

## REGISTRATION OF MICRONUCLEI IN THE HUMAN BUCCAL MUCOSA CELLS ON THE SOUTH VIETNAM TERRITORY

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Micronucleus test (MNT) is one of the most practicable and quick cytogenetic tests in toxicogenetics used for screening and monitoring clastogens in the environment. The MNT allows to reveal the cytogenetic anomalies in persons exposed to different chemical agents, ionizing radiation, medicine treatment and vaccination<sup>1,2,3</sup>). The sizes of MN may be used as markers of alterations in the chromosome set. The appearance of large MN (2-3  $\mu\text{m}$  in diameter) is associated with the genome disturbances, the appearance of small MN - with the chromosome's structure breaks<sup>4</sup>). This study was carried out to estimate the perspective of MN test application for an express diagnosis of the environmental factors influence on the South Vietnam population, where the alterations in genetic material had been observed<sup>5</sup>) on the territory sprayed with phytotoxicants during the 2nd Indochina war.

### Material and methods.

During the epidemiological study in the Song Be province (South Vietnam)<sup>6,7</sup>) two hundreds eighty women (aged > 40) were randomly selected from 2 villages (one control - Tan An, and one sprayed by phytotoxicants during the 2-nd Indochina war - Binh My village). In 1994 these women were analyzed once again and questionnaires were obtained with special attention to their reproductive history. The slide preparations of buccal mucosa cells were obtained from 100 of these women and proceeded and stained as according to the standard protocol<sup>8</sup>).

The slides were analyzed under the immersion objective (100x) and the frequencies of micronucleated cells were registered. Statistical examination was done with the help of "Statgraphics, v.3.0" и "Excel v.4.0".

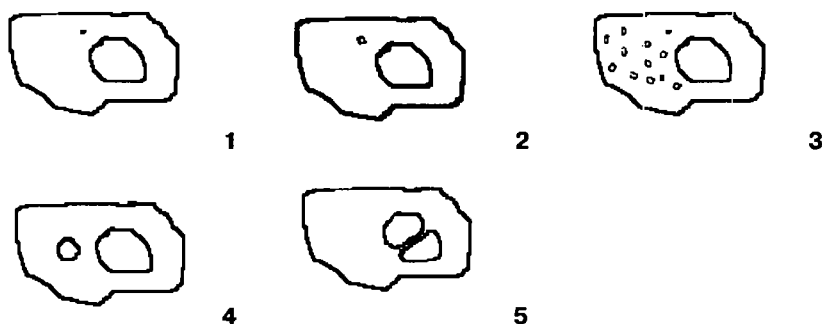
### Results and discussion.

The micronuclei of 4 types were registered in the epidermal cells of buccal mucosa (Fig.1): 1-st type - one small MN (~1/40 of main nucleus size) near main nucleus; this type MN is formed by a chromosome fragment; 2-nd type - one larger MN (~1/15-1/10 of main nucleus size) formed by several small fragments of several chromosomes, or by 1-2 large chromosome fragments. The 3-rd type of MN

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represents several small formations (from 2 to 10) of the same size as MN of the 1-st and 2-nd types. We consider it necessary to divide this MN type into two variants because in some cells these formations looked like small "vacuoles" (round structures with determined border and fine dispersed chromatin) although they were stained as normal chromatin structures. The 4-th type - large formation with the 1/4 size of main nucleus, and bearing 1-3 whole chromosomes and, possibly, many fragments of other chromosomes.

Many cells bearing two nuclei had been registered also. These nuclei sometimes had normal size, and sometimes - the size was only half of that one. Apparently only nucleus was divided in these cells. These binucleated cells were registered separately.



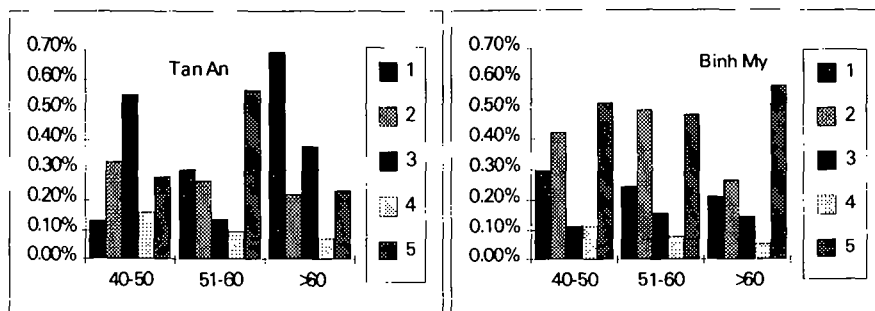
**Fig.1. Cells with different types of micronuclei and binucleated cells observed on the slides of buccal mucosa from women (South Vietnam, Song Be province):** 1 - cells with one small micronucleus; 2 - cells with larger micronucleus; 3 - cells with several small micronuclei; 4 - cells with one large micronucleus; 5 - cells with two nuclei; ordinate axes - percentage of cells with defectec nuclei.

Table 1. Frequency of cells with chromatin structure defects in buccal mucosa cells of women. Two villages with different level of environmental chemicals, Song Be province, South Vietnam.

Index	Village	Tan An (Control region)	Binh My (Sprayed region)
Persons studied		47	47
Cells examined		23684	30867
Cells with micronuclei (types 1,2,3,4)	N	247	230
	%	1,04	0,75
Binucleated cells	N	81	147
	%	0,31	0,48
"Vacuolated" chromatin (type 3a)	N	114	770
	%	0,48	2,49
"Defected" cells	N	470	1170
	%	1,98	3,79

The cells with all types MN and those binucleated were considered as "defected". Analogous types of MN had been described in erythrocytes of *Rana ridibunda*<sup>9</sup>). We suppose to carry out a morphometric examination of different types of chromatin structure damage in human buccal mucosa cells.

There were no significant differences between women from these villages according to percentages of micronucleated cells ( $p > 0.05$ , Table 1). However the values of these parameters have been wide and differently directed (vectored). At the same time it is well known that the frequencies of micronucleated cells increase with aging<sup>10</sup>). That's why we stratified the examined persons according to 3 age groups (40-50, 51-60 and >60 years) and considered the cytogenetic anomalies separately by age groups and by types of nucleus damage. Fig.2. presents the distribution of defected cells by age groups.



**Fig.2. Distribution of cells with defected nucleus in buccal mucosa of women from different age groups in two South Vietnam villages. A - Tan An village (control region), B - Binh My village (sprayed with Agent Orange).**

Legend - age groups; absciss axes - types of micronuclei and nuclei's defects: 1 - cells with one small micronucleus; 2 - cells with larger micronucleus; 3 - cells with several small micronuclei; 4 - cells with one large micronucleus; 5 - cells with two nuclei; ordinate axes - percentage of cells with defected nuclei.

The alterations of defected cells' frequency among age groups were registered. A natural increase of cells with the 1-st type MN had been observed (approximately from 0.1% up to 0.7%) with aging in Tan An village. At the same time, in the sprayed region the decrease of these cells' rates had been observed (up to 0.2%).

The frequency of cells with 2nd type MN in the control village was practically the same in all age groups ( $p > 0.05$ ); in the sprayed region their frequency was increased in the groups elder then 60 years.

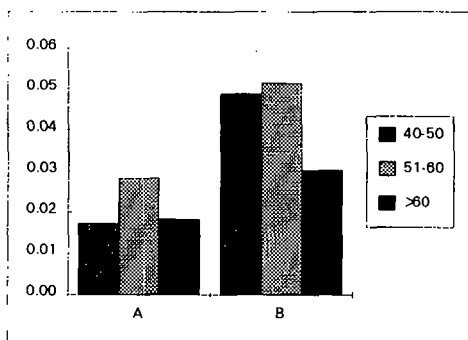
The frequency of cells with several small MN was rather high among women aged 40-50 from referent group (~ 0.55%), decreased (up to 0.1%) in the group of 51-60, and again become high in the elder group. In exposed group the level of these cells had not exceed 0.15% in all groups studied.

The cells with large MN (4th type) had been present almostly at the same levels in all age groups and in both villages.

The comparative analysis of binucleated cells' frequencies allowed to reveal one more interesting phenomenon (Fig.2) - relative increase of these cells' frequencies in women of the control region aged 51-60 (up to 0.55%,  $p=0.08$ ). In the sprayed region these cells had been much more frequent in all age groups and that had been 5-6 cells per 1000 in average.

The distribution of total rate values of the cells with defected chromatin structures (all the above mentioned types of MN, binucleated cells, and cells with "vacuolated" chromatin in age groups is presented on Fig.3.

Thus, analyzing the buccal mucosa exfoliated epithelial cells we observed the significant increase in frequencies of the cells with chromatin structure damage.



**Fig. 3. Distribution of total defected cells in buccal mucosa among women of three age groups living in two villages with different spraying history (South Vietnam). A - Tan An village (control region), B - Binh My village (Agent Orange sprayed region).**

Legend - age groups; ordinate axe - percentage of defected cells.

In buccal mucosa the intensive replacement of cells bearing liaisons for young cells should be very intensive. That's why an increase in women younger than 60 years old of the values of defected cells (particularly with chromatin destruction) indicates the tissue functional insufficiency. An increased frequency of binucleated cells in the contaminated village may be exactly associated with the disturbances of the genetic material in the blast and /or stem cells as the result of former contacts with the defoliants. The observed decrease of micronucleated cells of the 3rd and 4th types might be connected with their elimination as the last viable, and some increases in 2nd type MN cells' frequencies might be the result of modern environmental factors influence. Alterations in the frequencies of 1st type MN cells with age may be considered as background in the control region, and in the sprayed region - their transformation into the much more serious (rude) breakage could be proposed.

Thus, the results of MN-test in the Song Be province prove the previous data on the alterations of the cytogenetic parameters in the cells of people inhabiting the dioxin-contaminated provinces of Vietnam and provide additional systemic information for the affection of separate stem cells, that couldn't produce new generations of

functionally normal cells because of beared abnormalities and reparation abilities' exhaustion<sup>11)</sup>.

#### References

1. Norppa H., Luomahaara S. et al. Micronucleus assay in lymphocytes as a tool to biomonitor human exposure to aneuploidogens and clastogens //Environ. Health Perspectives. - 1993. - 101, S.3. - pp. 139-143.
2. Hogstedt B. Micronuclei in lymphocytes with preserved cytoplasm. A method for assessment of cytogenetic damage in man //Mutat. Res. - 1984. - 130. - pp. 63-72.
3. Ilyinskikh N.N., Ilyinskikh I.N., Nekrasov V.N. Micronucleus test application for screening and monitoring of mutagens //Cytology and genetics. - 1988. - 20, N1. - pp. 67-71 (In russ.).
4. Yamamoto K.J., Kikuchi Y.A. A comparison of diameter of micronuclei induced by clastogens and by spindle poisons //Mutat. Res. - 1980. - 71, N1. - pp. 127-132.
5. Oumnova N., Bourakov V., H. T. Kim Chi, N. Q. An, Roumak V., Cytogenetic Study of Peripheral Blood Lymphocytes in the South Vietnam Population Living in the Villages with Different Spraying History with Agent Orange. Abstracts of the XI International Symposium on Chlorinated Dioxins and Related Compounds, //DIOXIN' 91", USA, 1991, P - 40, p. 250; Oumnova N.V., Roumak V.S., Huynh Thi Kim Chi, Nguyen Quoc An. Genetico-toxicological studies of dioxin-containing ecotoxicants long-term health consequences in the South Vietnam. Cytogenetic parameters of cultured peripheral blood lymphocytes of rural inhabitants. DIOXIN'94 (November, Japan). - 1994. - v. 21. - pp. 383-388.
6. Roumak V.S., S.P. Poznyakov, V.V. Antonyuk, Tran Xuan Thu, and Sofronov G.A. Medico-Biological Foundation for Estimation of Long-Term Health Consequences of Agent Orange in Vietnam. 2nd Int Symp: Herbicides in War, Hanoi. Scientific Reports //Hanoi. - 1993. - pp. 147-163
7. Huynh Thi Kim Chi, Oumnova N.V., Nguyen Quoc An, Roumak V.S. Genetico-Toxicological Studies in the South Vietnam. 3. Reproduction Function Disturbances in the South Vietnamese Rural Inhabitants. The 2nd Int. Symp. "Herbicides in War", Hanoi, Vietnam, 1993, Abstr. book //Hanoi. - 1993. - pp. 215-218.
8. Schmid W. The micronucleus test //Mutat. Res. - 1975. - 31, N1. - pp. 9-16.
9. Zhuleova L.Yu., Dubinin N.P. Analysis of micronuclei in erythrocytes of *Rana ridibunda* for evaluation of environmental pollution in Astrakhanh area //Genetics (Moscow). - 1994. - N6.
10. Ilyinskikh N.N., Ilyinskikh I.N., Bocharov E.F. Cytogenetic homeostasis and immunity //Novosibirsk. - 1986. - 246 p. (In russ.).
11. Roumak V.S., Oumnova N.V., Poznyakov S.P., An N.Q., Sofronov G.A. Disadaptive effects in humans after exposure to chemicals containing dioxin //DIOXIN'94 (November, Japan). - 1994. - v. 21. - pp. 379-381.

