

ORIGINAL ARTICLE

CHROMOSOMAL ABERRATIONS IN LYMPHOCYTES OF INDIVIDUALS WITH CHRONIC EXPOSURE TO GAMMA RADIATION

Hossein Mozdarani PhD^{*}, Ashkan Hejazi MSc, Peyman Hejazi MSc

Department of Radiology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran

Abstract

Background-Apart from direct dose assessment of radiation exposure, analysis of chromosomal aberrations using the standard method has been widely used in monitoring the cytogenetic effects of long-term occupational radiation exposure. Chromosomal damage in human somatic cells may serve as an early indicator of hazard, thus enabling prevention of adverse effects.

Methods-The frequency of chromosomal aberrations were evaluated in the peripheral blood lymphocytes obtained from 51 nuclear medicine technologists (NMT) who were exposed to chronic doses of gamma rays and 15 healthy controls. Lymphocytes were cultured in RPMI-1640 and metaphase plates were prepared using standard cytogenetic method. One hundred mitoses were analyzed for the presence of chromosomal aberrations for each sample. Radiation exposure was measured using film badge and thermoluminescent dosimeter (TLD).

Results-The monthly dose equivalent received by the nuclear medicine technologists was between 0.03 to 2.80 mSv as measured by TLD and film badges. The frequencies of all types of aberrations (gaps, breaks and dicentrics) in exposed subjects were higher than those observed in the control group ($p < 0.0001$). This study showed a linear dose-response relationship for total chromosomal deletions and dicentrics ($p < 0.0001$).

Conclusion-The findings in this study are important, because radiation workers who have been exposed to doses well below accepted standards for exposure to radiation also show elevated levels of all types of chromosomal aberrations. Therefore, these results might imply that workers receiving very low doses of radiation during a long period of time could be considered as individuals at risk.

Keywords • Occupational radiation exposure • nuclear medicine • chromosomal aberrations • lymphocytes

Introduction

The importance of cytogenetic study of peripheral lymphocytes in persons exposed to ionizing radiation has been reported for more than 30 years.¹ During this time, numerous papers have been published studying the effects of low-level radiation for the occupationally exposed hospital workers²⁻⁸, nuclear plant personnel⁹⁻¹¹, accidental exposures^{1,12-15} or more recently, for the Chernobyl accident clean-up

workers¹⁶ by means of conventional metaphase chromosome analysis. It is now well established that chromosomal aberrations in human lymphocytes provides the most sensitive and reliable method for biological dosimetry.^{17,18}

Although it is now apparent that cytogenetic biodosimetry can be used reliably to reconstruct radiation dose after acute uniform whole body exposure within certain dose ranges¹⁹, additional data are required to fully validate cytogenetic biodosimetry for the chronic doses of external irradiation.

The baseline frequency of chromosomal aberrations from the normal population has a wide

^{*} Correspondence: H. Mozdarani PhD, School of Medical Sciences, Tarbiat Modarres University, P.O.Box: 14115-111, Fax: +98-21-8006544, E-mail: Mozdara@Net1cs.modares.ac.ir.

range of variation and these might reflect inter-laboratory differences in culture conditions or sample characteristics. The aberration yields were analyzed by considering some influencing factors such as absorbed dose. However, it is evident that in all individuals exposed to ionizing radiation, higher frequency of chromosomal aberrations was reported compared to controls, even if their exposure was lower than permissible level.^{3,7,20-22} The permissible level of radiation received by exposed persons is also estimated on the basis of the frequency of chromosomal aberrations in order to prevent the occurrence of significant changes in the genetic pool of the whole population.

The purpose of this study was to provide more information on the genetic hazards of occupational exposure to chronic doses of gamma rays, hence its potential risks and biological consequences. Ionizing radiation is known as a classical mutagen capable of inducing various kinds of stable and unstable chromosomal aberrations. Therefore, prevention of cytogenetic damage may lead to providing better radiation protection and reduced potential carcinogenic hazards of exposure to ionizing radiation.

Materials and Methods

Blood samples were obtained from 51 male nuclear medicine technologists (NMT) ranging in age from 22 to 49 years (mean±SD 32.1±6.5 years) who were exposed to gamma rays. They were monitored by wearing film badges based on their annual film badge reports. They were divided into two groups; one in the range of accepted permissible level and the second group of over-exposed individuals. All participants were supplied with thermoluminescent dosimeter (TLD) badges and were monitored for a period of one month, and their monthly equivalent doses were estimated.

Fifteen matched healthy persons ranging in age from 23 to 50 years (mean±SD 33.2±8.8 years) who were not working with radiation or in a radiation working area were chosen as controls. In both the control and study groups, only individuals without concurrent infections and medication and no general and dental radiography in the last 4 months were admitted. None of the subjects were cigarette smokers.

Cell culture: Venous blood was drawn into heparinized tubes and samples coded and culture established the same day. To culture lymphocytes, 0.51 mL whole blood was added to 4.5 mL of

PRMI 1640 (Sigma, USA) containing 15% fetal calf serum and 0.1 mL phytohemagglutinin M (PHA-M) (Sigma). All cultures were incubated at 37° C for 48 hours and colchicine was added at a final concentration of 0.2 µg/mL for three hours until harvesting. After hypotonic treatment with 0.075 mol/L KCl for 10 minutes, the lymphocytes were fixed in Carnoy's fixative and transferred onto glass slides.²³ After staining with 4% Giemsa solution, 100 mitoses were analyzed for each sample. For some overdose samples, duplicate culture were set up and 100 mitoses were scored for each culture, but because there was no significant difference in aberration frequencies, results are shown per 100 cells. Lesions were classified according to the International System of Cytogenetic Nomenclature (ISCN) for acquired chromosomal aberrations.²⁴ Chromosomal aberrations were divided into chromatid and chromosome types. Chromosomal lesions including chromosomal breaks, interstitial deletions (minutes) and exchange figures were analyzed. Chromatid gaps were defined as achromatic lesions less than the width of the chromatid, whereas chromatid deletions were scored if the separation was greater than the width of a chromatid and if there was displacement of chromatid arms.²⁵

The Wilcoxon signed rank test was used to compare the frequency of chromosomal aberrations in radiation workers and control subjects. Poisson regression analysis was performed to determine the correlation between the frequency of aberrations and monthly equivalent dose as well as association between dose and aberration induction.

Results

The results of this study are summarized in Table 1 and shown in Figures 1 and 2. As seen, both chromatid (gaps and deletions) and chromosome (isogap, breaks and exchanges, mainly dicentrics) type aberrations are observed. The frequency of all types of aberrations was higher in NMT compared to control subjects (Table 1) and increased with radiation dose (Figures 1 and 2). The majority of chromosomal aberrations were either chromatid deletions or achromatic lesions (gaps) in particular at doses below 2 mSv per month, but at higher doses, the frequency of breaks and dicentrics in chromosome are also increased (Figure 1). The frequency of

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Table 1. Frequency of chromosomal aberrations observed in nuclear medicine technologists (NMT) exposed to chronic doses of gamma rays.

Subjects	Monthly measured radiation dose (mSv)	Annual estimated radiation dose (mSv)	No. of subjects	No. of cells analyzed	Chromatid gap (%)	Chromatid deletion (%)	Chromosome isogap (5)	Chromosome break (5)	Dicentrics (5)	Percent aberrant cells
Control NMT exposure*	0	0	15	1500	12 (0.8)	5 (0.03)	0	0	0	1.13
Below dose limit	0.01-0.2	<20	15	1500	59 (3.39)	17 (1.13)	14 (0.93)	3 (0.20)	0	6.20
Dose limit	0.2	~20	20	2000	169 (8.45)	92 (4.60)	44 (0.20)	14 (0.70)	26 (1.30)	17.25
Over dose	1.71-2.88	>20	16	1600	131 (8.18)	97 (6.06)	39 (3.18)	35 (2.18)	53 (3.31)	22.18

*Exposure of NMT to low doses of gamma rays.

chromatid gaps and breaks in the irradiated group was statistically different compared to the controls ($p < 0.001$). When the frequency of chromosomal aberrations was assessed, based on average annual dose, it is clearly seen that the total chromosomal deletions and gaps increased with increasing average annual exposure. The frequency of dicentrics increased above control level at an annual dose of about 20 mSv (Figure 2).

Discussion

Several studies have shown elevated frequency of chromosomal aberrations in lymphocytes of nuclear medicine technologists^{6,26,27} as well as for other radiology technologists exposed to chronic doses of sparsely ionizing radiation.^{3,5,7,20-22} Almost

all radiation technologists in Iran use film badges for their monthly and yearly radiation monitoring. The present paper and many previous reports indicate that cytogenetic analysis is a more sensitive and reliable method for biomonitoring.

As seen in Figures 1 and 2, a higher frequency of chromatid type aberrations (deletions and gaps or achromatic lesions) was observed for all dose ranges received by the NMTs. Ionizing radiation induces various types of DNA damage, which may lead to chromosomal aberration formation. Very low doses of X or gamma rays might not produce DNA double strand breaks, which presumably are responsible for formation of unstable aberrations such as dicentrics and rings. But it might cause DNA single strand breaks (SSB) or base damage, which can be expressed as chromatid type aberrations

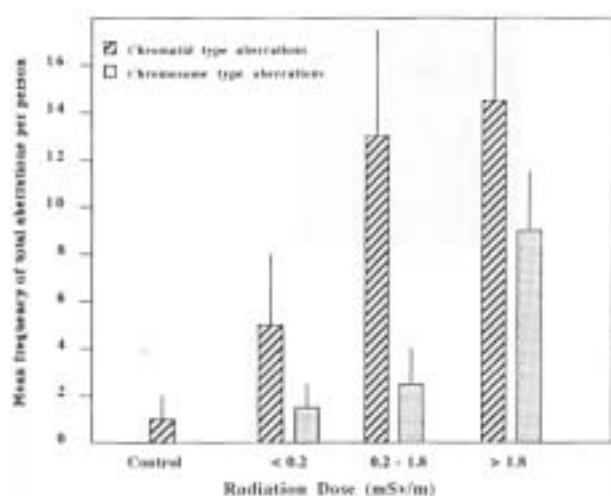


Figure 1. Comparison of total chromosome and chromatid type aberrations in peripheral lymphocytes of nuclear medicine technologists exposed to various levels of gamma rays received based on monthly dosimetry.

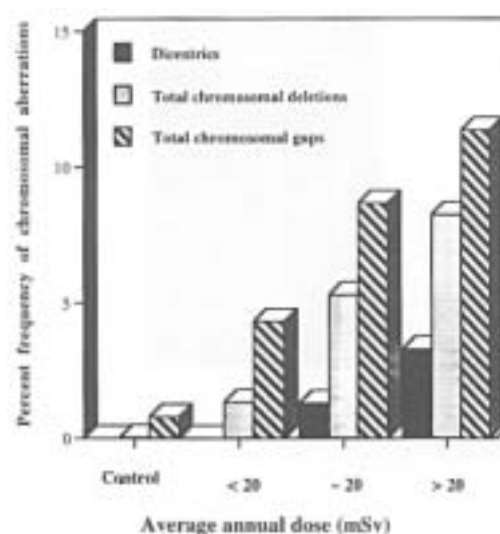


Figure 2. Frequency and types of chromosomal aberrations in nuclear medicine technologists receiving various doses of low level gamma radiations annually.

after an intervening S-phase. This might be the case in the present study, where frequency of chromosome type and chromatid type aberrations increased and decreased with increasing radiation dose, respectively. This observation is in agreement with other reports which show elevated frequency of chromatid aberrations where film badges did not detect radiation.^{7,10,21} On the other hand, persistent chromosomal abnormality such as reciprocal translocations have been reported following low or acute doses of ionizing radiation.^{11,28-30} It is known that formation of unstable chromosome aberrations such as ring and dicentrics lead to cell death; therefore other kinds of DNA damage which are not lethal for the cell might be responsible for persistent chromosomal abnormalities. Based on the mononeme theory in which chromosomes in eukaryotes are thought to contain a single DNA double helix molecule running through the chromosome, it has been suggested that gaps and deletions arise from single and double strand breaks in DNA, respectively.^{31,32} The high frequency of chromatid gaps and deletions observed in this study (Table 1, Figures 1 and 2) for very low doses of radiation might be due to the conversion of single-stranded base damage sites into SSB, or may represent unrepaired deletions. Therefore, chromatid gaps and deletions should not be excluded from results obtained by biomonitoring of occupationally-exposed people to low dose radiation.

An increased frequency of chromosomal aberrations in a population may be considered to indicate an increased risk for cancer. Mutational events are key steps in carcinogenesis and many types of neoplasms are associated with chromosomal rearrangements.^{33,34} Chromosomal instability may be associated with increased cancer susceptibility so it can be considered as a predictor of cancer risk.³⁵ It is also known that genetic predisposition to cancer is associated with certain chromosomal instability syndromes³⁶, suggesting the possible health significance of chromosomal breakage at the individual level. On the basis of cytogenetic analysis of lymphocytes of NMT, it can be concluded that health personnel exposed to low doses of gamma rays in nuclear medicine departments represent a group with increased exposure to radiation.

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