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CYTOGENETIC BIOMONITORING OF 65 RADIOLOGY TECHNOLOGISTS OCCUPATIONALLY EXPOSED TO CHRONIC DOSES OF X-IRRADIATION IN IRAN

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ABSTRACT

The frequency of chromosomal aberrations was studied in the peripheral blood lymphocytes of 65 radiology technologists (RT) working at hospitals chronically exposed to x-rays. Although film dosimetry did not show the maximal annual permitted dose in any of the examined subjects, cytogenetic analysis detected fairly high levels of chromosomal aberrations in RT compared to unexposed controls. The mean frequencies of structural chromosome aberration per 100 lymphocyte metaphases of workers and the controls were 2.93 and 0.54, respectively, excluding the high level of achromatic lesions registered. The difference between them was statistically significant with a P-value of <0.05.

Keywords: Cytogenetic biomonitoring, Chromosomal aberrations, Lymphocytes, Low-dose x-irradiation, Occupational exposure.

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INTRODUCTION

Ionizing radiation induces various kinds of DNA damage which may lead to chromosomal aberrations. Chromosomal aberrations are indicators of mutagenic activity and are widely used as end points in testing for mutagens and carcinogens.[1,2] The importance of cytogenetic studies in peripheral lymphocytes of persons occupationally exposed to ionizing radiation dates back to 1966 when for the first time Bender and Gooch carried out dose estimation of three men exposed during the Recuplex accident.[3] Since then numerous papers have been published studying the effects of exposure to high radiation doses due to accidental exposure[4-7] or occupationally exposed workers receiving low-level radiation doses[8-10] by the method of chromosomal aberration analysis. In spite of the growing importance in the risk assessment, the dose-yield kinetics of chromosomal aberrations and their implications for dose assessment are not well established in exposures to low-level radiation. The development of nuclear energy and the growing use of ionizing radiation in medical practice has created deep concern regarding the long-term effects of low-dose radiation on humans. Some reports indicate a higher frequency of chromosomal aberrations in people exposed to low doses of radiation than in control.[8,11-14] The effect of radiation on the human body depends on numerous factors[15] and a definite link between an increased frequency of chromosomal aberrations and the absorbed radiation dose often cannot be determined.[14] The main conceptual basis for using cytogenetic assays for biological monitoring is that genetic damage in a non-target tissue–most often peripheral blood lymphocytes–reflects similar events in cells involved in the carcinogenic process.[16] Therefore, chromosomal damage in human somatic cells may represent events in a process that eventually lead to manifestations of ill health such as cancer. Thus cytogenetic surveillance may serve as an early indicator of hazard, thus enabling prevention of adverse effects.

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MATERIALS AND METHODS

Subjects

Cytogenetic analysis of peripheral lymphocytes was

Table I. Frequency of chromosomal aberrations in radiology technologists occupationally exposed to low doses of x-rays.

Subjects	Years of Experience	Number of Cases	No. of Cells Analyzed	Chromatid Breaks	Chromosome Breaks	Chromosomal Exchanges	Breaks per 100 Cells	Gaps
Control								
Male		18	1520	4 (0.26)*	5 (0.33)	–	0.59	16 (1.05)
Female		11	1053	–	5 (0.48)	–	0.48	9 (0.85)
Radiology Technologists								
Male	5-15	14	1400	22 (1.57)	6 (0.43)	1 (0.07)	2.14	76 (5.43)
	16-25	19	1900	34 (1.79)	10 (0.53)	2 (0.11)	2.42	111 (5.84)
	26-32	4	400	11 (2.75)	5 (1.25)	1 (0.25)	4.25	34 (8.50)
Female	5-15	11	1100	20 (1.82)	4 (0.36)	1 (0.09)	2.27	33 (3)
	16-25	14	1400	32 (2.29)	10 (0.71)	2 (0.014)	3.14	72 (5.07)
	26-32	3	300	6 (2.0)	4 (1.33)	–	3.33	24 (8)

* = Values in parentheses indicate the number of aberrations per 100 cells.

employed as a group biological exposure test. A total of 65 occupationally-exposed persons working at departments of radiology at hospitals situated in Tehran were examined using this method. Hospitals were randomly selected and blood samples were collected from technologists whom their film dosimetry did not show the maximal annual permissible dose. The exposed group under observation consisted of 37 men and 28 women. A cytogenetic sheet was completed for every examined person. Subjects who had complaints due to genetic disorders in the family or had x-ray examination, smoking habits or used drugs within one month prior to the examinations were excluded from the study. 18 healthy males and 11 females with conditions similar to the exposed workers were chosen as controls. Thus, the chromosomes of 65 RT and 29 controls were analyzed.

Cytogenetic methods

Cell Culture: Venous blood was drawn into heparinized tubes and the samples coded and cultures established the same day. To culture lymphocytes, 0.5 ml whole blood was added to 4.5 ml of RPMI 1640 (Sigma) containing 15% fetal celf serum (Sigma) and 0.1 ml phytohemagglutinin M (Sigma). All cultures were incubated at 37°C for 48 hours, but few mitoses were seen which were not sufficient for analysis. This might be due to the delayed response of lymphocytes to PHA or the culture condition. Therefore an incubation period of 72 hours was preferred and used for all cultures. 3 hours prior to harvesting, colchicine was added at a final concentration of 0.2 μg/ml. After hypotonic treatment with 0.075 mol/L KCl for 10 minutes the lymphocytes were fixed in a mixture of methanol and acetic acid with a ratio of 3:1 and then transferred onto glass slides.[17]

After staining with 4% Giemsa (Merck) solution, 100 mitoses were analyzed for each sample. Lesions were classified according to the International System of Cytogenetic Nomenclature for acquired chromosome aberrations.[18] 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 chromatid and if there was displacement of the chromatid arm.[19,20]

The frequency of gaps was registered but not included for calculating the frequency of aberrations per 100 cells. The intergroup differences were statistically evaluated using Student's t-test and variance analysis.

RESULTS AND DISCUSSION

On the basis of cytogenetic analysis of lymphocytes, it can be concluded that health personnel exposed to low doses of radiation at radiology departments represent a group with increased exposure to radiation. Human peripheral blood lymphocytes are suitable for use in surveillance studies because they are easily accessible and can integrate exposures over a relatively long life span.

In the present study, chromosomal aberrations in peripheral lymphocytes from 65 RT and 29 unexposed controls were analyzed because exposure of lymphocytes results in an increased frequency of chromosomal aberrations in which the extent of damage is an indicator of exposure level. It was found that the frequency of aberrations was considerably higher in RT than in controls. The majority of chromosomal aberrations were either chromatid deletions or achromatic lesions (gaps). Very low dose x-irradiation might not produce DNA strand breaks capable of forming unstable aberrations such as dicentrics and rings. But it may cause DNA base damage which can be expressed after the first mitosis in forms of chromatid type aberrations. This observation is in agreement with the recent findings of Kubelka et al.[10] who found more chromatid aberrations than dicentrics and rings in lymphocytes of workers in the hot zone of a nuclear power

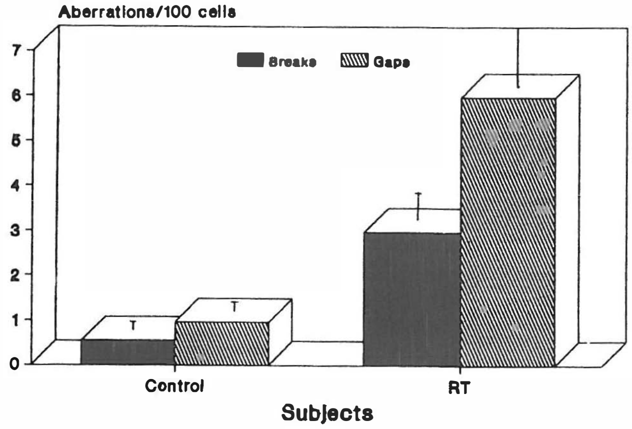

Fig. 1. Mean frequency of chromosomal aberrations observed in male and female control and radiology technologists. Error bars show standard deviation of mean values.

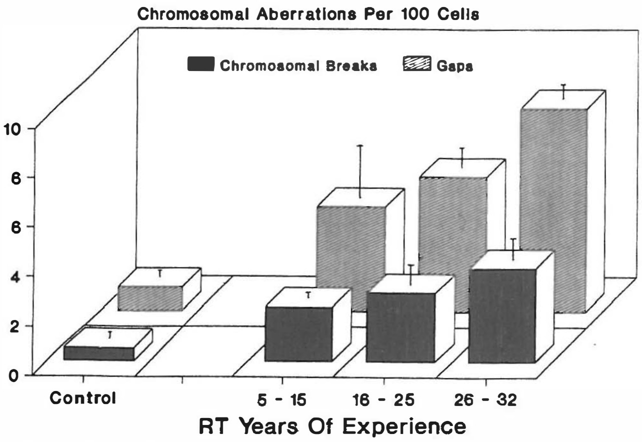

Fig. 2. Mean frequency of chromosomal aberrations observed in radiology technologists (male and female) with different job experience. Error bars show standard deviation of mean values.

plant whose film badges did not detect radiation.

Table I shows the type and frequency of chromosomal aberrations identified in both groups. The higher frequency of aberrations observed among RTs is statistically significant compared to controls ($P<0.05$) (Fig. 1). These data also show a similar response of male and female radiation workers to chronic x-ray exposure (Table I) which is in agreement with the observation of Kumagai et al.[8] who studied the long-term effects of low-dose radiation on the frequency of stable and unstable aberration formation in lymphocytes of radiology technicians.

When the frequency of chromosomal aberrations are assessed based on job experience, it is seen that the frequency of chromatid deletions and gaps increase with increasing years of experience (Fig. 2). This may be due to an accumulation of episodes of cell damage in people exposed to continuous long-term low-dose radiation. Nevertheless, the frequency of chromosomal aberrations is not always proportional to the cumulative dose,[21] because the effect of radiation on the human body depends on numerous factors.[15]

Based on the mononeme theory of Bender et al. in which chromosomes in eukaryotes are thought to contain a single DNA double helix molecule running all through the chromosome,[22,23] it has been suggested that gaps and deletions arise from single and double strand breaks in DNA, respectively. The high frequency of gaps observed in this study (Figs. 1 & 2) might be due to the conversion of single-stranded base damage sites into ssb, or represent unrepaired deletions. Therefore gaps 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. The chromosome aberrations induced by higher doses of radiation are very damaging to the cells, such that they cannot continue to divide and form viable colonies.[24] For this reason, such aberrations are sometimes termed unstable. Thus it is unlikely that diploid cells with unstable damage in them could become cancerous. However, numerous reports indicate that most neoplasms are associated with chromosomal rearrangements.[25-27] It is also known that genetic predisposition to cancer is associated with certain chromosomal instability syndromes such as ataxia telangiectasia and Fanconi's anemia, suggesting the possible health significance of chromosomal breakage at the individual level.[28]

In conclusion, cytogenetic monitoring is a valuable tool versus film dosimetry following low-dose radiation exposure and for risk assessment of personnel believed to be exposed to such radiation.

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