Обзорные статьи

A.K. NERSESYAN
Institute of Cancer Research, Medical University of Vienna,
Vienna A-1090, Austria
E-mails: armen.nersesyan@meduniwien.ac.at; armenn@freenet.am

BIOMONITORING OF CYTOGENETIC EFFECT OF ANTITUMOR THERAPY BY MEANS OF MICRONUCLEUS ASSAY IN EXFOLIATED EPITHELIAL CELLS

The data concerning possibility to use micronuclei (MN) level as a biomarker of cytogenetic effects in exfoliated epithelial cells of cancer patients under therapy are presented. The number of MN in buccal cells of cancer patients under chemotherapy are very contradictory. Significant dose-dependent increment of MN in tumor and normal epithelial cells due to radiotherapy was shown in most investigations. Evaluation of MN induced by radiotherapy in exfoliated tumor cells can potentially identify radiosensitivity of tumors and the treatment outcome after the first fractions of irradiation. This technique is almost completely non-invasive and easily done in accessible primary cancers (oral cavity and uterine cervix).

It is well established that radio- and chemotherapy widely used for treatment of cancer patients induce chromosomal aberrations and micronuclei (MN) both in tumor [1–4] and normal (healthy) cells [5, 6]. Because of some technical difficulties (invasive procedure to obtain tumor cells during and after treatment) cytogenetic disturbances are mostly studied in lymphocytes [7, 8]. These cells are the most used target for biomonitoring of cytogenetic effects of cancer treatment.

Of course, it would be of interest to monitor cytogenetic alterations not in surrogate tissue (lymphocytes) but in tumor and epithelial cells. It is noteworthy that about 90 % of all human tumors are derived from epithelial tissues [9, 10]. MN assay in exfoliated human epithelial cells is used to study clastogenic/aneugenic effects of agents of various origin [11, 12]. It has been shown that exposure of persons to some environmental and occupational pollutants can lead to increased level of MN in inepithelial cells. Some lifestyle habits, such as tobacco smoking, khat, areca nut and betel chewing could also be the causes of MN induction in oral mucosal cells. In some diseases, including precancerous ones and cancer, increment of MN was also frequently observed [11, 12].

The aim of this paper is to evaluate the data concerning MN level in exfoliated epithelial cells of cancer patients as possible biomarkers of cytogenetic effect of antiblastic chemo- and radiotherapy. In the review paper by Majer et al. [11] three articles were cited concerning radiotherapy of oral cancer (totally 8 subjects, with MN increase in not affected by tumor cells in all cases), one – concerning treatment of thyroid cancer with ^{131}I (31 subjects, negative result), and one paper concerning cancer chemotherapy (7 subjects, positive results in 5 persons, and correlation of MN data in lymphocytes) [11]. It should be added to the last cited paper [13], that the increment of MN both in exfoliated epithelial cells and lymphocytes were not observed in two subjects treated only with interferon. Hence, correlation was observed between the responsibility of two types of cells to genotoxic action of chemotherapeutic drugs.

The most important data concerning MN induction in exfoliated epithelial cells by radio- and chemotherapy are presented in Tables 1 and 2 (age and sex of subjects, the type of stain used and the number of cells studied). On the accuracy of scoring and evaluation of number of MN in mucosal cells the most important impact could

Table 1

Micronuclei frequency in buccal cells of cancer patients under antiblastic chemotherapy

Country	Treatment	Number of subjects, sex (age)	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks	Reference
Armenia	Chemotherapy (cancer of various sites, various schedules)	10m + 12f (54) Control – the same pts before therapy	3.2 1.0 0.8	Feulgen + fast green 2000	Effect of exposure: ↑ x 3.2	[17]
Mexico	Chemotherapy (cancer of various sites, isophosphamide+epirubicin)	13m + 8f cancer patients (48.9) Control – the same pts before therapy	2.6 1.2	Orcein 2000	Effect of exposure: ↑ x 2.3	[18]
Mexico	Chemotherapy (cancer of various sites, carboplatin+5-fluorouracil)	9m + 5f cancer patients (49.7) Control – the same pts before therapy	1.3 1.0	Orcein 2000	Effect of exposure: ↔	[18]
Mexico	Chemotherapy (cancer of various sites, cisDDP+5-fluorouracil)	6m cancer patients (61) Control – the same pts before therapy	2.7 1.5	Orcein 2000	Effect of exposure: ↕	[18]

Note. In table 1–3: ↑ – significant increase; ↔ – no effect; ↕ – either increase or decrease, but not significant; pts – patients; f – female, m – male.

have only the stain used (DNA-specific or not) and the number of studied cells [14–16].

Only 3 papers were found in Medline concerning MN induction in exfoliated epithelial cells due to antiblastic chemotherapy. One of them was already analysed by Majer et al. [13]. In the paper by Nersesyan et al. [17] 4 males and 5 females with lymphogranulomatosis, 4 males with lung cancer and 7 females with breast cancer were analysed a week after various schedules of antiblastic chemotherapy. Significantly increased number of MN was observed. In 21 Mexican patients with various localization of tumors treated with isophosphamide+epirubicin the significantly increased number of MN was registered (from 1.2 ‰ before to 2.6 ‰ after treatment) [18]. In 14 patients (mostly with oral and penis cancer) treated with carboplatin + 5-fluorouracil and 6 patients (3 with penis, 1 with prostate, and 2 with oral cancers) treated with cisDDP+5-fluorouracil no such effect was registered. In this paper both primary patients and patients subjected to the second and even the third courses of chemotherapy were studied. Based on the data presented by the authors, the number of cells with MN only in primary cancer patients treated with three chemotherapeutic schedules were calculated. Totally among them 19 were primary, and MN frequencies were 1.6 ± 0.4 ‰ before and 2.6 ± 0.7 ‰ after treatment ($p > 0.05$, Mann Whitney test). In 10 patients treated with carboplatin + 5-fluorouracil MN numbers were 0.95 ± 0.12 ‰ before and 1.35 ± 0.42 ‰ after treatment, and in 7 patients treated with isophosphamide+epirubicin the frequencies were 2.9 ± 1.0 ‰ (before) and $4.9 \pm \pm 1.6$ ‰ (after treatment) ($p > 0.05$ in both cases, Mann Whitney U-test). In all cases not significant increase of MN was observed. Torres-Bugarin et al. [18] mentioned that many patients under chemotherapy had signs of toxicity – the presence of a lot of buccal cell with karyolysis. Hence, 2 papers reported about significant increment of MN induced by antiblastic chemotherapy and 1 the same effect only due to one schedule of therapy. Correlations between MN level induction in somatic epithelial

Table 2

Micronuclei frequency in normal (not cancerous) epithelial cells of cancer patients under radiotherapy

Country	Treatment (dose of radiation)	Number of subjects, sex (age)	Type of cells	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks	Reference
Armenia	Radiotherapy of cervix cancer (70.0 Gy)	14f (50) Control – the same subjects before therapy	Cervix	8.0 – (35.0 Gy) 7.4 – (70.0 Gy) 2.9 – before therapy	Feulgen + fast green (2000)	Effect of exposure: ↑ x 2.0 (35 Gy) and ↑ x 1.8 (70 Gy)	[23]
Armenia	Radiotherapy of oral cavity cancer (25 Gy)	8m+6f (61) Control – the same pts before therapy	Buccal	4.8 (25 Gy) 6.2 (15 Gy) 0.9 – before therapy	Feulgen + fast green (2000)	Effect of exposure: ↑ x 5.3	[24]
Brazil	Radiotherapy of head and neck cancer 6MeV linear accelerator (X-ray, equivalent body dose 3.3 Gy)	25m, 6f (59) Control – the same subjects before therapy	Buccal	2.3 0.8 – before therapy	Feulgen + fast green (4000 during therapy, 2000 before)	Effect of exposure: ↑ x 2.9. No effect of smoking on MN level, although there were 17 heavy smokers (more than 30 cigarettes per day consumers)	[25]
China	Radiotherapy of nasopharyngeal cancer (68.0 Gy)	9m (36) Control – the same subjects before therapy	Buccal	7.7 – (28 Gy) 8.8 – (48 Gy) 7.6 – (68 Gy) 2.3 – before therapy	Acridine orange (1000)	Effect of exposure: ↑ x 3.3 (28 Gy), ↑ x 3.8(48 Gy), ↑ x 3.3 (68 Gy). Positive results were obtained in MN, CAs, and comet assays in lymphocytes with less doses of radiation (4–10 Gy)	[26]

cells and treatment results are unknown because no data were published about the treatment outcome of the patients after chemotherapy.

Although the contradictory results were published concerning chemotherapy action on MN level in buccal mucosa cells, it is noteworthy that in two studies the significantly increased level of MN was observed in buccal cells of nurses handling cytostatic drugs (1.6–2.0-fold) [19–21], and in one case 2-fold not significant increase [22].

The data concerning the frequencies of MN induced by radiotherapy in normal and tumor

Table 3

Micronuclei frequency in tumor cells of cancer patients under radiotherapy

Country	Treatment (dose of radiation)	Number of subjects, sex (age)	Type of cells	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks	Reference
Armenia	Radiotherapy of oral cancer (25 Gy)	8m + 6f (61)	Buccal	5.6 (25 Gy) 6.8 (15 Gy)	Feulgen (1000)	Effect of exposure: ↑ x 5.3	[24]
		Control – the same pts before therapy		0.9			
India	Radiotherapy of oral cancer (28.8 Gy)	31sex and age not specified	Buccal	19.5	Giemsa (1000)	Effect of exposure: ↑ x 7.0	[27]
		Control – the same pts before therapy		2.8			
India	Radiotherapy of oral cancer (24.0 Gy)	49 sex and age nor specified	Buccal	25.2 (21 sensitive to treatment pts) 15.0 (28 resistant to treatment pts)	Giemsa (1000)	Effect of exposure: ↑ in both resistant (x 3.7) and sensitive (x 6.1) to therapy pts. The number of MN was significantly higher in sensitive to treatment pts	[28]
		Control – the same pts before therapy		4.1			
India	Radiotherapy of cervix cancer (50.0 Gy)	25f (52)	Cervix	42	May-Grunwald-Giemsa (1000)	Effect of exposure: ↑ x 2.8. The significant increase of MN in cancer cells after first week could predict for a local better response and survival	[29]
		Control – the same pts before therapy		15			
India*	Radiotherapy of oral cancer (38.5 Gy)	68 (sex not specified) (62)	Buccal	43 – (7.0 Gy) 55 – (17.5 Gy) 71 – (28 Gy) 78 – (38.5 Gy)	Giemsa (500)	Effect of exposure: linear, maximally at maximum dose ↑ x 7.7	[30]
		Control – the same pts before therapy		11			

*Total number of MN, but not cells with MN were measured.

epithelial cells are presented in Tables 2 and 3, respectively. The most important data of 4 papers in Table 2 are presented concerning the studied cells with no sign of pathology [23–26], e.g. they were obtained from the opposite site of timor localization, or from the same site, close to the tumor. In four other papers the results of studies of tumor cells during and/or after radiotherapy are

presented (Table 3) [27–30]. Both in normal and tumor cells a significant increase of MN was observed due to radiation. It is important that in normal cervical [23] and buccal [25, 26] cells of patients under radiotherapy, MN level increased linearly until the certain dose (mostly about 25–35 Gy), and then even decreased after additional doses of radiation. In cervix cells the frequency of cells with MN was significantly higher after 35 Gy (8‰) than the level after 70 Gy (7.4 ‰) [23]. In buccal mucosa 8.8 ‰ cell with MN were observed after the dose of 48 Gy, and only 7.6 ‰ after 68 Gy [26]. This phenomenon was observed also in lymphocytes of head-and-neck and cervix cancer patients during radiotherapy where the frequencies of micronuclei increased during the first half of therapy and declined thereafter, reaching, in some patients, values below the pre-treatment level [26, 31]. In all mentioned cases no attention was paid to outcome of the therapy. The oral mucosa cells of cancer patients under radiotherapy were studied along with the cervix cells. Unlike the cervix cells, even after receiving of high local dose of radiation (about 70 Gy), no significant increase was observed in the oral cells [23].

In 4 papers MN level was studied in cervix and oral mucosa tumor cells (Table 3) [24, 27–30]. In some cases MN frequencies in the tumor cells were higher than in the normal mucosa cells (e. g., 15 ‰ [27] and 11 ‰ [28] compared with the healthy subjects from India – 0.7–4.0 ‰ [32]). In two studies the linear increase of MN number in cancer cells with the dose of radiation was observed [24, 30]. In the exfoliated oral tumor cells of the patients with good outcome of radiotherapy the number of cells with MN was significantly higher than in the resistant ones to therapy [28]. In the cervix tumor cells MN have good predictive value after one week of therapy – high number of MN compared to the background level predict good response to radiotherapy [29]. The same results were obtained by the research group of Wiedel – they investigated the tumor cells obtained with biopsy instead of exfoliated tumor cells [3, 4].

Some groups of investigators studied along with MN in exfoliated cells also MN chromosomal aberrations and DNA damage (by means of the comet assay) in lymphocytes [25, 26]. Good correlation was observed with these genotoxicity endpoints, but all of them were more sensitive to radiation than MN assay in exfoliated cells. Two-three months after the end of the radiotherapy the level of MN in buccal cells but not in lymphocytes decreased. The number of cells with MN in buccal mucosa was higher than in the negative control, but not statistically significant [25]. In the same paper the authors paid attention to the influence of smoking on the MN level induced by radiotherapy, and found no effect even in heavy smokers (30 or more cigarettes per day).

It is well known that in lymphocytes of primary cancer patients, independent of stage and localization of the tumor, the significantly increased number of chromosomal aberrations and MN was reported [33–35]. In the normal buccal cells of the patients from Armenia with oral cavity cancer [24] and from Brazil with head and neck cancer [25] almost the same frequencies of MN were observed (0.8–0.9 ‰). This level did not differ significantly from the data obtaihed on the healthy persons of the same ethnicity – the negative control in both papers – were 0.7 ‰ in Armenians and 0.5 ‰ in Brazilians [17, 25]. In Chinese patients with nasopharyngeal cancer 2.3 ‰ cells with MN were observed [26] which is very close to the data reported concerning the healthy persons from China [36]. As for Mexican cancer patients, in all three presented groups the number of cells with MN significantly increased compared with 43 healthy persons from the same study (0.6 ‰) [18]. But at the same time 37 primary cancer patients with various localization of tumors in the same study used as a control did not show any increase compared with healthy subjects (0.8 ‰). It could be because of the previously treated with chemotherapy patients were included in the mentioned three groups along with the primary patients. Anyway, the data concerning background level of MN in epithelial cells of primary cancer patients are very contradictory. In head-and-neck patients from Brazil no difference was observed in MN frequency in the patients and the control subjects, and also between the patients at various stages of tumor [25]. But the results of some investigations witness about significantly increased number of MN in cancer patients. In 12 breast, 10 lung, 16 lymph nodes, and 21 cervix cancer patients from Armenia [37] and 30 oral cancer patients from Brazil [38] 2–3-fold significant

increase compared with the healthy subjects was reported. In the normal cervix cells of the patients with cervix cancer from Armenia the level of MN is significantly higher (3.9 ‰) than in healthy females with normal menstrual function, and also in pre- and postmenopausal ones [39, 40]. The background level of MN in epithelial cells of cancer patients and even in the healthy persons also warrants further investigations [41].

Hence, on this stage of knowledge it is not possible to predict the sensitivity of the patients to cytostatic chemotherapy based on MN level, and even the data on MN level in exfoliated epithelial cells of the patients under therapy are contradictory. In contrast, all studies showed significant increment of MN in tumor and normal mucosal cells due to radiotherapy. This increment was dose-dependent. It is extremely important that serial cytological assay of MN induction can potentially identify radiosensitivity of tumors and the treatment outcome. This technique is almost completely non-invasive and easily done in accessible primary cancers like of oral cavity and uterine cervix. In other sites, a fine needle cytology can be tried.

In conclusion, MN assay in epithelial tumors can be very useful in prognosis of sensitivity of tumors to radiotherapy. As for MN assay in the tumor patients treated with cytostatics, the further investigations in this area are certainly warranted to evaluate the application of this test for prognosis of treatment outcome.

РЕЗЮМЕ. Представлены данные относительно возможности использования теста на микроядра (МЯ) в эксфолиативных эпителиальных клетках онкологических больных, получающих противоопухолевую терапию, как биомаркера цитогенетического эффекта. Количество МЯ у больных, получавших химиотерапию, крайне противоречиво. Почти все исследователи показали, что как в опухолевых, так и в нормальных эпителиальных клетках после радиотерапии число МЯ достоверно увеличивается. Оценка числа МЯ, индуцированных радиотерапией в эксфолиативных эпителиальных клетках, потенциально может выявить радиочувствительность опухоли и эффективность лечения уже после первых сеансов терапии. Этот метод практически неинвазивен и может быть применен в оценке лечения опухолей ротовой полости и шейки матки.

РЕЗЮМЕ. Представлено дані відносно можливості використання тесту на мікроядра (МЯ) в ексфоліативних епітеліальних клітинах онкологічних хворих, що отримували протипухлинну терапію, як маркера цитогенетичного ефекту. Кількість МЯ у хворих, що отримували хіміотерапію, дуже суперечлива. Майже всі дослідники показали, що і в пухлинних клітинах, і в нормальних епітеліальних клітинах після радіотерапії число МЯ вірогідно збільшується. Оцінка числа МЯ, що індуковані радіотерапією в ексфоліативно епітеліальних клітинах, потенційно може виявити радіочутливість пухлини та ефективність лікування вже після перших сеансів терапії. Цей метод практично неінвазивний і може бути використаний для оцінки лікування пухлин ротової порожнини та шийки матки.

REFERENCES

1. *Schlomm T., Gunawan B., Schulten H.J., Sander B., Thangavelu K., Graf N., Leuschner I., Ringert R.H., Fuzesi L.* Effects of chemotherapy on the cytogenetic constitution of Wilms' tumor // Clin. Cancer Res. –2005. – **11**. – P. 4382–4387.
2. *Yin C.C., Glassman A.B., Lin P., Valbuena J.R., Jones D., Luthra R., Medeiros L.J.* Morphologic, cytogenetic, and molecular abnormalities in therapy-related acute promyelocytic leukaemia // Amer. J. Clin. Pathol. – 2005. – **123**. –P. 840–848.
3. *Widel M., Kolosza Z., Jedrus S., Lukaszczyk B., Raczek-Zwierzycka K., Swierniak A.* Micronucleus assay in vivo provides significant prognostic information in human cervical carcinoma; the updated analysis // Int. J. Radiat. Biol. – 2001. – **77**. – P. 631–636.
4. *Widel M., Jedrus S., Owczarek S., Konopacka M., Lubecka B., Kolosza Z.* The increment of micronucleus frequency in cervical carcinoma during irradiation in vivo and its prognostic value for tumour radiocurability // Brit. J. Cancer. – 1999. – **80**. – P. 1599–1607.
5. *Kutsuki S., Ihara N., Shigematsu N., Okamoto S., Kubo A.* Relation between chromosomal aberrations and radiation dose during the process of TBI // Radiat. Med. – 2005. – **23**. – P. 37–42.
6. *Lee R., Yamada S., Yamamoto N., Miyamoto T., Ando K., Durante M., Tsujii H.* Chromosomal aberrations in lymphocytes of lung cancer patients treated with carbonions // J. Radiat. Res. (Tokyo). – 2004. – **45**. – P. 195–199.
7. *Kopjar N., Garaj-Vrhovac V., Milas I.* Acute cytogenetic effects of antineoplastic drugs in peripheral blood lymphocytes in cancer patients chromosome aberrations and micronuclei // Tumori. – 2002. – **88**. – P. 300–312.
8. *Silva L.M., Takahashi C.S., Carrara H.H.* Study of chromosome damage in patients with breast cancer treated by two antineoplastic treatments // Teratog. Carcinog. Mutagen. – 2002. – **22**. – P. 257–269.
9. *Cairns J.* The cancer problem // Sci. Amer. – 1975. – **233**. – P. 64–72.
10. *Cairns J.* Mutation selection and the natural history of cancer // Nature. – 1975. –**255**. – P. 197–200.

11. *Majer B.J., Laky B., Knasmuller S., Kassie F.* Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials // Mutat. Res. – 2001. – **489**. – P. 147–72.
12. *Nersesian A.K.* The micronucleus test in human exfoliative cells as a method for studying the action of mutagens/carcinogens // Tsitol. Genet. – 1996. – **30**. – P. 91–96.
13. *Sarto F., Tomanin R., Giacomelli L., Canova A., Raimondi F., Ghiotto C., Fiorentino M.V.* Evaluation of chromosomal aberrations in lymphocytes and micronuclei in lymphocytes, oral mucosa and hair root cells of patients under antiblastic therapy // Mutat. Res. – 1990. – **228**. – P. 157–169.
14. *Casartelli G., Monteghirfo S., De Ferrari M., Bonatti S., Scala M., Toma S., Margarino G., Abbondandolo A.* Staining of micronuclei in squamous epithelial cells of human oral mucosa // Anal. Quant, Cytol. Histol. – 1997. – **19**. – P. 475–481.
15. *Nersesyan A.K.* Strange phenomenon, i.e., an antimutagenic effect of areca nut chewing // Mutat. Res. – 2005. – **582**. – P. 163–164.
16. *Nersesyan A.K.* Letter to Editor // Carcinogenesis. – 2001. – **22**. – P. 679.
17. *Nersesian A.K., Zil'fian V.N., Kumkumadzhian V.A., Nersesian A.K.* An analysis of the micronuclei in the oral mucosa of cancer patients for assessing the clastogenic effect of chemical preparations // Tsitol. Genet. – 1993. – **27**. – P. 77–80.
18. *Torres-Bugarin O., Ventura-Aguilar A., Zamora-Perez A., Gomez-Meda B.C., Ramos-Ibarra M.L., Morga-Villela G., Gutierrez-Franco A., Zuniga-Gonzalez G.* Evaluation of cisplatin + 5-FU, carboplatin + 5-FU, and ifosfamide + epirubicine regimens using the micronuclei test and nuclear abnormalities in the buccal mucosa // Mutat. Res. – 2004. – **565**. – P. 91–101.
19. *Machado-Santelli G.M., Cerqueira E.M., Oliveira C.T., Pereira C.A.* Biomonitoring of nurses handling antineoplastic drugs // Mutat. Res. – 1994. –**322**. – P. 203–208.
20. *Odio A.D., Duharte A.B., Carnesoltas D., Garcia L., Loaces E.L., Cabrera L.G.* Cytogenetis effect of occupational exposure to cytostatics // Rec. Med. IMSS. – 2004. – **42**. – P. 487–492.
21. *Cavallo D., Ursini C.L., Perniconi B., Francesco A.D., Giglio M., Rubino F.M., Marinaccio A., Iavicoli S.* Evaluation of genotoxic effects induced by exposure to antineoplastic drugs in lymphocytes and exfoliated buccal cells of oncology nurses and pharmacy employees // Mutat. Res. – 2005. – **587**. – P. 45–51.
22. *Burgaz S., Karahalil B., Bayrak P., Taskin L., Yavuzaslan F., Bokesoy I., Anzion R.B., Bos R.P., Platin N.* Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics // Mutat. Res. – 1999. – **439**. – P. 97–104.
23. *Vartazaryan N.S.* Investigation of cytogenetic disturbances in exfoliated human cells by means of micronucleus assay. PhD thesis. – Yerevan, Armenia, 2003.
24. *Nersesyan A.K.* The study of micronuclei frequency in buccal mucosa cells of oral cancer patients under radiotherapy // Proc. XVI Intern. Cancer Congress (New Delhi, India) / Ed. R.S. Rao. – Bologna, 1994. – P. 95–98.
25. *Minicucci E.M., Kowalski L.P., Maia M.A., Pereira A., Ribeiro L.R., de Camargo J.L., Salvadori D.M.* Cytogenetic damage in circulating lymphocytes and buccal mucosa cells of head-and-neck cancer patients undergoing radiotherapy // J. Radiat. Res. (Tokyo). – 2005. – **46**. – P. 135–142.
26. *Cao J., Liu Y., Sun H., Cheng G., Pang X., Zhou Z.* Chromosomal aberrations, DNA strand breaks and gene mutations in nasopharyngeal cancer patients undergoing radiation therapy // Mutat. Res. – 2002. – **504**. – P. 85–90.
27. *Bhattathiri N.V., Bindu L., Remani P., Chandralekha B., Nair K.M.* Radiation-induced acute immediate nuclear abnormalities in oral cancer cells: serial cytologic evaluation // Acta. Cytol. – 1998. – **42**. – P. 1084–1090.
28. *Bhattathiri N.V., Bharathykkutty C., Prathapan R., Chirayathmanjiyil D.A., Nair K.M.* Prediction of radiosensitivity of oral cancers by serial cytological assay of nuclear changes // Radiother. Oncol. – 1998. – **49**. – P. 61–65.
29. *Singh S., Datta N.R., Krishnani N., Lal P., Kumar S.* Radiation therapy induced micronuclei in cervical cancer—does it have a predictive value for local disease control? // Gynecol. Oncol. – 2005. – **97**. – P. 764–771.
30. *Bindu L., Balaram P., Mathew A., Remani P., Bhattathiri V.N., Nair M.K.* Radiation-induced changes in oral carcinoma cells – a multiparametric evaluation // Cytopathology. – 2003. – **14**. – P. 287–293.
31. *Widel M., Jedrus S., Lukaszczyk B., Raczek-Zwierzycka K., Swierniak A.* Radiation-induced micronucleus frequency in peripheral blood lymphocytes is correlated with normal tissue damage in patients with cervical carcinoma undergoing radiotherapy // Radiat. Res. – 2003. – **159**. – P. 713–721.
32. *Nersesyan A.K.* Arsenic and drinking water in West Bengal // Cancer Epidemiol. Biomarkers Prev. –**14**. – 2005. – P. 757–759.
33. *Venkatachalam P., Paul S.F., Mohankumar M.N., Prabhu B.K., Gajendiran N., Kathiresan A., Jeevanram R.K.* Higher frequency of dicentrics and micronuclei in peripheral blood lymphocytes of cancer patients // Mutat. Res. – 1999. – **425**. – P. 1–8.
34. *Duffaud F., Orsiere T., Digue L., Villani P., Volot F., Favre R., Botta A.* Micronucleated lymphocyte rates from head-and-neck cancer patients // Mutat. Res. – 1999. – **439**. – P. 259–266.

35. *Baciuchka-Palmaro M., Orsiere T., Duffaud F., Sari-Minodier I., Pompili J., Bellon L., De Meo M., Digue L., Favre R., Botta A.* Acentromeric micronuclei are increased in peripheral blood lymphocytes of untreated cancer patients // Mutat. Res. – 2002. – **520**. – P. 189–198.
36. *Ying C.J., Yan W.S., Zhao M.Y., Ye X.L., Xie H., Yin S.Y., Zhu X.S.* Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class // Biomed. Environ. Sci. – 1997. – **10**. – P. 451–455.
37. *Nersesyan A.K., Vartazaryan N.S., Gevorgyan A.L., Arutyunyan R.M.* Micronucleus level in exfoliated buccal mucosa cells of cancer patients // Arch. Oncol. – 2002. – **10**. – P. 193–194.
38. *Ramirez A., Saldanha P.H.* Micronucleus investigation of alcoholic patients with oral carcinomas // Genet. Mol. Res. – 2002. – **1**. – P. 246–260.
39. *Nersesyan A.K.* Micronuclei and other nuclear anomalies in exfoliated cells of gynaecological cancer patients // Polish J. Environ. Studies. – 2002. – **11**. – P. 58–61.
40. *Nersesyan A.K., Vartazaryan N.S., Arutyunyan R.M.* Micronuclei and other nuclear anomalies in exfoliated buccal and uterine cervical cells of healthy Armenian women // Central Eur. J. Occup. Envir. Med. – 2002. – **8**. – P. 39–43.
41. *Nersesyan A.K.* Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test // Genet. Mol. Res. – 2005. – **4**. – P. 174–176.

Received 25.04.06