Chromosomal Aberrations and Micronuclei in Lymphocytes of Medical Radiation Workers Exposed to Ionizing Radiation Below Dose Limit

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Abstract

Introduction: Ionizing radiation induces various kinds of DNA damage in which may lead to chromosomal aberrations (CA). Inspite of growing importance in the risk assessment, the dose yield kinetics of CA and their implications for dose assessment are not well established in exposures to low level radiation.

In the present study, cytochalasin-B blocked micronucleus assay and metaphase analysis were used as test systems to monitor hospital radiation workers who received chronic low dose ionizing radiation below dose limit.

Materials and Methods: Heparinized blood samples were taken from healthy non-smoker radiology and radiotherapy workers occupationally exposed to X and gamma rays and healty population whose duties do not expose them to radiation sources and chemical agents. The whole body dose was measured by film badge.

Lymphocytes were cultured in RPMI-1640 supplemented with 15% FBS, and metaphase spread was prepared using standard cytogenetic method. Cytochalasine-B (3μg/ml)treatment was used for binuclei micronuclei assay. 100 mitoses and 1000 binuclei lymphocytes were scored for CA and micronuclei (MN) respectively.

Results: Results show a high frequency of CA mainly deletions and simple breaks in radiation workers compared to control($p<0.001$).Also results show the mean MN/cell was significantly ($p<0.001$) higher in radiation workers(0.035) when compared to non exposed individuals(0.022).

Conclusion: A relatively high frequency of MN and CA formation in lymphocytes of radiation workers compared to non exposed individuals might be due to an accumulation of initial DNA damage in people exposed to chronic doses of radiation leading to detectable genetic damages. These observations might imply that the current occupational exposure levels might be inadequate to prevent an increase in chromosome damage rate.

Key Words: Micronuclei, Chromosome aberration, Low dose radiation, Peripheral lymphocyte

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Introduction

Physical procedures to estimate radiation dose are based on personal dosimeters which have the capability to assess the amount of radiation exposure with adequate accuracy and over a sufficiently wide dose range. The personal dosimeters, measure the radiation dose to themselves only and under non-uniform field, the personal dosimeters could record a very low absorbed dose to the body, while a substantial dose is received by some parts of the body. Also there is no absolute guarantee that those involved in a radiation accident will be wearing a personal dosimeter. These kinds of limitations have led to the search for finding suitable biological indicators for dosimetry.

Because DNA is a critical radiation target and any damage to DNA is generally repaired with high efficiency, bio-indicators based on cytogenetics will measure biologically relevant dose, whereas those based on non-repairable lesions or non-adaptive responses will measure absorbed dose(1,2).

After an accidental over-exposure with low LET radiation where the dose is uniformly distributed over the whole body, the frequency of aberrations induced - blood lymphocytes is a reliable measure of the mean dose to the body (3). Even in cases of partial-body exposure, cytogenetic changes in blood lymphocytes are indicators of the average absorbed dose to the whole body (4).

The common cytogenetic observed end-points are chromosomal aberrations and micronuclei. Because of its rapidness, simplicity and potential for automation, the measurement of micronucleated cells might be a good candidate assay to replace in vitro chromosomal aberration assay, especially for screening of large populations. The development of cytokinesis-block micronucleus assay has enabled direct and valid comparisons with chromosome aberration assays (5).

Micronuclei are formed from the condensation of lagging acentric chromosome or chromatid fragments or entire chromosomes. Previously the frequency of micronuclei was usually calculated on the basis of all interphase cells, but the use of cytochalasin B for blocking cells at cytokinesis, produces binucleated cells which allows unequivocal identification of cells which had gone through at least one cell division (6).

In this paper we present results from a comparative study on biological dosimetry of medical radiation workers with two techniques of metaphase analysis of chromosomes and cytokinesis-blocked micronucleus assay (CBMN).

Materials and Methods

* *Sampling*

Heparinized blood samples were drawn by venopuncture from 37 healthy non-smoker individuals, 24 radiology and radiotheraphy workers, occupationally exposed to X and gamma rays [18 males and 6 females aged 24-63 years(mean 36.5±9.6 years) and occupational experiences of 2-25y] and 13 individuals as a control population [9 males and 4 females aged 25-59 years(mean 36.7±10y)] whose duties do not expose them to radiation sources or chemical agents.

No medicines were taken by the donors for at least one month before sampling. Also subjects who had complaints due to genetic disorders in the family or had X-ray examination within one month prior to the examination were excluded from the study. The whole body doses received by the radiation workers group as measured by film badges showed that none of them had annual doses more than the permissible limits as defined by the International Commission for Radiation Protection(ICRP).

* *Cell culture*

Each blood sample was divided into two parts;one for CBMN and the other for metaphase preparation.

Whole blood cultures were prepared by adding 0.5ml blood to 4.5ml culture medium consisting of RPMI-1640(Gibco BRL) supplemented with 0.2mM L-glutamine,15% heat-inactivated fetal bovine serum(Gibco BRL), 100μg/ml penicilline and 5μg/ml phytohemagglutinin (PHA) was used to stimulate division of lymphocytes in culture.

For MN preparation, cytochalasin B (Sigma) was added to cell cultures 44h after PHA stimulation, at a concentration of 3 μg/ml (3) to block cells at cytokinesis. After an incubation period of 64h at 37° C,

the cells were collected by centrifugation and treated with a hypotonic solution containing 0.075 M KCl, for 4-6min to obtain good preservation of the cytoplasm. After centrifugation at 1500 r.p.m. The cells were fixed in a 3:1 methanol: glacial acetic acid mixture diluted 1:1 in Ringer's solution. Final fixation was made by a mixture of 6:1 methanol: glacial acetic acid. Cells were dropped on to cooled, clean slides with a Pasteure pipette and air dried.

For metaphase preparation blood cultures were incubated at 37° C for 48 hours and 2 hours prior to harvesting, colcemid (Gibco BRL) was added at a final concentration of0.1μg/ml. After hypotonic treatment with 0.075 M KCl for 10 minutes, lymphocytes were fixed in a 3:1 mixture of methanol and Glacial acetic acid. Cells were dropped from suitable distance onto cooled, clean slides with a Pasteure pipette and air dried.

A 4% Giemsa (Merck) solution was used for staining of micronuclei and metaphases slides.

The number of cells scored varied depending on the techniques used for cell culture. One hundred mitoses were analysed for each sample on the basis of different chromosomal aberrations.Lesions were classified according to the international system of cytogenetic nomenclature for acquired chromosome aberrations (ISCN 1995).Chromosomal aberrations were divided into chromosome and chromatid types. Micronuclei were scored in binucleate cytokinesis-blocked cells using 100x magnification. The criteria described by Fenech in 1993 (5) were followed for identification of binucleate lymphocyts and MN. One thousend binucleate cells were scored for each sample.

Kolmogorov-Smirnove, Student *t*-test, and multiple regression analysis were used for statistical evaluation. These analyses were done with Spss software.

Results

The mean frequencies of chromosomal aberrations in lymphocytes of radiation workers and healthy controls are shown in table 1.

In both groups, the majority of aberrations were either simple chromatid deletions (ctd) or achromatic lesions (gaps). The mean frequencies of different aberrations are higher in radiation workers than controls, and these differences except for chromosomal exchanges (cse) are statistically significant.

Table 1: Frequencies of chromosomal aberrations per cell in radiation workers and controls

	Aberration	Radiationworkers	Controls	Pvalue
Chromosome type	Exchanges	0.021±0.039	0.005±0.007	>0.05
	Chromosome del.	0.027±0.016	0.012±0.007	0.001
	Total Chromosomal ab.	0.058±0.040	0.025±0.010	0.002
Chromatid type	Gaps	0.044±0.018	0.024±0.013	0.002
	Chromatid del.	0.079±0.030	0.037±0.019	0.000
	Total Chromatid ab.	0.124±0.044	0.061±0.028	0.000
	Total aberrations	0.182±0.068	0.085±0.035	0.000

del.= deletions, ab.= aberration

There were significant relations between total aberrations and also total of chromosome aberrations (cst) with the years of chronic exposure (fig 1). Also there were significant relationships for some types of aberrations in control group and the age of them.

$cse/cell = 3.83\times10^{-4}A - 8.71\times10^{-3}$ $r^2=0.33$ P=0.04 (1)

$ctd/cell = 1.45\times10^{-3}A - 0.016$ $r^2=0.56$ P=0.003 (2)

$cht/cell = 1.99\times10^{-3}A - 0.013$ $r^2=0.49$ P=0.008 (3)

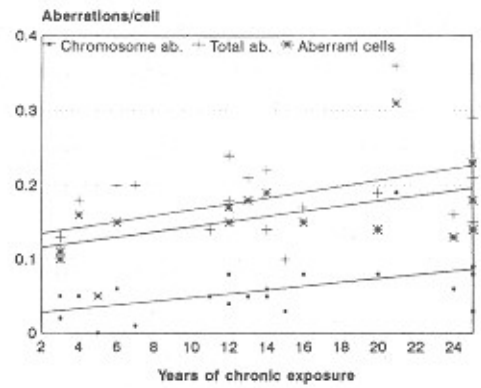

Fig. 1: Relations between deferent types of aberration and the years of cronic exposure

The mean frequencies of spontaneous MN/cell was significantly (P<0.001) higher in radiation workers (0.035) when compared with non-exposed individuals (0.022) (table 2, for complete results see reference 7). There was a relationship between spontaneous MN/cell and the years of chronic exposure of radiation workers (Y) (eq. 4).

Table 2: Frequencies and distribution of MN in blood lymphocytes of radiation workers and controls

group	No. of scored binucleated cells	MN distribution				TotalMN	MN/cell
		0	1	2	3		
R.W.	24000	23172	695	65	1	828	0.035±0.008
C.G.	13000	12711	257	16	—	289	0.0022±0.004

R.W.= Radiation workers, C.G.: Control Group

$MN/cell=0.028+5.3\times10^{-4}Y \quad r^2=0.29, \quad P=0.006$(4)

A linear regression for the MN culture of radiation workers (R.W.) and control group (C.G.) Yields the following relations between the spontaneous MN per binucleated cells and donor age(A):

$MN/cell(R.W.)=0.023+3.33\times10^{-4}A \quad r^2=0.19 \quad P=0.03$(5)

$MN/cell(C.G.)=0.013+2.61\times10^{-4}A \quad r^2=0.41 \quad P=0.02$(6)

The ratio of micronuclei to the different aberration types are shown in table 3. These ratios for radiation workers are generally lower than control group.The nearest ratio to one for radiation workers and control group is MN/chromosome exchanges and deletions.

Table 3: The ratio of micronuclei to the different aberration types

Ratio of MN to:	Radation workers	Controls
Exchanges	1.67	4.4
Chromosome del.	1.3	1.83
Exchanges + chr.del.	0.60	0.88
Chromatid del.	0.44	0.60
Ctd.del. + chr.del.	0.33	0.45
Exchanges + ctd.del. + chr.del.	0.83	1.22

chr. = chromosome

ctd. = chromatid

Discussion

In 1969 for the first time Bender and Gooch used cytogenetic indicators for dose estimation of three men exposed during Recuplex accident(8). Since then numerous papers have been published, studying the effects of low or high doses of radiation on the level of chromosomal aberrations (8,9,10,11,12).

All of these studies indicate that the level of these aberrations in exposed people are more than non-exposed one. Also in this study we found that occupational exposure to low levels of X or gamma rays increased the frequency of different types of aberrations. Very low doses of X ray might not produce DNA strand breaks capable of forming unstable aberrations such as dicentrics and rings, for this reason there was not a significant difference in the rate of exchanges/cell between R.W. and C.G. The low level of radiation can cause DNA base damage which may be expressed as chromatid aberrations(11). This idea is in agreement with our results that show a significant increase in chromatid types of aberrations (Table 1 and references 11,14). In the other part of this study the frequencies of MN in binucleated cells were significantly higher in radiation workers compared to control group. These identical findings indicate that exposure to chronic low doses of radiation could lead to detectable genetic effects and might imply that current exposure limits are inadequate to prevent an increase in genetic damage rate or probably techniques and physical instruments for detecting occupational recieved dose are not very accurate.

The observed increase in frequency of total aberrations/cell, chromosome aberrations/cell and also MN/cell with increasing years of chronic exposure in radiation worker group indicates that frequency of genetic damages to the cells which expressed as chromosomal aberration and formation of micronuclei, depends partly on cumulative dose. Existance of this kind of relation between years of experences of R.W. and chromatid aberration (14) and also with total deletions and aberration (10) previously reported, but because of dependence of radiation effect to human body is depended on numerous factors (15) like inter-individual differences in sensitivity to radiation (16), the frequency of aberrations or MN are not always depended on integrated dose. DNA breakage with endogenous origin or exposing to environmental clastogenes and natural background radiation may increase the frequencies of aberrations of MN formation with increasing the age of human (17,18). Also decreasing the capibility of cell for repairing damages to DNA with increasing the age is probably the other reason for this phenomenon (19). In this study we found such relations for exchanges, chromatid aberrations and MN formation which especially for the last one is in agreement with the findings of other authors such as Fenech and Morley (6), Thierens et.al. (20) Fenech (5). Of course there is a

few paper which did not reports this relationship (21).

The similarities between results from chromosome study and MN scoring suggest that using of CBMN which is a more easier and faster technique can substitute for metaphase study. Results indicate that in radiation workers the number of MN is lower than simple chromatid deletions, chromosome exchanges & deletions, simple chromosome and chromated deletions & exchanges (total aberrations). Except for the total deletions, the same results valied for control group, of course the ratios of MN to different aberration are higher for control group than R.W. It is thought that any aberration or even a whole chromosome might become a micronuclei. Some of published results showed that by increasing the dose, the ratio of MN to total aberration decrease (3, 21, 22). Because we showed that the radiation workers have recieved radiation doses higher than control group, maybe we can conclude that the lower ratios in R.W. is the result of these doses. There is also some published paper that have presented not constant relation between dose and ratios of MN to total aberration (23, 24). It is suggested that with increasing the radiation dose and consequently the number of aberrations, there is a greater probability of two or more aberrations being enclosed in a single micronuclear membrane.

In this paper we have shown that the micronuclei assay with cytokinesis blocked method is a suitable and sensetive indicator of the frequency of chromosomal aberrations induced by ionizing radiation and it is useful for monitoring populations at risk such as radiation workers.

We should also insist on lowering the dose recieved by radiation workers and improving the physical methods for estimation of recieved dose by this group.

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