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THE INFLUENCE OF RADIATION THERAPY ON THE LEVELS OF MICRONUCLEI AND OTHER NUCLEAR ANOMALIES IN EXFOLIATED BUCCAL AND CERVIX CELLS OF CANCER PATIENTS

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Abstract

The micronucleus (MN) and other nuclear anomalies levels in exfoliated buccal mucosa and cervix cells of 10 women with uterine cervix cancer before, during, and after radiotherapy were investigated. The data were compared with basal accepted parameters, parameters of 10 healthy women of corresponding age were used as negative control.

Significantly increased levels of MN and cells with MN along with some nuclear anomalies were found in both buccal and cervical cells of patients compared with healthy women.

The significant increase of frequencies of MN and most of nuclear anomalies were found in cervical cells of patients during the radiation therapy and immediately after it. Two weeks after the end of therapy, almost all endpoints recovered to the basal levels.

In contrast, no significant changes were observed in buccal cells of patients under the therapy.

Keywords: *Micronuclei; nuclear anomalies; exfoliated cells; cervix cancer; radiotherapy*

INTRODUCTION

Micronuclei (MNs) are DNA-containing bodies in cell cytoplasm which are either chromosomal fragments or whole chromosomes. It was shown that their presence indicate chromosomal aberrations (either structural – clastogenic effect, or numerical – aneugenic effect) and can reflect genotoxic effects of different environmental, occupational and life style factors (mutagens/carcinogens) [*Rosin M., 1992*].

The MN assay in exfoliated epithelial cells is more economic, widely used and minimally invasive method for estimation of genotoxic and acute toxic effects of different factors. The increased level of cells with MN was observed in cells of cancer patients before and after local radiotherapy [*Holland N. et al., 2008; Bonassi S. et al., 2009; Thomas P. et al., 2009*].

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Significantly increased frequencies of cells with MN (MNC) in patients with cancers of different organs (e.g., breast, uterus, cervix of uterus, lungs) were revealed compared with healthy persons [*Rajeswari N. et al., 2000; Nersesyan A., 2002; Nersesyan A., Adamyan R., 2004; Vardazaryan N., 2004*].

Recently N. Vardazaryan [*Vardazaryan N., 2004*] studied the changes of MN and other nuclear anomalies in exfoliated oral mucosa and cervix cells of patients with cancer of cervix uterus and hirsutism. In this study the cells were evaluated before, during and after the radiation therapy.

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

MATERIAL AND METHODS

In the present work 10 women with diagnosed uterine cervix cancer of the first degree were studied. The patients were diagnosed and treated at the National Center of Oncology, Yerevan.

The control group was comprised of 10 healthy women volunteers of the corresponding age. Each of volonteers was interviewed about their life-style habits, which could influence MN level (smoking, alcohol, medication, hereditary diseases, viral infections). Subjects, who did not meet mentioned criteria, were excluded from the study. Written consents from all study participants were obtained.

Buccal mucosa and cervix cells were collected from both patients with cancer and healthy women with wooden spatula. Cells from cervix were collected from the opposite site of tumor location. Both types of cells were collected before the initiation of radiation therapy according to the internationally accepted protocol [*Toita T., 2009*], after receiving radiation doses of 25 *Gy*, 50 *Gy,* and 15-20 days after the end of therapy.

Feulgen staining technique with some modifications was used. As counter-stain fast green was used. During the analysis of cells other nuclear anomalies such as karyolysis (KL), pycnosis (P), karyorhexis (KR), condensed chromatin (CC), binucleares (BN), "broken egg" (BE) were studied as well [*Tolbert P. et al., 1992; Holland N. et al., 2008; Bonassi S. et al., 2009; Thomas P. et al., 2009*]. The criteria of scoring cells with MN and other nuclear anomalies described in the protocols standardized in the frame of $HUMN_{XL}$ project were applied [*Holland N. et al., 2008; Bonassi S. et al., 2009; Thomas P. et al., 2009*].

Investigation was performed in 20000 buccal and 20 000 cervical cells from healthy women and 80000 buccal and 80000 cervical cells from patients. Totally, 200000 cells were evaluated in this study.

The statistical analyses were carried out by means of Mann-Whitney U-test (GraphPad-Prism, version 5.03).

RESULTS AND DISCUSSION

In exfoliated cells collected from buccal cavity and cervix of cancer patients the significant increase of of MNC and MN frequencies was observed compared with healthy women. In buccal cells of patients along with MN some other nuclear anomalies probably connected with genotoxicity were also significantly increased, namely: BE, BN and KR. In cervical cells frequencies of all nuclear anomalies, except CC and P, were significantly elevated (Tables 1-2).

No significant changes in number of MN and all nuclear anomalies were observed in buccal cells of patients during the radiotherapy and after it. Therefore, these data are not presented.

Studies of the cervical cells after receiving half the radiation dose (25 *Gy*) showed that the frequencies of MNC and MN were significantly increased showing well-known genotoxic effects of radiation. Along with mentioned endpoints the number of BN was significantly increased as well (Table 3).

In contrast, immediately after the end of the therapy, all nuclear anomalies, except CC and P, were increased along with MNC and MN. It is noteworthy that the frequencies of MNC and MN after the end of therapy were significantly lower than after receiving half the radiation dose (Table 3).

Two weeks after the therapy only frequencies of KR and KL were significantly increased compared with the basal levels of patients.

In the present study, we found significantly increased numbers of MN in exfoliated buccal and cervical cells of gynecological cancer patients; this latter support our data [*Nersesyan A. et al., 2002*] and data obtained by other groups of investigators [*Vardazaryan N., 2004*].

It is noteworthy that many investigators mentioned that in cells of patients exposed to half the radiation dose, the number of MN is significantly higher than in cells of patients after

Table 1.

The levels of micronuclei and other nuclear anomalies in exfoliated buccal mucosa cells of healthy women and patients

Parameters in buccal cells	Healthy subjects (‰)	Patients (‰)
cell with micronuclei (CMN)	1.1±0.1	2.5±0.7*
micronuclei (MN)	1.5±0.2	2.8±0.7*
karyorhexis (KR)	1.7±0.2	4.4±0.4*
karyolysis (KL)	0.7±0.1	1.4±0.3
pycnosis (P)	0.4±0.1	0.7±0.3
binucleates (BN)	4.4±0.9	7.0±0.8*
condensed chromatin (CC)	5.6±0.9	5.9±0.8
broken egg (BE)	0.5±0.2	1.5±0.1*

** - p<0.02*

Table 2.

The level of micronuclei and other nuclear anomalies in exfoliated cervical cells of healthy women and patients

Parameters in cervical cells	Healthy subjects (‰)	Patients (‰)
cell with micronuclei (CMN)	1.6±0.1	3.0±0.7*
micronuclei (MN)	1.9±0.2	3.4±0.7*
karyorhexis (KR)	2.4±0.2	8.2±0.4*
karyolysis (KL)	1.7±0.1	5.4±0.6*
pycnosis (P)	0.3±0.1	0.7±0.2
binucleates (BN)	4.9±0.9	6.8±0.8*
condensed chromatin (CC)	5.0±0.9	5.9±0.8
broken egg (BE)	0.5±0.1	1.2±0.3*

** - p<0.02*

Table 3.

The levels of micronuclei and other nuclear anomalies in exfoliated cervical cells of patients during the therapy

Parameters in cervical cells (‰)	Time points			
	Before the therapy	During the therapy	Immediately after the end of therapy	Two weeks after the end of therapy
cell with micronuclei (CMN)	3.0±0.7	6.4±0.6*	5.2±0.5*	4.2±0.3
micronuclei (MN)	3.4±0.7	7.5±0.8*	5.7±0.6*	4.9±0.4
karyorhexis (KR)	8.2±0.4	8.6±0.7	15.8±0.5*	11.1±0.9*
karyolysis (KL)	5.4±0.6	5.1±0.4	9.7±0.4*	7.9±0.6*
pycnosis (P)	0.7±0.2	1.2±0.3	0.7±0.5	1.3±0.4
binucleates (BN)	6.8±0.8	9.6±0.4*	11.0±0.9*	7.3±0.7
condensed chromatin (CC)	5.9±0.8	6.3±0.5	8.0±0.9	3.9±0.4
broken egg (BE)	1.2±0.3	1.2±0.2	6.3±0.2*	2.1±0.6

** - p<0.02*

the end of therapy [*Bhattathiri N. et al., 1998a; 1998b; Cao J. et al., 2002; Nersesyan A. et al., 2002; Vardazaryan N., 2004*].

The absence of MN and most nuclear anomalies 2 weeks after the therapy is due to change (turnover) of cells, because the cell cycle of exfoliated cells is between 15 and 21 days [*Holland N. et al., 2008; Bonassi S. et al., 2009; Thomas P. et al., 2009*].

It is noteworthy that our results support data presented by the research group of N. Bhattathiri and co-authors [*Bhattathiri N. et al., 1998a; 1998b*] that at half the radiation dose only few nuclear anomalies were significantly increased along with MN. But after receiving the complete dose of radiation accumulation of the number of all nuclear anomalies was registered.

Also, N. Bhattathiri and associates [*Bhattathiri N. et al., 1998a; 1998b*] showed that in patients sensitive to radiation therapy the number of MN is higher than in non-sensitive ones. We also noted such phenomenon, but because of low number of patients under study, we do not stress this circumstance because in this case it will be very speculative.

In conclusion, MN assay in exfoliated cells is very promising in the gynecological oncology. Further investigations in this area are certainly warranted.

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