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DETECTION OF GENOTOXIC EFFECT OF CHEMOTHERAPY IN PATIENTS OF THE GASTROENTEROLOGY CLINIC IN A HOSPITAL IN PRISHTINA

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Summary

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The micronuclei assay (MA) in exfoliated buccal cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens. Micronuclei are suitable internal dosimeters for revealing tissue-specific genotoxic damage in individuals exposed to carcinogenic mixtures. In the present investigation, 15 patient and 15 control subjects were enrolled for DNA damage analysis in buccal cells by micronucleus assay. Patients showed a significant increase in micronucleated cells when compared to controls groups.

Key words: induced genotoxic effect, micronuclei, medicaments

INTRODUCTION

The study of DNA damage in exfoliated cells collected from the oral cavity holds great promise as a minimally invasive method for monitoring populations exposed to genotoxic agents. The presence of micronuclei (MN) and other nuclear anomalies within these cells has been shown to be associated with genetic defects in genome maintenance, accelerated ageing, exposure to genotoxic agents, oral cancer risk and neurodegenerative diseases and was also used in chemopreventive studies. While these results indicate the suitability of this assay for measuring chromosomal damage in human populations, the mechanism linking MN in buccal exfoliated cells to chromosome damage in progenitor cells is difficult to prove given the difficulty in performing chromosome metaphase analysis in this cell type. However, given the extensive evidence from *in vitro* studies of cultured epithelial cells and lymphocytes demonstrating that MN originate from either lagging chromosome fragments or whole chromosomes, it is reasonable to infer that MN in buccal cells originate mainly via these mechanisms.

MATERIAL AND METHODS

The study population consisted of 15 patients (8 males and 7 female) and control group of 15 healthy subjects. The youngest patient was 21 years of age and the oldest – 74 years of age. The patients are subjected to chemotherapy to cure tuberculosis.

Buccal cells were sampled with toothbrush from the inside of the cheeks and

Table 1. Frequency of micronuclei (MN) in patients after chemotherapy, scored to 1,000 buccal cells

	MN in chemotherapy patients	MN in control group	Significance
Total MN number (female and male)	122 MN	46 MN	
Mean MN (female and male)	8.25 MN	3.06 MN	P=0.004
Total MN number (females; n=7)	50 MN	40 MN	
Mean MN (females)	7.14 MN	2.85 MN	P=0.083
Total MN number (males; n=8)	66 MN	25 MN	
Mean MN (males)	8.25 MN	3.12 MN	P=0.003
Oldest group	7.5 MN	5.12 MN	
Youngest group	5 MN	3.28 MN	

placed in physiological solution (NaCl 0.9%). The cells were washed thrice in the buffer solution by centrifugation.

After centrifugation (10 min at 1000 rpm), the pellet was fixed in 3:1 methanol/acetic-acid, for 10 minutes. Cell suspension was dropped onto clean slides. Five slides were prepared for each subject and 1000 cells (at 100× magnification), from each subject were examined. The cells were stained in 10% Giemsa solution.

RESULTS

The frequency of micronuclei in buccal cells after chemiotherapy of patients was 8.13 MN/1000 buccal cell it was statistically significantly (P=0.004) higher compared with MN in control group (3.06 MN/1000 buccal cells) (Table 1; Fig. 1–3).

When we analysed the frequency of MN after separating the patients according to the gender we found that the frequency of MN it was higher at male (8.25 MN/1000 cells) compared with female (7.14 MN/1000 cells), but not statistically significant.

Also, MN frequency in sick males was statistically higher (8.25 MN/1000 cells) compared with male at control group (3.12 MN/cells; P=0.003).

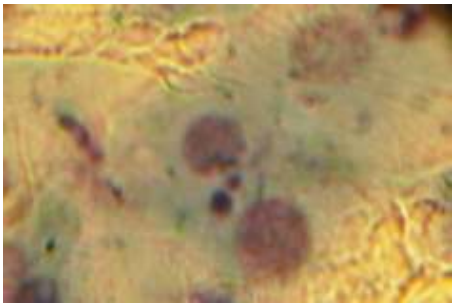

Fig. 1. Micronucleus in buccal cells.

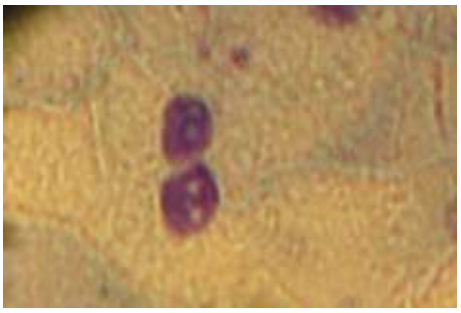

Fig. 2. Kariolysis and pyknosis.

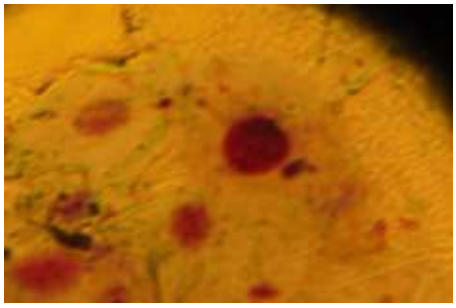

Fig. 3. Karrhyorhexis.

Differences between sick and control females were not statistically significant (P=0.083).

DISSCUSION

Human buccal mucosa is composed of progenitor and maturing cell populations (Ten Cate *et al.,* 1998). Exfoliated cells of buccal mucosa are good indicators of chromosomal damage and other nuclear abnormalities such as binucleates, karyorrhexis and karyolysis (Tolbert *et al.,* 1992). Buccal MN test is preferred over cytokinesis-block micronucleus (CBMN) or human capillary blood lymphocytes micronucleus (HCBL-MN) methods by researchers worldwide to detect genotoxicity induced *in vivo* by environmental carcinogens (Martino-Roth *et al.,* 2003), chemotherapeutic agents (Rekhadevi *et al.,* 2007) and pesticides (Susana *et al.,* 2002), as this test is a minimally invasive, less time consuming and can be easily applied on interphase cells with no requirement for cell culture or metaphase preparations (Holland *et al.,* 2008).

CONCLUSION

According to this investigation, we can conclude that: 1) Chemotherapy induced increased number of micronuclei in the buccal cells but not statistically significantly compared with control group; 2) Males had greater average number of MN (8.25 MN) compared with females (7.14); 3) The frequency of micronuclei in the oldest group was insignificiantly higher compared to that of the youngest group (7.6 and 5 MN respectively).

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