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Micronucleus level in exfoliated buccal mucosa cells of cancer patients

Micronucleus levels in exfoliated buccal mucosa cells of patients with primary breast, lung, cervix uteri cancer, and patients with Hodgkin's disease were studied (n=59). Significantly increased number of micronuclei in cells of cancer patients was observed compared with healthy persons (n=45). The evaluation of micronuclei number in buccal mucosa cells shows genomic instability in somatic cells of humans.

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Numerous investigations have shown that in normal somatic cells of primary cancer patients chromosomal aberration level is significantly increased compared with healthy subjects (1-3). One of the modern techniques to study the impact of environmental, genetic and life-style factors on genetic stability in human population is a micronucleus (MN) test. MN originates from chromosomal fragments and/or whole chromosomes that are not included in the main daughter nuclei during nuclear division (4). Hence, MN provides a measure of both structural and numerical chromosomal aberrations. MN analysis in exfoliated human cells gives a possibility to study genomic changes directly in target organs affected by tumor (4). In some investigations significant correlation between the level of chromosomal aberrations in lymphocytes and MN in exfoliated buccal mucosa cells of subjects exposed to environmental mutagens have been shown (5-7).

The aim of the present paper was to evaluate the MN level in exfoliated buccal mucosa cells of primary cancer patients.

Exfoliated buccal mucosa cells were collected from 59 primary cancer patients who were under study at the Cancer Research Center, Ministry of Health of Armenia, Yerevan. There were 41 females (12 with breast cancer, 8 with Hodgkin's disease, 21 with cancer of cervix uteri) and 18 males (10 with lung cancer and 8 with Hodgkin's disease). All patients were at I-II stages of disease. The mean age of female and male cancer patients was 48.6 and 51.4 years, respectively. As a control we used the cells of volun-

teers (15 males and 30 females) of corresponding ages (with median age of 48.3 years for males and 44.0 years for females) who underwent a complete medical examination at the same Center and were considered healthy. Each volunteer was interviewed personally about his/her habits and health condition, using a detailed questionnaire. The purpose was 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 or health showed any factor that was likely to affect the induction or expression of MN. All subjects under study were non-smokers. 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 MN cells was performed on coded slides. Two thousand cells were scored from each individual. Only cells contained intact nuclei, that were not clumped, smeared or overlapped were included in the analysis. Cell with degenerative processes such as karyorrhexis, karyolysis, pycnosis, binucleates, condensed chromatin, "broken egg" were not considered. The criterion of scoring the cells with MN was the same as described by Rosin (8). Questionable MN were disregarded. A non-parametric criterion (Mann-Whitney U-test) was used to analyze the frequency of MN. The data obtained are presented in Table 1.

Analysis of the data showed significant increase of MN number in cancer patients' cells compared to control subjects (1.75 - 2.17 times increase). In all cancer patients except female ones with Hodgkin's disease the difference was at $p < 0.001$ level (in men-

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tioned case, significance was at $p < 0.05$ level). In both groups of patients the median number of MN did not differ significantly and it gave possibility to operate with the median number of pooled MN. In control subjects also there was no significant difference in males and females (1.07% and 0.95%, respectively; $p > 0.05$). It is very close to our previous results (9) and the data obtained from healthy subjects in Spain and Italy (5,7). The mean number of pooled data for all cancer patients was 1.98 times higher than that of controls ($1.97 \pm 0.02\%$ and $0.99 \pm 0.04\%$, respectively, $p < 0.001$). Corresponding data for male ($1.97 \pm 0.05\%$) and female patients ($2.01 \pm 0.02\%$) were also at high significance level ($p < 0.001$ in both cases). Hence, in primary cancer patients the level of MN reflected genetic instability in somatic cells increased significantly compared with healthy persons.

Table 1. Micronucleus level in exfoliated buccal mucosa cells in primary cancer patients

Tumor site	Sex (mean age in years)	Number of patients	Number of control subjects	MN level in exfoliated oral cells (%)	Range of cell with MN
Breast	F (46.7)	12	-	$2.05 \pm 0.28^*$	1-4
Lung	M (52.2)	10	-	$2.30 \pm 0.43^*$	1-5
Lymph nodes	M (50.4)	8	-	$1.88 \pm 0.27^*$	0-2
Lymph nodes	F (42.3)	6	-	$1.75 \pm 0.49^{**}$	0-3
Cervix uteri	F (51.0)	21	-	$2.07 \pm 0.24^*$	1-5
-	M (48.3)	-	15	1.07 ± 0.15	0-2
-	F (44.0)	-	30	0.95 ± 0.05	0-1

* Significant difference with corresponding control data at significance level of $p < 0.001$ and ** at $p < 0.05$

It is noteworthy that some investigators have shown that in cancer patients' lymphocytes the MN level is two-fold higher than in corresponding healthy both males and females (1,2).

MN level in exfoliated cells of breast cancer patients has been studied recently in India (10). It shows the increased level of MN in their cells compared with healthy subjects although mentioned increase was surprisingly high (see comments to this article - 11). As for patients with other localization of tumor, MN in their buccal cells has not been studied yet. But in lymphocytes of all mentioned groups of patients the chromosomal aberration level is shown to be significantly increased (3). It is well known that there is correlation between the chromosomal aberrations level in lymphocytes and MN level in exfoliated buccal mucosa cells of persons exposed to environmental mutagens or carcinogens (5-7). Thus, the evaluation of MN number in oral mucosa cells can show genomic instability in somatic cells of organism. Our data confirm the opinion of the group of investigators that MN assay is more rapid, economic, and at least as sensitive an indicator of chromosome damage as classical metaphase chromosome analysis (4).

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