

### Comparative analysis of buccal mucous cells defects in residents of three Vietnamese villages

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A comparative analysis was carried out to reveal the differences between the results of micronucleus (MN) tests for residents of three vietnamese villages.

Two villages - Binh My (BM) and Tan An (TA) - are situated in the South Vietnam and differ by dioxin exposure. Residents of these villages were the subjects of JRVTC complex study during 1988-1995 [1]. The third village (Bach Hong, BH) is in the northern province Dong Anh and its residents were used as general external comparison [2]. The ecogenetic investigations revealed the development of a new level of functional associations between the genetical parameters and general health status of persons from the exposed village [3,4]. The results of epidemiological study of males [2,5] and females [5] proved the fragility of health in exposed region.

#### Materials and methods

Common MN-test [6] was carried out on oral cavity mucous smears of females from three villages - 50 persons (aged 24-56) in each village. Medical groups examined all individuals, the epidemiological questionnaires [7] were received also. Mucous smears were collected at the day of examination, proceeded at the laboratory and analysed according to cellular defects types presented in our previous publication [6,7].

To stratify the influence of different factors the comparative analysis was designed and an analysis of different nucleus anomalies was launched.

Statistical and other analyses was fulfilled with the help of "Statistica, 4.3" and "Excel, 7.0".

#### Results and discussion

The cells with different types of anomalies were registered in all three exposure risk groups. That are the following: micronucleated cells (1-4th types, [6]); binucleated cells; cells in different stages of apoptotic elimination; cells with destroyed nucleus; cells with perforated nucleus; cells with abnormal nucleus morphology.

The frequencies of different nucleus aberrations are presented in Table 1. It's interesting to stress the absence of perforated nuclei in smears of BH residents. There were 40 such cells per 1000 in TA village, and 140 per 1000 - in exposed village.

The following analysis is based on two parameters - the frequency of micronucleated cells and the frequency of total nucleus defects. The latter value summarises all the cells with abnormal nucleus' morphology. On the cellular level these two characteristics manifest the disturbances

and alterations produced in the organism by the unfavourable factors [8]. Fig.1. presents the results for MN frequencies and defected nuclei frequencies. It demonstrates the increase of both values in the exposed BM village ( $p < 0.0001$ ).

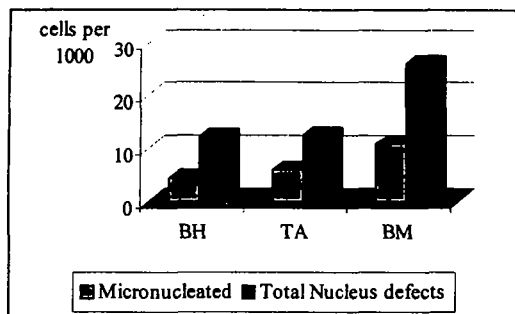


Fig. 1. Cellular defects in examined residents of several vietnamese villages

The comparative analyse of persons without smoking habits and any contacts with pesticides revealed the difference between these three groups to be significant ( $p < 0.001$ ) only for those above 40 years old (table 2).

Table 1. Percentage and range of nuclei defects in oral cavity epitheliocytes of persons from three Exposure Risk Groups (North and South Vietnam)

PARAMETERS	Exposure Risk Groups			p
	BH	TA	BM	
Persons examined	47	50	50	
Cells examined	47000	50000	50000	
Age	44.08±5.71	38.46±8.27	38.24±8.16	
Cells with micronuclei				0.000
Type 1	0.13 0÷9	0.29 0÷8	0.57 0÷19	
Type 2	0.12 0÷4	0.06 0÷4	0.17 0÷6	0.001
Type 3	0.09 0÷16	0.16 0÷30	0.21 0÷17	0.307
Type 4	0.10 0÷4	0.08 0÷4	0.14 0÷6	0.033
Binucleated cells	0.40 0÷11	0.32 0÷11	0.46 0÷12	0.026
Cells with fragmented chromatin	0.18 0÷44	0.11 0÷9	0.24 0÷15	0.354
Cells with abnormal nucleus form	0.08 0÷7	0.02 0÷3	0.06 0÷4	0.102
Cells with destroyed nucleus	0.15 1÷16	0.20 0÷18	0.78 0÷39	0.01
Cells with perforated nucleus	0	0.08 6÷34	0.28 0÷30	

Table 2. Average frequency of micronucleated cells and total defects of nucleus in oral cavity epitheliocytes of non-smoking persons (above 40 years old) from three Exposure Risk Groups without contacts with pesticides

PARAMETERS	Exposure Risk Groups			p
	BH	TA	BM	
Persons examined	9	18	6	
Age	49.44±3.88	48.44±4.19	46.83±4.92	n.s.
Micronucleated cells	3.00±2.12	4.39±2.09	10.50±6.12	0.000
Total defects of nucleus	11.22±4.49	9.28±3.69	32.33±21.27	0.000

The examined parameters were practically the same in two control villages – BH and TA. The difference was observed only in MN-types spectrum. Among the northern village residents there were much more micronucleated cells with single micronucleus, represented by rather large amount of chromatin (type 2,  $p=0.014$ ). In the south the smaller MN variants (type 1) dominated ( $p=0.001$ ). No difference has been seen for the younger persons (up to 40 years old).

The contact with agriculture pesticides was registered only in two villages – BH and BM. But the results of internal comparative study for each village demonstrated the following. No pesticides' influence was observed in the northern village even in the older age group (Table 3). The situation was the same in BM village (table 4), except the type 1 micronuclei ( $p=0.40$ ).

Table 3. Frequency of nuclear defects in oral cavity epitheliocytes according to contacts with pesticides in older persons from Bach Hong village

	Pesticides		p
	No	Yes	
Persons examined	9	21	
Age	49.44±3.88	46.38±4.54	
Micronucleated cells	3.00±2.12	4.57±4.86	n.s.
Total defects of nucleus	4.22±4.49	14.95±14.87	n.s.

Table 4. Frequency of nuclear defects in oral cavity epitheliocytes according to contacts with pesticides in older persons from Binh My village

	Pesticides		p
	No	Yes	
Persons examined	6	4	
Age	46.83±4.95	49.25±4.11	
Micronucleated cells	10.50±6.12	19.50±3.11	n.s.
Total defects of nucleus	32.33±21.27	48.75±18.15	n.s.

Thus, in the northern village without dioxin contamination in the past and any current proximate stress the frequency of defected cells among oral cavity epitheliocytes is on the normal level and dose not differ from the generally registered in several populations [9,10]. Even

professional contacts with agricultural pesticides don't alter seriously the number of micronucleated cells, as well as other nucleus parameters. Practically healthy organism of these women could manage with such stress and control the homeostasis.

The analyzed morphological and cytogenetic parameters are slightly higher in TA residents. Tan An village is far from the territory sprayed with Agent Orange, but could be probably influenced by the secondary dioxins [11]. Special investigations of this region showed the appearance of new toxic factors in unsprayed regions of South Vietnam. However, this is the subject for the future study.

The examined persons in Binh My village are exposed to current DEF [1], their organism exhibits altered homeostasis with multilevel shifts [1-4]. The analyzed cellular parameters demonstrate the prominent exceed of those registered in two control regions. So, the high amount of cells with abnormal nucleus material as well as fragile health of these women support the thesis of deep systemic reorganisation leading to early exhaust and inability to manage with the additional factors influence [3]. The latter is confirmed by an increase of health disturbances [4,5] in persons with high degree of contacts with chemicals, in smoking persons, and by lesser ability to repair lesions on the level of genetic apparatus [12].

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