

Assessment of cytogenetic effect of antineoplastic therapy by means of micronucleus assay in exfoliated epithelial cells

Review Article

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Summary

Literature data concerning possibility to use micronuclei (MN) level in exfoliated epithelial cells of patients under radio- and chemotherapy as a biomarker of cytogenetic effect are presented and discussed. The number of MN in buccal cells of patients under chemotherapy are very few and contradictory. Significant dose-dependent increment of MN in tumor and normal epithelial cells due to radiotherapy of cancer patients was shown by almost all investigators. 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. MN assay is almost completely non-invasive and easily done in accessible tumors (oral cavity and uterine cervix).

I. Introduction

It is well established that radio- and chemotherapy widely used for treatment of cancer patients induce chromosomal aberrations and micronuclei (MN) both in tumor (Widel et al, 1999, 2001; Schlomm et al, 2005, Yin et al, 2005) and normal (healthy) cells (Kutsuki et al, 2005, Lee et al, 2004). Because of technical difficulties (invasive procedure to obtain tumor cells before, during and after treatment) cytogenetic disturbances in organism due to the therapy are mostly studied in lymphocytes (Kopjar et al, 2002, Silva et al, 2002). These cells are the most used targets for biomonitoring of cytogenetic effect of cancer treatment.

Of course, it would be of interest to monitor cytogenetic alterations not in surrogate tissue – lymphocytes, but in the target, tumor cells. It is possible to obtain with minimal invasion and then to study exfoliated epithelial cells (EEC). It is noteworthy that about 90% of all human tumors are derived from this tissue (Cairns 1975a,b).

MN assay in EEC is used to study clastogenic/aneugenic effects of agents of various origin (Nersisyan et al, 1996; Majer et al, 2002). It has been shown that exposure of persons to many environmental

and occupational pollutants can lead to increased level of MN in epithelial cells. Also some lifestyle habits, such as tobacco smoking, khat, areca nut and betel chewing can be reasons of MN induction in oral mucosal cells. In some diseases, including precancerous ones and cancer, increment of MN was also frequently observed (Nersisyan et al, 1996; Majer et al, 2002).

The aim of this paper was to evaluate the data concerning MN level in EEC of cancer patients as possible biomarkers of cytogenetic effect of antineoplastic chemo- and radiotherapy.

In review paper by Majer et al, 2002 three articles were cited concerning radiotherapy of oral cancer (totally 8 subjects, with MN increase in not affected by tumor cells in all cases), treatment of thyroid cancer with ¹³¹I (31 subjects, negative result), and one paper concerning cancer chemotherapy (7 subjects, positive results in 5 persons, and correlation with MN numbers in lymphocytes) (Majer et al, 2002). It should be added to the last cited paper (Sarto et al, 1990), that the increment of MN both in EEC and lymphocytes were not observed in two subjects treated only with interferon which is absolutely non-genotoxic. Hence, good agreement (and in one case correlation) was observed between the responsibility of two types of cells

(oral mucosa and lymphocytes) to genotoxic action of chemotherapeutic drugs.

The papers available via Medline and Scopus were analysed. The most important data concerning MN induction in EEC by radio- and chemotherapy are presented in **Tables 1-3** (age and sex of subjects, 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 have only stain used (DNA-specific or no) and the number of studied cells (Casartelli et al, 1997; Nersesyan 2001, 2005).

II. MN in cells of patients under chemotherapy

Only 4 papers were found concerning MN induction in exfoliated epithelial cells due to antitubercular chemotherapy. One was already analysed by Majer et al 2002, others are presented in **Table 1**. In paper by Nersesyan et al, 1993, 6 males and 4 females with lymphogranulomatosis, 4 males with lung cancer and 7 females with breast cancer were analysed a week after various schedules of antitubercular chemotherapy. Significantly increased number of MN (3.2-fold) was observed. In 21 Mexican patients with various localization of tumor treated with isophosphamide+epirubicin significantly increased number of MN was registered (from 1.2‰ before to 2.6‰ after treatment) (Torres-Bugarin et al, 2004). 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 second and even 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 $1.6 \pm 0.4\%$ before and $2.6 \pm 0.7\%$ after treatment ($p > 0.05$, Mann Whitney test). In 10 patients treated with carboplatin+5-fluorouracil MN numbers were $0.95 \pm 0.12\%$ before and $1.35 \pm 0.42\%$ after treatment, and in 9 patients treated with isophosphamide+epirubicin the frequencies were $2.9 \pm 1.0\%$ (before) and $4.9 \pm 1.6\%$ (after treatment) ($p > 0.05$ in both cases, Mann Whitney U-test). In another paper Torres-Bugarin and colleagues used in 1998 cells of 10 cancer patients after course of antitubercular chemotherapy as a positive control in their study, but they did not report about the sites of tumors, age and sex of the patients. In this investigation the number of cells with MN was increased significantly 3.3-fold.

Hence, two papers (Nersesyan et al, 1993; Torres-Bugarin et al, 1998) reported about significant increment of MN induced by antitubercular chemotherapy and one the same effect only due to one schedule of therapy (Torres-Bugarin et al, 2004). Two other schedules used for treatment of the patients did not increase the number of MN in buccal cells. But as it was mentioned earlier, there is no possibility to evaluate real increment of MN (if any) because many patients were not primary, i. e. they received polychemotherapy previously (before the last

treatment) and then cells were collected for the investigations. Correlations between MN level induction in somatic epithelial cells and treatment results are unknown because no data were published about the treatment outcome of the patients after chemotherapy.

Although contradictory results were published concerning chemotherapy action on MN level in buccal mucosa cells, it is noteworthy that in four studies significantly increased level of MN was observed in buccal cells of nurses handling cytostatic drugs (1.6-2.0-fold) (Machado-Santelli et al, 1994; Odio et al, 2004; Cavallo et al, 2005, 2007), and in one case 2-fold not significant increase (Burgaz et al, 1999). It is really surprising result because nurses are exposed to antitubercular drugs during preparation of the drug solutions for injections by inhalation and possibly via skin. Of course, the doses of cytostatics received by the nurses are significantly lower than that received by the patients, but all cases the number of MN was increased in nurses unlike the patients. Torres-Bugarin and colleagues mentioned in 2004 that many of patients under chemotherapy had signs of toxicity, and this circumstance could influence on MN induction in EEC. Anyway, this phenomenon warrants further investigations.

III. MN in cells of patients under radiotherapy

The data concerning the frequencies of MN induced by radiotherapy in healthy (normal) and tumor EEC are presented in **Tables 2 and 3**, respectively. In **Table 2** are presented the most important data of six papers concerning studied cells with no sign of pathology (Cao et al, 2002, Guzman et al, 2003, Mehrotra et al, 2004a, Minicucci et al, 2005, Nersesyan 1994, Vartazaryan 2003), e.g. they were obtained from opposite site of tumors localization, or from the same site, close to the tumor. In other papers the results of studies of tumor cells during and/or after radiotherapy are presented (**Table 3**) (Bhattathiri et al, 1998a,b; Bindu et al, 2003; Mehrotra et al, 2004b; Rimpu et al, 2005; Singh et al, 2005). Both in normal and tumor cells significant increase of MN was observed due to radiation. It is important that in normal cervical (Vartazaryan 2003) and buccal (Cao et al, 2002; Guzman et al, 2003; Minicucci et al, 2005) 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‰) (Vartazaryan 2003). In buccal mucosa 8.8‰ cell with MN were observed after the dose of 48 Gy, and only 7.6‰ after 68 Gy (Vartazaryan 2003). This phenomenon was observed also in lymphocytes of head-and-neck and cervix cancer patients during radiotherapy where the frequencies of MN increased during the first half of therapy and declined thereafter, reaching, in some patients, values below the pre-treatment level (Tolbert et al, 1991; Nersesyan 1994; Bhattathiri et al, 1998a,b; Cao et al, 2002; Bindu et al, 2003; Guzman et al, 2003, Mehrotra et al, 2004a,b; Minicucci et al, 2005; Rimpu et al, 2005; Singh et al, 2005). In all mentioned cases no attention was

paid to outcome of therapy. In the study of Vartazaryan in 2003 oral mucosa cells of cervix uterus cancer patients under radiotherapy were studied along with cervix cells (to check possible vulnerability of cells located on long distance from irradiated zone due to circulating reactive oxygen species and/or other genotoxic substances appeared due to irradiation). Unlike cervix cells, even after receiving of high local dose of radiation, no significant increase was observed in oral EEC (Vartazaryan 2003).

In seven papers MN levels changes were reported in cervix and oral mucosa tumor cells of cancer patients under radiotherapy (**Table 3**) (Bhattathiri et al, 1998a,b, Bindu et al, 2003, Mehrotra et al, 2004b, Rimpu et al, 2005, Singh et al, 2005). In some cases MN frequencies in tumor cells were higher than in normal mucosa cells (e.g., 15‰ (Bhattathiri et al, 1998a) and 11‰ (Bhattathiri et al, 1998b) compared with healthy subjects from India – 0.7‰-4.0‰ (Nersesyan, 2006). In all studies linear increase of MN number in cancer cells with dose of radiation was observed (Bhattathiri et al, 1998a,b, Bindu et al, 2003, Mehrotra et al, 2004b, Rimpu et al, 2005, Singh et al, 2005). It is very important that in EEC of oral tumors of patients with good outcome of radiotherapy the number of cells with MN was higher than in resistant to therapy, significant in one case (Bhattathiri et al, 1998b) and not

significant in another (Bhattathiri et al, 1998b). In 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 (Singh et al, 2005). The same results were obtained by the research group of Widel – but they instead of EEC of tumor investigated tumor cells obtained with biopsy (Widel et al, 1999, 2001).

Some groups of investigators studied with MN in EEC also MN, chromosomal aberrations and DNA damage (by means of the comet assay) in lymphocytes (Cao et al, 2002, Guzman et al, 2003, Minicucci et al, 2005). Good correlation was observed with these genotoxicity endpoints, but all of them were more sensitive to radiation than MN assay in EEC. Two-three months after the end of 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 negative control, but not statistically significant (Minicucci et al, 2005). In the same paper the authors paid attention to the influence of smoking on MN level induced by radiotherapy, and found no effect even in heavy smokers (30 or more cigarettes per day consumers) (Minicucci et al, 2005).

Table 1. Micronuclei frequency in buccal cells of cancer patients under antitubercular chemotherapy.

Treatment	Number of subjects, (age)	of sex, cells	Type of cells	Number of cells MN (‰)	of with studied per subject)	Stain	(cells per subject)	Remarks	Reference
Chemotherapy (cancer of various sites, various schedules)	10m+12f (54) control – the same pts before therapy		Buccal	3.2 1.0 0.8		Feulgen fast green (2000)	+	Effect of exposure: ↑ x 3.2	Nersesian et al, 1993
Chemotherapy (cancer of various sites, isophosphamide+e pirubicin)	13m+8f patients (48.9) control – the same pts before therapy	cancer	Buccal	2.6 1.2		Orcein (2000)		Effect of exposure: ↑ x 2.3	Torres-Bugarin et al, 2004
Chemotherapy (cancer of various sites, carboplatin+5-fluorouracil)	9m+5f patients (49.7) control – the same pts before therapy	cancer	Buccal	1.3 1.0		Orcein (2000)		Effect of exposure: ↔	Torres-Bugarin et al, 2004
Chemotherapy (cancer of various sites, cisDDP+5-fluorouracil)	6m cancer patients (61) control – the same pts before therapy		Buccal	2.7 1.5		Orcein (2000)		Effect of exposure: ↓	Torres-Bugarin et al, 2004
Chemotherapy (cancer of various sites, not specified; drugs used – cyclophosphamide, cytosine-arabioside, epirubicin)	10 (sex not specified) control – the same pts before therapy	not specified		3.7 1.1		Feulgen fast green (2000)	+	Effect of exposure: ↑ x 3.3	Torres-Bugarin et al, 1998

Symbols: ↑ - significant increase; ↔ - no effect; ↓ - either increase or decrease, but not significant; pts – patients; f – female, m – male

Table 2. Micronuclei frequency in normal (not cancerous) epithelial cells of cancer patients under radiotherapy.

Treatment (dose of radiation)	Number of subjects, sex, (age)	Number of cells with MN (%)	Stain (cells studied per subject)	Remarks	Reference
Radiotherapy of cervix cancer (50.0 Gy) ¹	14f (50) the same pts	8.0 – (35.0 Gy) 7.4 – (70.0 Gy) 2.9	Feulgen + fast green (2000)	Effect of exposure: ↑ x 2.0 (25 Gy) and ↑ x 1.8 (50 Gy) [significantly less than in the cells of pts who received 35.0 Gy]	Vartazaryan 2003
Radiotherapy of squamous cell carcinoma of oral cavity	8m+6f (61) the same pts	4.8 (25 Gy) 6.2 (15 Gy) 0.9	Feulgen + fast green (2000)	Effect of exposure: ↑ x 5.3 (25 Gy), ↑ x 6.8 (15 Gy)	Nersesyan 1994
Radiotherapy of head and neck cancer 6MeV linear accelerator (X-ray, equivalent body dose 3.3 Gy)	25m, 6f (59) the same pts	2.3 0.8	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)	Minicucci et al, 2005
Radiotherapy of cancer of cervix uterus ¹	39f (age nor specified) the same pts	0.9 0.6	Feulgen + fast green (2000)	Effect of exposure: ↓ x 1.5	Guzman et al, 2003
Radiotherapy of nasopharyngeal cancer (68.0 Gy)	9m (36) the same pts	7.7 – (28 Gy) 8.8 – (48 Gy) 7.6 – (68 Gy) 2.3	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)	Cao et al, 2002
Radiotherapy of squamous cell carcinoma of oral cavity	78m, 33f (age nor specified) the same pts	4.4 - (24 Gy) 1.8 - (6.0 Gy) 0.9	Giemsa (1000)	Effect of exposure: ↑ x 2.0 (6 Gy) ↑ x 4.4 (24 Gy)	Mehrotra et al, 2004a

¹ – cervix cells were studied, in all other cases buccal cells were studied

↑ - significant increase; ↔ - no effect; ↓ - either increase or decrease, but not significant; pts – patients; f – female, m - male

Table 3. Micronuclei frequency in tumor cells of cancer patients under radiotherapy

Treatment (dose of radiation)	Number of subjects, sex, (age)	Number of cells with MN (%)	Stain (cells studied per subject)	Remarks	Reference
Radiotherapy of squamos cell carcinoma of oral cavity	31 (sex and age not specified) the same pts	19.5 (24 Gy) 2.8	Giensa (1000)	Effect of exposure: ↑ x 7.1	Bhattathiri et al, 1998a
Radiotherapy of squamos cell carcinoma of oral cavity	49 (sex and age not specified) the same pts	25.2 (24 Gy, 21 sensitive to treatment pts) 15.0 (24 Gy, 28 resistant to treatment pts) 4.1	Giensa (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	Bhattathiri et al, 1998b
Radiotherapy of cervix cancer	25f (52) the same pts	42.0 15.0	May-Grunwald-Giensa (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	Singh et al, 2005
Radiotherapy of squamos cell carcinoma of oral cavity	68 (sex not specified) (62) the same pts	43 – (7.0 Gy) 55 – (17.5 Gy) 71 – (28 Gy) 78 – (38.5 Gy) 11	Giensa (500)	Effect of exposure: linear, maximally at maximum dose ↑ x 7.7	Bindu et al, 2003
Radiotherapy of squamos cell carcinoma of oral cavity	78m, 33f the same pts	6.0 - (24 Gy) 1.1	Giensa (1000)	Effect of exposure: ↑ x 5.4 (24 Gy)	Mehrotra et al, 2004a
Radiotherapy of squamos cell carcinoma of oral cavity	102 m the same pts	14.1 1.6	Giensa (1000)	Effect of exposure: ↑ x 8.8	Mehrotra et al, 2004b
Radiotherapy of head and neck cancer (squamos cell carcinoma of oral cavity -6, carcinoma of base of tongue-12 and others)	27m, 3 f the same pts	7.7 (4 Gy) 8.8 (14 Gy) 12.8 (24 Gy) 3.5	Giensa (750)	Effect of exposure: ↑ x 3.7 at 24 Gy Linear increase of the number of MN with the dose	Rimpu et al, 2005
Radiotherapy of epidermoid carcinoma of oral cavity (buccal mucosa - 21, gingival – 8, palate – 3: one site; 12 – more than one site)	34m, 10f the same pts	27.7 (28 Gy, sensitive) 18.3 7 (28 Gy, resistant) 2.0	Giensa (750)	Effect of exposure: ↑ x 13.9 sensitive to therapy tumors, ↑ x 9.1 resistant Linear increase of the number of MN with the dose. No significant difference between two groups of pts.	Bhattathiri et al, 1998b

↑ - significant increase; ↔ - no effect; ↓ - either increase or decrease, but not significant; pts – patients; f – female, m - male

Hence, two papers (Nersesian et al, 1993; Torres-Bugarin et al, 1998) reported about significant increment of MN induced by antineoplastic chemotherapy and 1 the same effect only due to one schedule of therapy. Two

other schedules used for treatment of the patients did not increase the number of MN in buccal cells. But as it was mentioned earlier, there is no possibility to evaluate real increment of MN (if any) because many patients were not

primary, i. e. they received polychemotherapy previously (before the last treatment) and then cells were collected for the investigations. Correlations between MN level induction in somatic epithelial cells and treatment results are unknown because no data were published about the treatment outcome of the patients after chemotherapy.

Although contradictory results were published concerning chemotherapy action on MN level in buccal mucosa cells, it is noteworthy that in four studies significantly increased level of MN was observed in buccal cells of nurses handling cytostatic drugs (1.6-2.0-fold) (Machado-Santelli et al, 1994; Odio et al, 2004; Cavallo et al, 2005, 2007), and in one case 2-fold not significant increase (Burgaz et al, 1999). It is really surprising result because nurses are exposed to antitubercular drugs during preparation of the drug solutions for injections by inhalation and possibly via skin. Of course, the doses of cytostatics received by the nurses are significantly lower than that received by the patients, but all cases the number of MN was increased in nurses unlike the patients. Torres-Bugarin and colleagues mentioned in 2004 that many of patients under chemotherapy had signs of toxicity, and this circumstance could influence on MN induction in epithelial cells. Anyway, this phenomenon warrants further investigations.

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were studied along with cervix cells (to check possible vulnerability of cells located on long distance from irradiated zone due to circulating reactive oxygen species and/or other genotoxic substances appear due to irradiation). Unlike cervix cells, even after receiving of high local dose of radiation, no significant increase was observed in oral cells (Vartazaryan, 2003).

In seven papers MN levels changes were reported in cervix and oral mucosa tumor cells of cancer patients under radiotherapy (**Table 3**) (Bhattathiri et al, 1998a, b, c; Bindu et al, 2003; Mehrotra et al, 2004a; Rimpu et al, 2005; Singh et al, 2005). In some cases MN frequencies in tumor cells were higher than in normal mucosa cells (e. g., 15‰ (Bhattathiri et al, 1998c) and 11‰ (Bhattathiri et al, 1998a) compared with healthy subjects from India – 0.7‰-4.0‰ (Nersesyan, 2006b)). In all studies linear increase of MN number in cancer cells with dose of radiation was observed (Bhattathiri et al, 1998a, b, c; Bindu et al, 2003; Mehrotra et al, 2004a; Rimpu et al, 2005; Singh et al, 2005). It is very important that in exfoliated oral tumor cells of patients with good outcome of radiotherapy the number of cells with MN was higher than in resistant to therapy, significant in one case (Bhattathiri et al, 1998a) and not significant in another (Bhattathiri et al, 1998b). In 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 (Singh et al, 2005). The same results were obtained by the research group of Wiedel – but they instead of exfoliated tumor cells investigated tumor cells obtained with biopsy (Wiedel et al, 1999, 2001).

Some groups of investigators studied with MN in exfoliated cells also MN, chromosomal aberrations and DNA damage (by means of the comet assay) in lymphocytes (Cao et al, 2002; Minicucci et al, 2005). 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 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 negative control, but not statistically significant (Minicucci et al, 2005). In the same paper the authors paid attention to the influence of smoking on MN level induced by radiotherapy, and found no effect even in heavy smokers (30 or more cigarettes per day consumers) (Minicucci et al, 2005).

IV. Nuclear anomalies in exfoliated cells of subjects exposed to cytostatic drugs and under radiotherapy

It is well known that in EEC except the MN also other events called nuclear anomalies (NA) can be registered, e. i. karyorrhexis (nucleus broken to pieces), karyolysis (lysed nucleus which appears as a ghost), binucleated cells (cells with 2 nuclei), pycnosis (very small, shrunken nucleus), budded cells (cells with budded nucleus including so-called “broken egg” phenomenon – a MN attached to main nucleus with the stalk) and

condensed chromatin (Tolbert et al, 1991). Sometimes these NA can be wrongly considered as MN.

Although the pioneers of the investigations of NA Tolbert and colleagues proposed in 1991 that some of them, namely binucleates and “broken egg” phenomenon can be connected with genotoxicity, recently two papers were published stating that cells with “broken egg” phenomenon appear not due to genotoxic effect (Nersesyan, 2006a,b). Some investigators proposed that NA are consequences of cytotoxic effects and apoptosis (Cerqueira et al, 2004; Torres-Bugarin et al, 2004; Angelieri et al, 2007). The real meaning of NA is unknown although they should be registered separately from cells with MN because in some cases NA can mimic real MN (Nersesyan et al, 2006a).

Only two investigations concerning the effect of chemotherapy on NA in EEC are available. Torres-Bugarin and colleagues shown in 1998, 2004 that due to only certain schedules of chemotherapy some changes in NA frequencies can be detected. Namely, the number of cells with karyolysis increased significantly in patients treated with cisplatin + fluorouracil, isophosphamid + epirubicin. At the same time, the number of binucleates decreased significantly in buccal cells of patients under chemotherapy. In one study (Odio et al, 2004) was shown that in oral EEC of nurses handling cytostatic drugs the frequencies of all NA were increased compared with non-exposed subjects.

Unlike chemotherapy, almost all investigators registered substantial increase of NA frequencies in EEC of cancer patients under radiotherapy. Studying EEC of patients under radiotherapy, Indian investigators proposed some new features of cells, both normal (healthy) and tumor ones, e. g. multinucleated cells (Bindu et al, 2003; Mehrotra et al, 2004b; Rimpu et al, 2005) and cytoplasmic granulation (Bindu et al, 2003; Mehrotra et al, 2004b). Since they did not present the photos of so-called granulation it is not possible to understand what they mean, and is it the same as condensed chromatin.

As it was mentioned above, the real meaning of all of these NA is unknown. Recently the group of Indian investigators proposed that binucleates and multinucleation in exfoliated cells could be due to viral infection (Mehrotra et al, 2006). de Almeida and colleagues proposed in 2004 that the cells with ‘broken egg’ phenomenon observed in human liver affected with hepatitis C virus were due to viral infection (de Almeida et al, 2004).

Anyway, it is noteworthy that in all cases the number of all nuclear anomalies was increased significantly compared with the levels before the treatment and this increase was linear.

V. Conclusions

In this review an attempt was carried out to analyse all available papers concerning the effect of chemo- and radiotherapy on MN induction in EEC of patients under antitublastic therapy.

Based on the small number of papers concerning an effect of cytostatic drugs it is not possible to come to certain conclusions. Absence of changes in MN number in

EEC of patients receiving some schedules of chemotherapy could be explained by toxic effect of therapy because in nurses who were exposed to many times less doses of cytostatics the increment of cells with MN was registered.

In contrast, all studies showed significant increment of MN and other nuclear anomalies in tumor and normal EEC due to radiotherapy. This increase was dose-dependent. It is extremely important that serial cytological assay of MN induction can potentially identify radiosensitivity of tumors and the treatment outcome.

The technique to apply MN assay in EEC is almost completely non-invasive and easily done in accessible primary cancers (i.e. oral cavity and uterine cervix). In other sites, fine needle cytology can be applied.

It should be mentioned that although MN assay in EEC has many advantages it is less sensitive to register the effects of radiotherapy than conventional chromosomal aberrations and MN assays in lymphocytes and the comet assay in lymphocytes.

In conclusion, MN assay in EEC of tumors could be very useful in prognosis of sensitivity of tumors to radiotherapy unlike MN assay in patients treated with cytostatics. Further investigations in this area are certainly warranted to evaluate possibility of the application of this test for prognosis of treatment outcome.

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