

APPLICATION OF MICRONUCLEUS TEST FOR INDICATION OF THE ENVIRONMENTAL FACTORS CLASTOGENIC ACTIVITY ON THE TERRITORY OF SONG BE PROVINCE (SOUTH VIETNAM)

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The presence of cytogenetic damages has been registered by tests on chromosome aberrations, sister chromatid exchanges and micronuclei^{1,2,4}) in the cells of Binh My village (South Vietnam, Song Be province) residents. The territory of this village had been sprayed by Agent Orange and now it is characterized by the most high levels of dioxin residuals and formation of the new dioxin-containing ecotoxicological factor - DEF^{2,3}). The aim of this analysis was to study the distribution of several types of chromatin structure damage registered by the micronuclei (MN) test⁴). The induced by chemicals and/or other factors level of MN is known to return to normal values with time going, and MN persists with an incidence characterizing the ontogenetic features of an organism⁵). The present study of buccal mucosa cells was aimed on demonstration of the probable role of previous stem cells' disturbances in the observed frequencies of cytogenetic abnormalities.

Material and methods.

The slide preparations of buccal mucosa cells were obtained⁴) from 94 women in two villages of the Song Be province (South Vietnam): one sprayed by phytotoxicants during the 2-nd Indochina war - Binh My village, and one referent - Tan An. Personal questionnaires were also obtained with special attention to reproductive history. The slide preparations of exfoliated mucous cells were analyzed under the immersion objective (100x), and the frequencies of micronucleated cells were registered. Statistical analysis was done with the help of "Statgraphics, v.3.0" and "Excel v.4.0".

Results and discussion.

The micronucleated cells of four types were registered. They differed by size and structure of micronucleus (MN) - hence for the chromosome damage type⁴). In some cells the micronuclei's structures resembled mostly small vacuoles, although were stained as normal nuclei. Many binucleated cells were registered with nuclei sized from normal to half of normal. All cells with MN and those binucleated we defined as "defected" (tabl.1).

Table 1. Frequency of cells with defected nuclear structures in human buccal mucosa (two villages in the Song Be province, South Vietnam).

Village	Tan An (Control region)	Binh My (Sprayed region)	P
Persons studied	47	47	
Average age	57.44 ± 1.29	57.48 ± 1.24	
Cells examined	23684	30867	
Micronucleated cells (Types 1,2,3,4)	12.72 ^a 7.89 ÷ 17.55	8.83 6.05 ÷ 11.60	0.435
Type 1	4.51 1.33 ÷ 7.69	2.46 1.43 ÷ 3.49	0.224
Type 2	3.63 0.94 ÷ 6.32	0.13 -0.66 ÷ 0.92	0.012**
Type 3	3.61 1.34 ÷ 5.88	1.45 0.50 ÷ 2.40	0.075
Type 4	0.95 0.38 ÷ 1.57	0.80 0.39 ÷ 1.21	0.621
Binucleated cells (Type 5)	3.40 2.00 ÷ 4.80	5.18 3.58 ÷ 6.79	0.020*
Cells with "Vacuolated" chromatin (Type 3 a)	4.23 1.04 ÷ 7.41	30.20 16.31 ÷ 44.08	0.000008***
Defected cells	21.60 15.12 ÷ 28.08	44.59 29.21 ÷ 59.96	0.00731**

a - average per 1000 cells and 95% confidence interval;

P - non-parametric statistic test.

No significant differences were observed between two villages according to micronucleated cells' frequencies. However these differences were revealed⁴⁾ when the samples had been stratified by age (40-50, 51-60, and >60 years). The gerontology processes might have interfered into the results of this analysis⁶⁾ so we considered it possible to concentrate on the first two groups. Fig.1. presents the distribution of the defected cells by types of MN and by these age groups of women in two villages.

The level of the 1st and 2nd type MN in Binh My was significantly increased in the age group 40-50 as compared to reference group of the same age. The proportions of binucleated cells were also increased in middle aged women from Binh My.

The distribution analysis of defected cells' total values (all the above mentioned MN types, binucleated cells, and cells with "vacuolated" interphase chromatin) by age has shown the following. In exfoliated buccal mucosa cells in Binh My village an enhancement is registered in frequencies of cells with chromatin structure breakage. These alterations are pronounced in all age groups (tabl.2) but it became significant only in women aged 51-60 ($p < 0.01$). No association had been found for pesticide contacts and frequency of MN ($p > 0.05$).

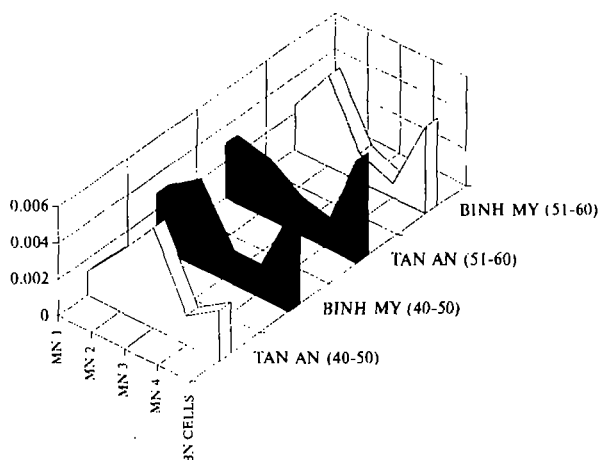


Fig.1. Distribution of cells with defected nucleus in buccal mucosa of women from different age groups (two South Vietnam's villages).

Legend - age groups; abscissa axes - types of micronuclei and nuclei defects: MN1 - cells with one small micronucleus; MN2 - cells with larger micronucleus; MN3 - cells with several small micronuclei; MN4 - cells with one large micronucleus; BN cells - cells with two nuclei; ordinate axes - rate of cells with defected nuclei.

The comparison of chromosome material different types' damage in age groups of *one village* did not reveal any significant alteration with age. Only some increase in cells with "vacuolated" chromatin was found in the age group of 51-60 years in the control region (tabl.2).

We observed the significant difference in tested parameters between villages when compared the groups aged above 50 years in relation to the rate of defected cells and binucleated cells (tabl.2). The main contribution to the first parameter is made by cells with "vacuolated" interphase chromatin. Their presence is enhanced up to 17-20 per 1000 cells in the group of 51-60 years old women, and up to 60 per 1000 cells in eldest group (above 60). In the control region the rates of these cells were low, and in the eldest group did not exceed 6 cells per 1000.

The observed alterations in frequencies of cells with different types of MN and binucleated cells, as well as in cells with primary signs of degradation (~ apoptosis, those with "vacuolated" chromatin) give further information for an evidence of the functional deviations in characteristics of these cells, and for the probability of stem cells among the residents of the area contaminated with DEF (tabl.1-2).

The MN types' distribution changes with aging indicate the possibility of transformation of smaller chromatin breaks into more serious genome damages (up to the large chromosome breaks and genome partial loss).

Stratification of other factors able to influence the destructive processes in cells (socio-economic standards, nutrition, professional contacts, morbidity and medications, etc.) did not reveal any significant association with the levels of different

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types of nucleus damage ($p>0.05$). At the same time, the tendency may be noted of MN enhancement in Binh My particularly, when dried fish and fish sause were predominating in the diet ($p=0.06$). This tendency became significant for the 1st type MN, and for the sum of 1st, 2nd and 4th types MN ($p=0.03$). On the other hand, the presence of meat products in the diet leads to somehow decrease in total rate of MN. There were no such associations in the control region. This may be very meaningful because the villagers could obtain dioxin residuals exactly with fish. So, the damages induced by DEF became pronounced just on the background of these residuals.

Table 2. Distribution of cells with different types on nuclear defects (per 1000) in age groups of women (two villages in the Song Be province, South Vietnam).

	Tan An		Binh My		P#
Index/Age group	Average per 1000 cells	95% C.I.	Average per 1000 cells	95% C.I.	
Cells analysed					
40-50	5799		5702		
51-60	7125		14822		
>60	12051		10643		
Total defected cells					
40-50	17.8	5.4 ÷ 30.1	50.0	16.4 ÷ 83.1	0.918
51-60	30.8	18.4 ÷ 43.1	49.0	27.5 ÷ 70.5	0.007**
>60	18.3	8.6 ÷ 28.0	32.4	3.2 ÷ 61.6	0.209
Binucleated cells					
40-50	2.6	-0.1 ÷ 5.2	3.3	-0.1 ÷ 6.8	0.683
51-60	6.0	3.3 ÷ 8.6	5.6	3.4 ÷ 7.8	0.020*
>60	2.3	0.2 ÷ 4.4	5.8	2.8 ÷ 3.9	0.021*
Micronucleated cells (1-4 types)					
40-50	12.2	3.0 ÷ 21.4	8.2	2.2 ÷ 14.3	0.308
51-60	11.9	2.7 ÷ 21.0	9.7	5.8 ÷ 13.6	0.435
>60	13.6	6.4 ÷ 20.8	7.7	2.4 ÷ 12.9	0.133
Cells with "vacuolated" chromatin (type 3a)					
40-50	2.1	-3.9 ÷ 8.2	17.4	-10.7 ÷ 45.5	0.076
51-60	5.8	3.4 ÷ 8.9	37.9	11.5 ÷ 65.5	0.001**
>60	0.9	-3.9 ÷ 5.6	59.3	34.6 ÷ 84.0	0.031*

P - Mann-Withney test, significance.

Besides, a significant increase in frequency of cells with serious functional and structural chromatin changes. That are up to the appearance of fine dispersed "vacuolated" interphase chromatin in the group of younger women. This proves the evident alterations in adaptive reactions of the cells in persons exposed to dioxin in youth.

The level 4th type MN is associated with division pathology - with separate chromosome lagging in meta- and anaphases. This provides the evidence of large MN formation by lagged chromosomes and small MN - by structure chromosome aberrations mainly⁷⁾.

Such "accumulation" in buccal mucosa of micronuclei, including "vacuolated", may reflect the cells' destruction processes (and chromatin's particularly) as the result of reserve potentials' exhaustion. Furthermore, the presence of many binucleated cells, each of which is smaller than normal nucleus' size, also may provide information on the loss of potentials either to synthesize the whole volume of genetic material during replication or to synthesize structures for constructing the membranes for daughter cells. We stress also the presence of cells being on the primary apoptotic stages. This is probably evident because of many "vacuolated" structures observed. Such "vacuoles" are bearing chromatin thrown away in an interphase and unable to condense in metaphase.

Thus, these results support the data previously obtained^{2,3,9)} on disturbance of cytogenetic parameters in persons inhabiting the dioxin exposed territories in Vietnam and give some new information on probability of different tissues stem cells' alterations.

The women's exposure with Agent Orange 20-30 years ago has led to the general disregulation and disadaptation of an organism as well as to the disturbances of cells' structure and functional abilities^{1,8)}. The exhaustion of the organism's reserve possibilities on different levels - from cells to organs' systems - is revealed in disability to support normal homeostasis and to restore the structures damaged during the metabolism.

We believe to have all bases for an opportunity to speak about the serious alterations in stem cells exposed to Agent Orange and DEF. These stem and/or blast cells have lost in persons studied the capability for self- restoration (repair). In addition to functional characters altered they are objected for self-degradation. The last can be fixed by the appearance of "vacuolated" interphase chromatin loose structures. So, the hypothesis stated in our previous publications^{1,3)} on different tissues stem cells' alterations is supported. The hypothesis was based on the results of analysis carried out on human lymphoid and erythroid cells^{2,3,9)}, keratinocytes¹⁰⁾, and on germinate cells of locusts¹¹⁾. Our postulate intends the search, development, and application of special tests and model experiments for an analysis of stem cells damage.

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