных со злокачественными опухолями.

РЕЗЮМЕ. Вивчено рівень мікроядер в эксфоліативних клітинах ротової порожнини хворих з доброякісними та злоякісними новоутвореннями жіночих репродуктивних органів і молочної залози. Вірогідно підвищений рівень мікроядер виявлено тільки у клітинах хворих із злоякісними пухлинами.

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Received 18.11.03