X-ray induced micronucleus frequency assay on radiology technicians' lymphocytes

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In this study, the effects of occupational exposure to X-rays were evaluated by using micronucleus (MN) frequency assay in peripheral lymphocyte culture. The study was carried out in 10 radiology technicians (group 1) and 10 healthy volunteers (group 2). The MN assay was performed according to the cytokinesis blocked method. The comparison of mean values of MN showed that there was not significantly differences between the two groups. [Turk J Med Res 1997; 15(2):56-59]

Key Words: Micronuclei frequencies, X-Rays, Human lymphocytes.

The micronucleus test is an effective method for the evaluation of genotoxic or clastogenic effects of physical and chemical agents since the micronuclei (MN) are formed from the condensation of lagging acentric chromosomes, chromatid fragments or entire chromosomes (1, 2). There has been a great deal of interest shown in this phenomenon but a major drawback of the system has been uncertainly regarding the cell cycle history of the cells chosen for analysis. This shortcoming has now been overcome, firstly by the bromodeoxyuridine/ Giemsa staining method of Pincu et al. (3) and more recently by the development of the cytokinesis-blocked MN technique which employs cytochalasin B to stop dividing cells before performing cytokinesis and thus allows cells that have completed one nuclear division to be recognised by their binucleated appearance (4, 5). As a consequence, the cytokinesis-blocked MN assay has been shown to be more accurate and more sensitive than conventional methods which do not distinguish between dividing and non-dividing cells {5, 6). This technique has been used to quantify the frequencies of radiation induced MN in human peripheral blood lymphocytes (4, 7). Increases in MN frequencies were most strongly correlated with the dose of ionising radiation, but age, alcohol consumption and smoking also affected MN frequencies. Despite these additional influences, MN frequencies can be useful as biological dosimeters (8).

The present study was carried out to obtain an insight into the clastogenic effects of radiation to the lymphocytes of radiology technicians who had been occupationally exposed to X-rays.

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MATERIALS AND METHODS

The study was carried out in ten male non-smoker radiology technicians (group 1) and ten male non-smoker healthy volunteers (group 2). Group 1 subjects aged between 25 and 35, had been occupational^ exposed to X-rays during the last six years. Group 2 subjects were selected among healthy volunteers who had the same age distribution as the workers. Heparinized blood samples were obtained from the subjects of both groups and standard lymphocyte cultures were performed. For the MN assay, cytochalasin B at a final concentration of 3 mg/ml was added to 44th of the culture of lymphocytes, according to the method of Fenech and Morley (4). Cytochalasin B is an inhibitor of microflament assembly and prevents cytop'asmic division after nuclear division occurred and does r ot itself produce MN (4, 5). To protect the cultures from the photolytic effect of the light, they were covered with aluminium folio. At the end of incubation peripheral blood lymphocyte cultures were centrifuged (1000 rpm, 10 min) at room temperature. Then cells were fixed in fresh fixative (methanol: acetic acid, 3:1). This procedure was repeated twice. The cell suspension was dropped onto cold slides and stained in 5% Giemsa solution for 5-7 minutes. Slides were coded and scored blind under a magnification of 200 X. A total of 1000 binucleated lym-

phocytes with preserved cytoplasm were scored for both groups. Scoring of MN frequencies was performed according to the criteria of Countryman and Heddle (9). Statistical evaluation was made using the Student's t test.

RESULTS

MN assay was conducted on the lymphocytes of 10 male non-smoker radiology technicians who had been occupational^ exposed to X-rays during the last six years and 10 male non-smoker healthy volunteers, according to the Fenech and Morley's cytokinesis blocked MN method. 1000 cytokinesis blocked binucleated cells of each cases scored for MN assay. A binucleated cell with MN obtained from lymphocyte cultures was shown in figure 1. The frequency of binucleated cells with MN obtained from cases were given in table 1. The mean value of MN/cell in radiology technicians and healty volunteers was 0.0089±0.0020 and 0.0082±0.0016, respectively. The comparison of mean values of MN/cell showed that there was not a significant statistical difference between the two groups (P>0.05).

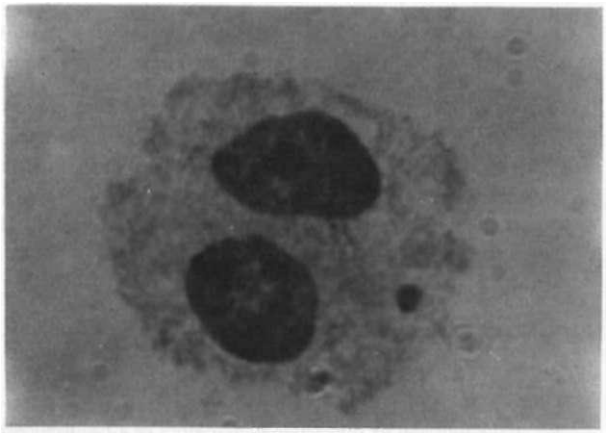

Figure 1. A binucleate lymphocyte with a micronucleus.

DISCUSSION

The frequencies of induced MN have been used as a quantitative indicator of X-rays induced chromosome damage in early studies with plant meristems (10). MN induction in polychromatic erythrocytes of treated mice are used to screen chemicals for genotoxicity in vivo (11). In human peripheral blood lymphocytes, induced MN have been used for quantitative comparison of frequencies of X-rays induced chromosomal aberrations in normal and Down syndrome individuals (9). Though there appears to be a good relationship between induced chromosomal aberrations and MN in experiments involving. Ramalho et al. (12) reported their recent work on MN induction in human lymphocytes using bromodeoxyuridine (BrdU) technique, and also with cytochalasin B inhibition of cytokinesis. They observed that the yield of chromosomal aberrations following irradiation with different doses of X-rays was determined parallel to the yield of MN in binucleated cells.

Table 1 shows the background MN levels measured in a sample of 10 individuals (control group) and 10 experimental group (radiology technicians). The frequency ranged from 0.005 to 0.010/cell in control group and 0.006 to 0.013/cell in experimental group which is narrower than the range of 0.002-0.032/cell published by Fenech and Morley (5) for donors aged between 20 and 40. Other typical average background values obtained using the earlier technique of fixation at a single culture time or at a time which gives maximum MN yield with no selection for second interphase cells are 0.014-0.022/cell (13). The variation in these figures probably reflects the kinetics of MN loss from cells undergoing successive divisions in culture. On the other hand, unknown factors can influence MN frequencies such that the accumulation of MN from non-radiation related causes may, with time, obscure the radiation induced frequencies. As MN result from chromosome breakage or aneuploidy induced MN tend to decrease in frequency with time as chromosomal

Table 1. Yield of micronuclei in control and radiology technicians' lymphocytes determined with the cytokinesis-blocked method.

		Control Group		Radiology Technicians	
No	Number of Cells	Number of Micronuclei	Micronuclei/Cell	Number of Micronuclei	Micronuclei/Cell
1	1000	7	0.007	8	0.008
2	1000	7	0.007	11	0.011
3	1000	9	0.009	7	0.007
4	1000	10	0.010	9	0.009
5	1000	9	0.009	10	0.010
6	1000	9	0.009	6	0.006
7	1000	10	0.010	8	0.008
8	1000	7	0.007	8	0.008
9	1000	5	0.005	13	0.013
10	1000	9	0.009	9	0.009
TOTAL		82	0.0082	89	0.0089
Mean Value±SD			0.0082±0.0016*		0.0089+0.0020*

*P>0.05

damage leads to cell death or MN are lost during cell division (14, 15). It seems likely that cells with more chromosomal damage, and hence more MN. As a result of the possible application of the cytokinesis-blocked MN assay in biological dosimetry following radiation accidents, several laboratories around the world have now established dose-response parameters following exposure to various sources of ionising radiation (12, 15, 16, 17, 18, 19, 20). In addition Fenech (8) suggested that spontaneous MN frequency in individuals was particularly important in a continuous monitoring programme of individuals who were occupational^ at risk of exposure to a powerful environmental mutagen such as radiation. In this case, individuals acted as their own controls; their MN frequencies were measured before and at regular intervals during their occupation. This situation was ideal since differences in life-style factors should not be a confounding factor. However, the value of this approach also depends on the reproducibility of the assay; in other words the assay should provide similar results when measurements were made on the same individual on different occasions separated by no known significant exposure to mutagenic agents.

Prosser et al. (7) have reported, by using the Fenech's cytokinesis-blocked method, that accurate dose estimation by means of MN induction was practicable for iow-LET radiation doses above about 0.1 Gy. In our experimental group, when the lymphocyte cultures of radiology technicians who had been occupational^ exposing to X-rays during the last six years and healthy volunteers were compared, it was seen that occupational X-rays exposure does not play an important role in MN induction. This proved that members of Radiology Department were under the efficient protection.

Radyoloji teknisyenlerinin lenfositlerinde X-ışınlarının uyardığı mikronükleusların incelenmesi

Bu çalışmada, X ışınlarına mesleki maruzatın insan periferal kan lenfosit kültürlerinde MN frekansını etkileyip etkilemediği araştırılmıştır. Radyoloji bölümünde çalışan teknisyenlerden bir deney ve gönüllüler arasından da bir kontrol grubu oluşturularak her iki gruptan elde edilen periferik kan lenfosit kültürlerinde sitokinezi blok metodu ile MN frekansı tesbit edilmiştir. İstatistiksel analizlerde deney grubundan elde edilen ortalama MN değeri ile kontrol grubundan elde edilen ortalama MN değeri arasında anlamlı bir farklılık görülmemiştir. [T Klin Araştırma 1997; 15(2):56-59]

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