УЛК 576.3.088

## Micronucleus level in exfoliated oral mucosa and cervix cells of cancer patients

G. Zalinyan<sup>1\*</sup>, G. Parsadanyan<sup>2</sup>, N. Vardazaryan<sup>1</sup>, A. Nersesyan<sup>1,3</sup>

<sup>1</sup>Yerevan State University, Yerevan, Armenia <sup>2</sup>Yerevan State Medical University, Yerevan, Armenia

<sup>3</sup> Institute of Cancer Research, Medical University of Vienna, Vienna, Austria;

Key words: micronucleus, buccal mucosa, cervix cells

## Միկրոկորիզների մակարդակը բերանի լորձաթաղանթի և արգանդի վզիկի բջիջներում քաղցկեղով հիվանդների մոտ

### Գ.Գ. Չալինյան, Գ.Գ. Փարսադանյան, Ъ. И. Циппидиприй, Ц.Ч. Ъблибирий

Հետազոտություն է կատարվել քաղցկեղով հիվանդ կանանց բերանի խոռոչի լորձաթաղանթի և արգանդի վզիկի էքսֆոլիատիվ բջիջների միկրոկորիզների մակարդակի (ՄՄ) որոշման նպատակով։ Այն կատարվել է մառագայթաթերապիայից առաջ, ընթացքում և ավարտելուց հետո։ Միկրոկորիզների մակարդակի բարձրացման միտում հայտնաբերվել է հիվանդների խմբում առողջ խմբի համեմասությամբ։ Ընդ որում, վիճակագրորեն հավաստի բարձրացում հայտնաբերվել է միայն արգանդի վզիկի էքսֆոլիատիվ բջիջներում ճառագայթաթերապիա անցնելու ընթացքում։

#### Introduction.

The micronucleui (MN) are chromosomal fragments or whole chromosomes, with are in cells cytoplasm just near of nuclei. It was shown that their presence indicates chromosomal aberrations (either structural – clastogenic effect. or numerical – aneugenic effect) (Rosin, 1992) and can reflect genotoxic effect of different factors (mutagens/carcinogens). It has been shown recently by Nersesvan et al. (2001: 2006) that the evaluation of MN in exfoliated buccal mucosa cells and increased frequency of chromosomal aberrations in lymphocytes of women with marked hirsutism can represent endogenous mutagenic process in organism of such women. The MN assay in exfoliated buccal mucosa and cervix cells is more economic, widely used and is a minimally invasive method for estimation of cytogenetic effect of different

## Уровень микроялер в клетках слизистой рта и шейки матки у пациенток с раком

Г.Г. Залинян, Г.Г. Парсаданян, Н.С. Вардазарян, А.К. Нерсесян

Исследован уровень микроядер (МЯ) в эксфолиативных клетках слизистой ротовой полости и в клетках шейки матки у женшин с раком до, в процессе и после прохождения ими радиотерапии. Обнаружена тенденция к возрастанию уровня МЯ у пациенток с раком по сравнению с контрольной группой. Было выявлено статистически достоверное повышение уровня МЯ в течение получения радиотерании только в клетках шейки матки.

factors and diseases. Increased level of cells with MN was observed in the cells of cancer patients after local radiotherapy (Tolbert et al, 1992; Nersesyan, 1996).

The increase in the number of cells with MN (MNC) in women with breast cancer compared with healthy women was revealed (Rajeswari et al., 2000).

Recently Nersesyan et al. (2002) have investigated women with uterine cancer and observed that the level of MN in patients was increased compared to healthy women.

The aim of the present work was to evaluate the level of MN and other nuclear anomalies in exfoliated buccal mucosa and cervix cells of gynecological cancer patients before, during and after radiotherapy.

#### Materials and Methods

In the present work we studied 5 females with diagnosed uterine cancer of first degree. The control group comprised 5 healthy women. Each of those women was interviewed about their habits concerning factors, that could influence MN level (smoking, alkohol, medication, hereditary diseases, viral infection). Subjects who did not meet the mentioned criteria were excluded from the study.

Buccal mucosa and cervix cells were collected both from patients with cancer and from healthy women. It was used standard Feulgen staining technique with some modifications (Nersesyan et al., 2006). As counterstain fast green was used. During the analysis of cells, other nuclear anomalies such as karyolysis, picnosis, karyorhexis, condensed chromatin, binucleares, **broken egg** also were studied (Tolbert et al, 1992). In our work it was used the same criteria of scoring the cells with MN and other nuclear anomalies, as it was suggested by Titenko-Holland et al. (1994) and protocols, which standardized in the frame of HUMNXL project (Holland et al., 2008; Bonassi et al., 2009).

## **Results and Discussion**

The results of the study are presented in Table 1 and Table 2. They show, that in cervix cells of patients with cancer it is observed the tendency of increasing the level of MN and CMN compared with corresponding healthy women. It is not statistically significant maybe because the group of investigated patients was not big enough.

#### Table 1

# The level of micronuclei and other nuclear anomalies in exfoliated buccal mucosa and cervix cells of healthy women

Parameter	Oral cells (‰)	Cervix cells (‰)	
CMN	1.16±0.25	1.29±0.19	
MN	1.34±0.25	1.46±0.25	
KR	1.04±0.19	2.24±0.34	
KI	0.23±0.12	0.93±0.19	
P	0.56±0.16	1.31±0.22	
BN	4.67±0.47	4.14±0.47	
	5.57±0.99	8.08±1.02	
BE	0.04±0.4	0.00±4.34	

<sup>\*</sup>CMN – cells with micronuclei; MN- micronuclei; KR – karyorhexis; KL - karyolysis; P -pycnosis; BN - binucleares; CC - condensed chromatin; BE - broken egg.

Results of the Table 2 show that there was statistically significant difference of MN, CMN in oral and cervix cells in patients with cancer during significant different radiotherapy.

The level of karyorhexis and karyolysis increased in the oral cells (after radiotherapy) and in cervix cells (during radiotherapy). Other nuclear anomalies were in the same level as in control group of healthy women. Some explanation can be offered for the increased level of MN and CMN in patient's two type cells – oral and cervix. As it is know radiotherapy is one of the methods mainly used in cancer treatment. Significant increase of nuclear anomalies in cervix cells during radiotherapy can be explained by the fact that it is target organ for radiation.

Г.Г. Залинян, Г.Г. Парсаданян, Н.С. Вардазарян, А.К. Нерсссян

Table 2

1						
Parameter	Oral cells (‰)		Cervix cells (‰)			
	before rt	during rt	after rt	before rt	during rt	after rt
CMN	3.0±0.28 *	3.7±0.28 *	3.1±0.28 *	3.4±0.14 *	5.9±0.28 *	4.9±0.14 *
MN	3.3±0.4 *	4.3±0.28 *	3.3±0.28 *	3.7±0.28 *	6.3±0.28 *	4.7±0.14 *
KR	2.1±0.3	2.7±0.14	1.7±0.43	2.9±0.28	3.6±0.42	1.7±0.43
KL	0.6±0.14	0.6±0.14	$0.7 \pm 0.14$	0.7±0.14	0.9±0.28	0.6±0.14
P	0.9±0.3	0.4±0.14	$0.4{\pm}0.14$	0.9±0.14	1.1±0.28	0.7±0.14
BN	5.3±0.3	5.3±0.43	5.3±0.14	5.6±0.28	6.6±0.42	5.1±0.28
CC	$5.3 \pm 0.43$	5.4±0.28	5.7±0.28	5.9±0.28	6.7±0.57	5.0±0.28
BE	0.3±0.14	$0.00 \pm 0.00$	$0.00 {\pm} 0.00$	0.14±0.14	0.00±0.00	0.00±0.00

The level of micronuclei and other nuclear anomalies in exfoliated buccal mucosa and cervix cells of women with cancer before, during and after radiotherapy

\* Significant statistical difference

In conclusion we may suggest that the evaluation of MN in exfoliated buccal mucosa and cervix cells can be very useful in oncogynecology. MN assay can represent ecogenous mutagenic process in such organisms as patients with cancer, whose chromosomes are more sensitive and unstabile for different mutagens action. Some investigators have the opinion, that MN test in exfoliated cells of cervix may be used in cancer screening programs as a routine test. So we support this opinion and suggest that MN assay, widely used as biomarker of cancer risk in humans, can be also used in Armenia especially for oncogynecologic patients.

### References

- Bonassi S., Biasotti B., Kirsch-Volders M., Knasmueller, Zeiger E., Burger S., Bolognesi K., Holland N., Thomas Ph., Fenech M. State of the art survey of the buccal micronucleus assay – a first stage in the HUMNXL project initiative. Mutation Research, 2009, v.24, N 4, p. 295-302.
- Holland N., Bolognesi C., Kirsch-Volders M., Bonassi S., Zeiger E., Knasmueller S. and Fenech, M. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutation Research, 2009, 659, p. 93-108.
- Nersesyan A., Vartazaryan N., Zalinyan G., Harutjunyan R. Micronucleus level in exfoliated buccal mucosa cells of women with hirsutism. Central Eur.Y. Occup. Environ. Med., 2001, 7, p. 39-44.

- 4. Nersesyan A., Vartazaryan N., Harutjunyan R. Micronuclei and other nuclear anomalies in exfoliated cells of gynecologycal cancer patients. NATO, Advanced research workshop, Human monitoring for genetic effects. Book of abstracts. 2002, p. 65.
- Nersesyan A.K. Micronucleus test in in exfoliated human cells as the method of study of the actions of mutagens/carcinogens. (In Russian). Tritol. Genet., 1996, 30, p. 91-97.
- 6. Nersesyan A., Kundi M., Atefie K., Shulte-Hermann R., Knasmueller S. Effect of staining procedures on the results of micronucleus assay with exfoliated oral mucosa cells. Cancer epidemiol biomarkers prev. 15(10), 2006, p. 1834-1840.
- Nersesyan A. Martirosyan A., Zalinyan G., Parsadanyan G. Chromosomal aberrations level in peripheral blood lymphocytes of women with polycystic ovary syndrome. LBUON 11, 2006, p. 477-480.
- Rajeswari N., Ahuja Y., Malani U., Chandrashekar S., Balakrishna N., Rao K., Khar A. Risk assessment in the first degree relatives of breast cancer patients using the alkaline comet assay. Carcinogenesis, 2000, 21, p. 557-561.
- 9. Rosin M., The use of the micronucleus test on exfoliated cells to identify anticlastogenic action in humans: a biological marker for the efficacy of chemopreventive agents 1992, p. 267, p. 265-276.
- Titenko-Holland N., Moore L., Smith M. Measurment and characterization of micronuclei in exfoliated human cells by fluorescence in situ hybridization with a centromeric probe, 1994, 312, p. 39-50.
- 11. Tolbert P., Shy C., Allen J. Micronuclei and other nuclear anomalies inbuccal smears: methods development. Mutat. Res., 1992, 271, p.69.