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THE MICRONUCLEUS ASSAY IN EXFOLIATED HUMAN CELLS: A MINI-REVIEW OF PAPERS FROM THE CIS

The critical analysis of the data concerning micronucleus assay in exfoliated epithelial cells presented by the investigators from the CIS is carried out. Twenty two articles are evaluated, and shortcomings of some of them are discussed. Presented results are compared whenever possible with literature data. The aim of the mini-review is a criticism of shortcomings of the papers for further improvement of the presentation of the data on micronucleus assay which will give the possibility to compare the results with the data presented by foreign investigators.

About 25 years ago, Stich and coworkers [1] developed a protocol for micronucleus (MN) assay with exfoliated human epithelial cells (EC) which was widely used in occupational and life style studies [2—4]. MN are formed by the exclusion of whole chromosome(s) or chromosomal fragments during cell division, and their presence in cells is a reflection of structural and/or numerical aberrations (clastogenic and/or aneugenic effects) [1, 2].

It was repeatedly emphasized that this non-invasive method might be a suitable biomonitoring approach for the detection of increased cancer risk in man since > 90 % of all human cancers are of epithelial origin [2]. Approximately 250 studies with EC have been published in the last 25 years and apart from mouth cells (buccal, tongue and lip mucosa), also EC from other organs such as nasal mucosa, cervix, bladder, oesophagus und bronchi were used as well [2]. Among the mentioned papers there are investigations carried out by the scientists from the CIS (22 of them). The papers were found using PubMed-MedLine, Scopus and Scholar. Google searching systems.

Recently one of these papers was criticised by Speit and Schmid [5] because of methodological errors and shortcomings. According to the request of Dr. M. Fenech, supervisor of «Human Micro-Nucleus Project» (SCIRO, Australia) we tried to evaluate and analyse the data on MN in EC published in Russian to include them in the review paper concerning MN assay in EC. In some cases it was not possible due to poor presentation of the results and methodological shortcomings.

The aim of the present paper is a critical evaluation and analysis of available publications carried out by the scientists from the CIS.

The most important information about the study with short comments is presented in Table. Nine studies were carried out in Armenia, 1 — in Georgia, 9 — in Russia, and 3 in Ukraine.

There are some methodological approaches concerning the preparation and staining, and also the criteria of registration of MN in EC.

1. About 10—14 days last between the MN formation in the basal level and migration of cells to the surface where they can be exfoliated and then studied [2]. Hence, EC should be collected and studied no earlier than 10 days after acute exposure.

2. It is important to wash EC some times in physiological saline or in the special buffer (0.1 EDTA, 0.01 Tris-HCl and 0.02 M NaCl, pH 7.0)

The main data from the papers of scientists from the CIS concerning MN assay in exfoliated epithelial cells

Country	Exposure or studied groups	Number of subjects, gender, (age)	Type of cellsstudied	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks and reference
1. Armenia	Various cytostatic drugs in cancer pts	10 m 12 f (44) Control—10 m 10 f (33)	Buccal	3.1 0.9	Feulgen 1000	Effect of exposure: ↑ × 3.2[11]
2. Armenia	Primary cancer pts (breast, lung, cervix uteri, lymph nodes)	20m (51) Control — 41 f (46)	Buccal	1.8—2.1 1.0	Feulgen 1000	In cancer pts: ↑ × 2.0[12]
3. Armenia	Women with hirsutism	20 f (32) Control — 20 f (29)	Buccal	2.3 1.1	Feulgen 2000	In pts: ↑ × 2.0[13]
4. Armenia	Women with normal menstrual function and postmenopausal	10 f (52) Control — 10 f (27)	Buccal Cervicalcal	1.3 1.0 1.5 1.2	Feulgen 2000	↔ [14]
5. Armenia	Gynecological cancer pts	27 f (cervix cancer, 54) 16 f (corpus uteri cancer, 56) 18 f (myoma, 47) control — 42 f (44)	Buccal	2.8 2.7 1.5 1.3	Feulgen 2000	In cancer pts: ↑ × 2.0; in pts with myoma: ↔ [15]
6. Armenia	Gynecological cancer pts	27 f (cervix cancer, 54) 16 f (corpus uteri cancer, 56) 18 f (myoma, 47) control — 42 f (44)	Cervical	2.8 2.9 1.8 1.5	Feulgen 2000	In cancer pts: ↑ × 1.8; in pts with myoma: ↔ [15]
7. Armenia	Primary cancer pts and pts with precancerous diseases	12 f (breast cancer, 47) 8 f (fibroadenoma of breast, 43) 28 f (cervix cancer, 51) 8 f (corpus uteri cancer, 46) 8 f (myoma, 45) Control — 15 f (49)	Buccal	2.1 1.1 2.1 3.0 1.0 0.9	Feulgen 2000	In cancer pts: ↑ × 1.8; in pts with myoma and breast fibroadenoma: ↔ [16]
8. Armenia	Radiotherapy 35.0 Gy	14f (50) Control — the same subjects before therapy	Cervix	8.0 3.9—before therapy	Feulgen + fast green 2000	Effect of exposure: ↑ × 2.0[17]
9. Armenia	Radiotherapy 70.0 Gy	14f (50) Control — the same subjects before therapy	Cervix	7.4 3.9—before therapy	Feulgen + fast green 2000	Effect of exposure: ↑ × 1.8[17]
10. Armenia	Primary cervix cancer pts	30f (50) Control — 20 (52)	Buccal	3.1 1.2	Feulgen + fast green2000	In pts: ↑ × 2.5[17]
11. Armenia	Primary cervix cancer pts	30f (50) Control — 20 (52)	Cervix	3.3 1.3	Feulgen + fast green 2000	In pts: ↑ × 2.5[17]
12. Armenia	Radiotherapy of oral cavity cancer pts (25 Gy)	8m+6f (61) Control — the same pts before therapy	Buccal	28.0 0.9	Feulgen + fast green 2000	Effect of exposure: ↑ × 31.1[18]
13. Armenia	Influence of age on MN incidence	60f (44)	Buccal	3.0 (60—69) 1.5 (20)	Feulgen + fast green 2000	Effect of age : direct correlation, r = +0.44[19]
14. Georgia	Exposure to arsenic in drinking water	50m+f (10) Control — 100m+f (11)	Buccal	22.4 4.4	Feulgen + fast green 1000	Effect of exposure: ↑ × 6.1 — fold. All exposed had bad health — acute respiratoryinfections [28]
15. Russia	Exposure to formaldehyde of teachers and students	8m+7f (51) Control — 7f (46) 6m+6f (18)	Buccal	1.2 m 2.9 f 0.64 0.8m 0.6f before exposure	Feulgen 2000	The results possibly showed other than chemical exposure, because the effect of exposure can be re-

Continue of table

Country	Exposure or studied groups	Number of subjects, gender, (age)	Type of cellsstudied	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks and reference
				2.0m 2.5f after 24 h 1.9m 2.6f after 48 h		gistered only in 14 days after exposure [32]
		Control — the same subjects before exposure		0.8m 0.6f		
16. Russia	Exposure to various chemicals in industry workers	59 m (23—74) Control — 70 m (18—66)	Buccal	0.8 0.4	Aceto-orceine 2000	Effect of exposure: ↔ [33]
17. Russia	Exposure to chlorophenoxy herbicides	52m + 49f (36)	Buccal	From 0.7 to 1.5 depending on work duration and work place	May-Gruenwald ORPannenheim (azur II-eosin) 2000	Effect of exposure: ↑ (MN level in controls was 0.0 so it not possible to compare increase in folds). Nuclear anomalies such as binucleates, karyorrhexis, pycnosis, broken egg, karyolysis were studied. In control anomalies were 2.03 %, in the most exposed group 8.76 %, in the less exposed group —4.36% ($p < 0.05$ in all cases) [34]
		Control — 13m+12f (40)		0.0		
18. Russia	Exposure to agent orange in Vietnam	47 f (50) Control — 47 f	Buccal	7.5 10.4	Giemsa 500	Effect of exposure: ↔ [38]
19. Russia	Exposure to dioxins	31 f (30) Control — 14 f	Buccal	0.45 0.46	Aceto-orceine 2000	Effect of exposure: ↔ [37]
20. Russia	Effect of antropogenic pollutants	383 m+f (11)	Buccal	8.1 in 1999	Aceto-orceine 500	Effect of pollution: ↑ [41]
		Control — 51 m+f (11)		3.3 in 2000—2001 in Voronezh city 3.9 in Novovoronezh city1.4 in «clean» region		
21. Russia	Effect of antropogenic pollutants	42 m+f (5) Control — 28 m+f (5)	Buccal	13.0 5.0	Orceine 400	Effect of exposure to pollutants:↑ × 2.6 [42]
22. Russia	Effect of antropogenic pollutants	65 m 65 f (5) Control — 22 m + 16 f 27 m 33 f(15) Control — 12 m 19 f	Buccal	0.8 0.4 0.7 0.7	Feulgen 2000	Effect of exposure to pollutants in children: ↑ Effect of exposure to pollutants in teenagers: ↔ [44]
23. Russia (Moscow and Novgorod)	The influence of scoliosis and environmental pollution	73 m+f (11) Control — 25 m+f Negative control (11) from clean area — 23 m+f	Buccal	0.19 0.13 0.00	Feulgen 1000	Effect of scoliosis and pollution: ↔ [45]

Continue of table

Country	Exposure or studied groups	Number of subjects, gender, (age)	Type of cellsstudied	Number of cells with MN, ‰	Stain (cells studied per subject)	Remarks and reference
24. Ukraine (Chernovtsi district — severely polluted ofwaists of che mical industry)	Exposure to various agents known (heavy metals) and unknown in air and water	Not specified (6) control — not specified (6) living in clean area	Buccal	MN index. 2.3—4.6 in polluted areas, 2.0 in clean	Aceto-carmine 1000	Effect of exposure: ↑ × 1.4—2.3. High correlation between the level of MN and content of heavy metals in soil and waterNot possible to calculate the real level of MN[46]
25. Ukraine (Dnepropetrovsk, Zheltie Vodi, and Marganets sities)	Exposure to various chemical agents in environment (waists of chemical and metal-producing industry)	221m+f (4) Control — 56m+f (4) living in clean area	Buccal	MN index. 0.0067—0.114 in polluted areas, 0.012 in clean	Aceto-orceine or Feulgen500	Effect of exposure: ↑ × 5.6—9.5. Not possible to calculate the real level of MN[47]
26. Ukraine (Dnepropetrovsk and Marganets sities)	Exposure to various chemical agents in environment (waists of chemical and metal-producing industry)	24m+f (4) 60m+f (4) 76m workers (no data) Control — the same persons before treatment with vitamin complexes	Buccal	MN index. 0.067—0.085 in infants before treatment, 0.046—0.043 after. In workers 0.094—0.15 before, 0.043—0.085 after	Aceto-orceine or Feulgen500	Infants received vitamin complexes and workers at harmful production received extract of echinocea. Effect of exposure: ↓ × 1.5—2.2 Not possible to calculate the real level of MN[47]
27. Ukraine	Influence of weather and some physical parameters in participants of Arctic expedition	12 m (46) No controls — study 14 times during the expedition	Buccal	2.2 mean (1.7—2.8)	Giemsa 2000	Effect of various conditions: ↔ [48]

Note. ↑ — significant increase; ↓ — significant decrease; ↔ — no effect; x — folds in increase or decrease; f — females; m — males; pts — patients.

to eliminate microbes which can resemble the MN in EC [2—4].

3. It is extremely important to stain EC with DNA-specific stains (Feulgen, acridine range, DAPI) to recognize the real MN, but not artefacts [6, 7].

4. Extrachromosomal cytoplasmic DNA fragments should be counted as MN under the following conditions: intact main nucleus and cytoplasm; diameter about 1/3 of the main nucleus or less; same staining and texture as the main nucleus; MN should be in the same focal plane as the main nucleus [2, 8].

5. In the first studies concerning MN assay in EC only 500 cells were evaluated. fterwards it has been stated that to obtain reliable results no less than 1000, but better 2000 cells ought to be studied [1—3, 9].

6. It is very important to register along with MN the so-called nuclear anomalies (or etanucleated cells), but separately from MN [8]. Many investigators disregard such EC considering them degenerative. These cells are connected with cytotoxicity, apoptosis and necrosis.

7. The MN in ideal should be indicated in two ways — number of EC with MN and total number of MN per 1000 cells.

8. According to the review of the «Human Micro-Nucleus Project», the average number of EC with MN in healthy subjects independent the type of EC is1-3 per 1000 EC [10].

Studies carried out in Armenia. In all studies [11—19] DNA-specific stain (Feulgen reaction) was used and the adequate numbers of cells were evaluated (1000-2000). In all cases, the number of MN in EC of healthy subjects was about or less than 1 per 1000 EC. Although EC were used without washing (direct smear on microscope slides) no effect of microbial contamination was registered because of use of DNA-specific stain.

In the mentioned publications strong effect of chemotherapy (in EC of buccal mucosa) [11] and radiotherapy (both EC of buccal mucosa and cervix) [17, 18] of cancer patients were registered. In was also shown that in primary cancer patients independently of the tumor site, the number of MN is significantly increased in buccal EC compared with healthy subjects [12, 15, 16]. But in women with benign tumors of breast and uterus no such effect was registered. Increased number of MN was observed both in cervix and oral mucosa EC of primary cervix and uterus cancer patients [12, 15, 16]. During radiotherapy of cervix strong increase of MN level was observed in cervix EC, although in buccal cells no effect was observed in cervix cancer patients [17].

The effect of chemo- and radiotherapy on MN induction in EC was supported by other investigations [20, 21]. Also significantly increased level of EC with MN was observed in primary cancer patients independent the site of tumor localization [22].

The increased frequency of MN in EC of buccal mucosa was also found in women with hirsutism [13] that was confirmed supported recently by Turkish investigators (due to reactive oxygen radicals formed in the organism) [23]. The impact of age on MN formation in females was shown ($r = +0.4$, $p < 0.05$) [19] although it is not supported by many investigators (for review see [24]), and the data concerning the effect of age are very contradictory.

It is noteworthy that almost in all papers published by Armenian investigators, along with MN all the types of nuclear anomalies were registered. Unlike Chakrabarti and Dutta [25] who reported about 4-fold increase in the number of MN in EC of cervix in postmenopausal females compared with women with normal menstrual function, Nersesyan [14, 15] showed no significant increase in MN level, but substantial increase in the number of degenerative cells. Vardazaryan [17] showed that after radiotherapy of cervix along with MN also the level of cells with karyorrhexis, binucleates and condensed chromatin was substantially increased. Bhattathiri et al [26] and Bindu et al [27] showed almost the same regularity in oral cancer cells during the radiotherapy, but in this case all kinds of nuclear anomalies were increased.

Study carried out in Georgia. The effect of increased level of arsenic in drinking water on MN in EC was evaluated [28]. It has been shown with adequate staining of EC and evaluation of 1000 EC that contamination of drinking water with arsenic led to 6-fold increase of EC with MN. It is in agreement with some recent publications where 3—7-fold increase of MN in EC of subjects exposed to arsenic (via drinking water or during glass production) was shown [29, 30]. But the number of EC with MN in control subjects is was very high 4.4 ‰, although it was noted that control subjects also had some health problems,namely acute respiratory infections. This is of course very bad control, and they ought to have another negative control — not exposed to arsenic healthy subjects. The problem of healthy control was discussed recently [31].

Studies carried out in Russia. In three papers the results of exposure to some chemical agents were presented. Kitaeva and co-workers studied the effect of acute exposure of students to formaldehyde and found significant increase [32]. But the samples of EC were collected from mouth 24 and 48 h after exposure, and the increase of MN of course was undoubtedly caused by the connected with other factors other than exposure to formaldehyde, because such effect could be registered only after 10-14 days after exposure. It was a main point of criticism of this paper by Speit and Schmid [5]. The number of MN was evaluated also in professors who were exposed to formaldehyde for about 20 years. Some surprizing results were obtained — significant effect in women and not significant in men (2.94 and 1.18 ‰, respectively — more than 2-fold difference depending on the gender). The control subjects were only women. The level of EC with MN was 0.6—0.8 ‰ after study of 2000 Feulgen stained cells.

Revazova et al. [33] evaluated the influence of some highly toxic agents (not specified) on MN induction and failed to show any significant difference between exposed and control subjects (0.8 and 0.4 ‰, respectively).

Chemikozova et al. [34] studied the influence of professional exposure to chlorophenoxy herbicides and found a significant increase of MN frequencies in EC of exposed persons. EC were stained with DNA-non-specific stains (May-Gruenwals-Giemsa and Pannenheim), and 2000 EC were evaluated. The number of MN was between 0.0 ‰ in control and 0.53—1.5 ‰ in exposed subjects. But the gradation of MN was extremely strange. The authors divided MN into four categories — from 1st to 4th (1st — one very small MN, 1/40th of the size of the main nucleus; 2nd — one MN, from 1/15th to 1/10th of the size of the main nucleus; 3rd — some MN with sizes of 1st and 2nd categories; 4th — one big MN, 1/4th of the size of the main nucleus). This gradation differs from the most publications because MN is MN if it meets all criteria of this phenomenon, e. g. should be only and only one category — MN. The authors have studied nuclear anomalies (except condensed chromatin), but the presentation of the results was far from presented in the most publications. In addition the authors added a new gradation to the recognized international classification, namely amitosis, and actually it is not understandable the difference between the new gradation and the recognized binucleates, which theyhave also considered. The analysis of nuclear anomalies differed from internationally recognized standards, although they showed a significant increase of nuclear anomalies levels.

The investigation carried out in India showed that in subjects exposed to pesticides during their production, a 3.6-fold increase was observed in EC stained with DNA-specific stain (DAPI) [35]. On the contrary, the studies carried out in Costa Rica and European Union, have shown that exposure to workers to various pesticides did not increase significantly the number of MN (in EC of workers from Costa Rica also the number of nuclear anomalies) [36, 37]. In both studies DNA-specific stains were used (Feulgen and DAPI, respectively) and enough EC were evaluated (1000 and 2000, correspondingly). Because of some methodological shortcomings it is not possible to compare the results of Chemikozova et al [34] with other publications.

Six papers presented the results of the effect of various kinds of pollutions on MN level. Zhuleva et al. [38] studied the effect of agent orange, used by US troops during the war in Vietnam many years after exposure. The authors did not observe any significant difference in the number of EC with MN in inhabitants of two villages — exposed and non-exposed. Along with MN the authors studied also binucleated cells and cells with «chromatin like vacuoles» (possibly the so-called cells with condensed chromatin used in western scientific literature). The gradation of MN into four categories (small MN, more bigger MN, some small MN and one big MN in EC) is unusual. This approach is far from common definition of MN used by most of scientists. Independently of size, MN should be considered as MN if it is in the frames of definition of this phenomenon. The level of EC with MN in control subjects was extremely high — 10 per 1000. The reason of this effect could be staining of EC with Giemsa (DNA-non-specific stain) and evaluation of 500 EC. In the photos of EC presented by authors, a lot of microbes are presented, which could be a source of bias.

Revazova et al. [39] studied the effect of dioxins contamination in Chapaevsk. Fifteen women workers of factory producing dioxins, 15 women living in the 1—3 km zone from the plant, and 14 ones living in the 5—8 km zone from the plant were examined for MN in EC. No effect was observed both in lymphocytes (chromosomal aberrations assay) and MN in EC. Along with MN the investigators evaluated also nuclear anomalies, such as broken egg, two-nuclei cells (also called binucleated cells), pycnosis, karyorrhexis and karyolysis. They presented «broken egg» and two-nuclei cells, and pycnosis and karyorrhexis together considering that the former two are of genotoxic origin, and the last two are of the mixed effect — both geno- and cytotoxic. Some authors also used similar approach although the real nature of nuclear anomalies is unknown [8, 40].

A group from Voronezh State University has published their studies concerning the influence of pollution on MN level in EC of children living in various district of Voronezh and found significant impact of pollution (increase of EC with MN 2.0-5.0-fold) [41, 42]. Such increase in the number of EC with MN is extremely high and suspicious because exposure to extremely biologically aggressive chemical agents cyclophosphamide elevated MN level 2.7-fold [43]. The reason of such results could be the use of DNA-non-specific stains and evaluation of no more than 500 EC. In addition,

the levels of EC with MN in control (non-exposed) subjects (5.0 ‰) is very high for healthy persons [10].

Maimulov et al. [44] studied the same problem in St. Petersburg and found no effect of pollution in teenagers and 2.0-fold increase of MN level in 5-years-old girls and boys. It is noteworthy that the number of EC with MN in subjects living both in clean and polluted areas was less than 1.0 ‰. It is close to the data reported by Revazova et al. [31, 36], but unlike Maimulov et al. [44], the authors used DNA-non-specific stain (carmine). In addition both groups studied 2000 EC.

Aleschenko et al. [45] studied both the influence of disease (scoliosis and kyphosis) in teenagers and also the environmental pollution on MN status in EC of buccal mucosa. No influence of both factors was obtained after evaluation of 1000 Feulgen stained EC from each subject (0.13; 0.19 and 0.0 ‰ in two polluted and clear areas, respectively). The authors studied also all nuclear anomalies as well, except condensed chromatin, and found significant impact of environmental pollution on karyorrhexis and karyolysis.

Studies carried out in Ukraine. Three papers were found [46—48]. Two of them described the effect of environmental pollution, and the last one the influence of weather and some physical parameters on MN in EC of buccal origin in participants of Arctic expedition. In both papers concerning the effect of pollution [46, 47] positive results were obtained, e.g. a significant increase of EC with MN in children due to living in polluted areas. In both papers the real number of MN is not possible to estimate. Rudenko et al. [46] studied the effect of environmental pollution in Chernovtsi region of Ukraine (which was polluted after Chernobyl disaster in 1986 and in 1988 a new disease of unknown reason was registered there in children which caused alopecia). From each subject 1000 EC stained with aceto-carmine were evaluated, but the number of participants in the study and their gender were not specified. Rudenko et al. [46] wrote that MN are presented as «MN index», but in the table it is written «The frequencies of cells with MN». If we consider the last point, the mean number of EC with MN in children living in polluted areas is 31.0 ‰ which of course is extremely high. The control level (MN in EC of children living in clean areas) is 19.0—23.0 ‰ (villages Mikhalkove and Lopushna). This investigation is very important because the children (and of course, all subjects) living in Chernovtsi region are continuously exposed to unknown genotoxic agent(s). The authors revealed the correlation between the level of soil and water contamination with heavy metals and aluminium and cytogenetic disturbances in EC. Independently of the mode of data presentation, as «index» or «frequency» of MN, the difference between the most and the least polluted areas is 2.4. It is close, but less than the data presented by two groups of Indian investigators who studied the effect of living in area contaminated with heavy metals and consumption of water polluted with heavy metals and other toxic substances on MN level in EC of urothelial and buccal origin — 5.2—7.7-fold [49—51]. The shortcoming of this very interesting paper is impossibility to compare the results with the data of other scientists investigating this problem.

In the paper by Gorovaya and Klimkina [47] the picture is an opposite one — in «Materials and methods» they wrote that MN were evaluated as «frequency», but in the table they are presented as «MN index». According to this paper it is not possible to estimate the real number of MN. The results of comparison of the data from the two papers [46] and [47] are surprising — MN index of control subjects (living in "clean" areas) are 2.1 and 0.012 (the difference is 175-fold!). In persons living in polluted areas the indexes are 3.1 and 0.107, respectively (29-fold difference!). In the paper by Gorovaya and Klimkina [47] it is not clear how many slides were stained with Schiff's reagent and how many ones were stained with aceto-orcein. It is noteworthy that only 500 cells were studied from each participant. Extremely important results were presented concerning the possibility to decrease the cytogenetic action of various agents by means of vitamin complexes and adaptogens [47]. But again because of the shortcomings of the presentation of the results obtained, it is practically impossible to compare them with the data of other scientists.

In the paper by Afanas'eva et al [48] some samples of EC from mouth were obtained and analysed from each participant of the Arctic expedition to evaluate the influence of weather and some physical parameters. No influence of various climatic conditions on MN level was studied. The mean le-

vel of EC with MN was 2.2 ‰ after Giemsa staining (2000 EC were studied). Since the subjects were participants of the Arctic expedition, all of them were with good health status.

Conclusion. Many papers analyzed in the present article have serious shortcomings which do not give a possibility to estimate the results obtained and to compare with the results obtained by other investigators.

There are two shortcomings in the paper by Gagoshidze et al. [28] from Georgia — very high level of MN in control and at the same time, not adequate negative control.

In some papers from Russia the problem of environmental (air) pollution was studied with significant impact of it on MN level (2—5-fold) [41, 42, 44, 46, 47]. Analysis of the literature showed that there should not be a significant impact of air pollution, as was shown in two of analized papers [39, 45] and the inverstigation carried out in Costa Rica [36]. In addition, 2—5-fold increase is too high for impact of air on buccal mucosa. Almost the same result (increase of EC of buccal origin with MN) depending on the air pollution was showed by Belowska et al in Poland (1.5—3.0-fold) [52]. The authors studied 1000 Giemsa stained cells. The presentation has some shortcomings, and some points of the investigation are not clear. In this kind of investigations a lot of variables are extremely important — the data on levels of pollution, proximity to gas station, use of gas appliances, ozone level in air, and application of distance-weighted traffic density evaluation approach [53]. Some other data are of high importance are some other data — smoking habit, alcohol intake, smoking habit of parents (for children), social-economic status of family, vitamin use, daily fruits and vegetables servings [51]. Investigations carried out in California showed significant correlation between MN and ozone levels, but not with other kind of air pollutants [53, 54].

It is noteworthy that in Russian population level of EC with MN is less than 1.0 ‰ (0.0—0.8 ‰) when 2000 EC were evaluated (very close to spontaneous level of MN in Armenians [11—19], inhabitants of Mexico [20], Costa Rica [34], European Union [4, 35], and the USA [51, 52]), and 1.4—5.0 ‰ when 400-500 EC were studied.

In some papers an unusual division of MN into the categories was done [34, 38]. Only in few papers the so-called nuclear anomalies were registered. But in all of them EC with condensed chromatin were not considered although some key investigators of this problem consider them as events connected with cancer [2].

In two Ukrainian papers it is not possible to evaluate the data presented because of methodological problems [44, 45]. Unclear is the fact that in the same population (healthy controls) MN levels (actually not possible what the authors mean — frequency or index) considerably differ. Very interesting results were obtained in infants living in polluted areas and workers exposed to some genotoxic agents at harmful productions after applications of vitamin complexes and adaptogens — significant decrease in MN frequency or index of MN [47].

The common shortcoming of a vast majority papers is the absence of general information or descriptive characteristics of control and exposed subjects. This part of the paper should include the data concerning the number of participants, mean age, gender, addictions — tobacco smoke or alcohol consumption, occupation (preferably in a separate table).

All investigators in the field of MN assay in EC must be in accordance with some rules listed above. If it will be the case, the results presented in the papers would be easily comparable with the results of other investigators.

РЕЗЮМЕ. Проведен анализ данных по микроядерному тесту в эксфолиативных клетках человека, представленных исследователями из стран СНГ. Критически рассмотрены данные 22 статьей, отмечены некоторые недостатки. Проведено сравнение с данными литературы. Целью обзора является демонстрация имеющихся недостатков в представлении результатов исследований по микроядрам в эксфолиативных клетках человека для последующего их исправления, что даст возможность сравнения результатов с результатами зарубежных исследователей.

РЕЗЮМЕ. Проведено аналіз даних по мікроядерному тесту в ексфоліативних клітинах людини, що представлені дослідниками з країн СНД. Критично розглянуті дані 22 статей, зазначені деякі недоліки. Проведено порівняння з даними літератури. Метою огляду є демонстрація недоліків в поданні результатів досліджень по мікроядрах в ексфоліативних клітинах людини для наступного їх виправлення, що дасть можливість порівняти результати зарубіжних дослідників і дослідників з країн СНГ.

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