Application of conventional chromosomal aberration and fluorescence *in situ* hybridisation (FISH) translocation in the assessment of occupationally derived irradiation

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ABSTRACT

Background: Most of our current understanding of the biological effects of exposure to ionising radiation is based on conventional cytogenetic techniques, which enable us to determine the relationship between chromosomal aberration and dose received by radiation workers. However, conventional techniques have numerous limitations and chromosomal aberrations can be easily missed. Since FISH plays an important role in detecting chromosomal changes, this method was used to reassess data derived from previous studies employing conventional techniques.

Materials and Methods: Two groups of radiographers were the subject of a study on conventional chromosomal aberration and fluorescence *in situ* hybridisation (FISH) for translocation. The first group was chosen following an accidental contamination incident in a nuclear medicine department. The second group was composed of six radiographers working in an X-ray department with a previous record of overdose as recorded by film-badges; these workers had been the subjects of a previous chromosomal study. Coded blood samples from 11 radiographers and 11 controls were analysed for chromosomal aberration and by FISH for translocation. 200 metaphases from the peripheral blood lymphocytes per subject were analysed to investigate possible frequencies of chromosome and chromatid type aberration and 2000 metaphases per subject were scored in FISH method.

Results: There was no significant difference between the radiographers and the control groups in conventional analysis; also there was no significant difference at the 95% level of confidence in FISH analysis. There was no correlation between levels of translocation and total lifetime doses from occupational (according film-badge and TLD) and/or background irradiation.

Conclusion: The overall conclusion is that the frequency of chromosomal damage in both groups of radiographers did not exceed that of the control group. *Iran. J. Radiat. Res., 2004; 1(4): 195-198*

Keywords: *FISH translocation, chromosomal aberration, accidental irradiation, radiation workers.*

INTRODUCTION

The objectives of this study were to verify an earlier report of excess chromosomal damage in lymphocytes of a group of radiation workers and to assess any possible chromosomal damage in the other group, which had been subjected to over-exposure in a medical centre.

Based on the previous study of the first group, the lymphocytes from the radiation workers exhibited a two-fold excess of chromosomal aberration, which may be associated with health effects due to their

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exposure to radiation. This study reassesses the previous work and compares the results of both conventional and FISH method in any possible chromosomal damage in two groups of radiation workers and a control group.

MATERIALS AND METHODS

Conventional chromosomal aberration and FISH analyses were carried out in two groups of radiographers suspected of receiving high levels of radiation during their work. A group of 5 (4 radiographers and a member of the cleaning staff) working in a nuclear medicine department were accidentally exposed to 350 mCi ^{99m}Tc for 7 hours. The second group of 6 radiographers worked in an X-ray department with a previous record of overdose as determined by film-badges: during a 4-month period their dosage was from 85 to 170 mSv, compared with the estimated lifetime background doses was about 120 mSv using the method applied by Reza-Nejad (2000).

Radiation workers were individually matched with controls. The controls were the same age (± 2 years), sex, with similar smoking habits and preferably the same social class and ethnicity; they were not currently taking medication, having good health histories with no excessive diagnostic radiology, and had not been exposed to organic solvents, pesticides and petroleum products at work or home (table 1).

Using standard techniques, 15 ml venous blood was drawn into heparinized tubes and transported in cooling packs to the laboratory; the samples were coded and refrigerated, and culture established after 24 hours. To culture lymphocytes, 0.5 ml whole blood was added to 4.5 ml of RPMI 1640 (Sigma) containing 15% fetal calf serum (Sigma) and 0.1 ml phytohaemaglutinin (PHA) M (Sigma). All cultures were incubated at 37ºC for 48 h.

Three 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 min, the lymphocytes were fixed in a mixture of 3 methanol: 1 acetic acid and then transferred onto glass slides (IAEA 1986).

After staining with 4% Giemsa (Merck) solution, 100 mitoses were analysed for each sample. Lesions were classified according to the International System of Cytogenetic Nomenclature for Acquired Chromosome Aberrations (ICSN 1985, Sorsa 1990).

Table 1. Details of matched pairs of two radiographer groups with control group.

Pair no.		1	2	3	4	5	6	7	8	9	10	11
Age (y)	Radiographer	**Group 1**					**Group 2**					
		39	42	38	29	41	43	39	50	48	44	38
	Control	40	41	37	28	41	44	41	51	47	43	39
Smoking		yes	yes	no	no	yes	no	no	yes	yes	ex	no
Working (y) radiographer		16	18	14	8	17	18	16	30	25	23	17

2000 metaphases per subject were stained by FISH method. Chromosomes 1, 2 and 4 were painted with orange spectrum and counterstained with diamidino-2-phenylindole (DAPI). A pancentromere probe was not routinely used but occasionally applied later if problems were encountered for particular slides in resolving centromeres with DAPI stain. Spreads were accepted for scoring if some painted material seemed to be absent provided centromeric material of chromosomes 1, 2 and 4 were present. All aberrations involving the painted chromosomes were recorded and categorised as shown in table 2. Simple one-way and two-way translocations were recorded separately. For the one-way types t(Ab) and t(Ba) respectively, the

presence of an accompanying foreshortened painted chromosome, (B), or an acentric, (b), were also noted. The FISH protocol used was according to Rooney (2001) and Dear (1997).

RESULTS AND DISCUSSION

In table 2 the results of scoring 200 metaphases from each person are shown. There is no significant difference in dicentric between radiographers and controls. No centric rings were observed. The total dicentric for radiographer and controls are 10 and 8 respectively. Although chromatid aberrations in blood lymphocytes are not normally considered to reflect exposure to radiation, they have been included in table 2. The number of chromatid gaps shows the greatest variability, but some of these are probably artefacts and not true aberration (Savage 1976). Table 2 also lists the FISH analysis results. The total translocation for radiographers and controls are 83 and 63 respectively.

Table 2. Conventional Giemsa-stained aberration scored in 200 cells and aberration involving painted chromosome scored in 2000 cells in radiographers and controls.

Pair and group no.		Radiographers			Controls			Two-way translocation		One-way translocation			
		Dic.	Chd. Ab.	Gap	Dic.	Chd. Ab.	Gap	RG.	Con.	t(Ab)		t(Ba)	
										RG.	Con.	RG.	Con.
1	1	1	1	9	0	0	8	3	2	1	0	0	0
	2	1	2	12	0	1	9	5	5	1	0	0	1
	3	0	1	11	1	1	11	3	3	2	1	0	1
	4	0	0	6	1	0	8	3	2	0	1	3	0
	5	2	2	12	2	2	12	5	5	1	3	0	2
2	6	1	2	8	0	0	6	4	2	2	1	2	0
	7	0	0	5	1	1	6	5	4	0	1	1	1
	8	2	1	12	1	2	7	11	6	3	2	0	1
	9	2	3	14	0	1	6	7	6	4	3	4	1
	10	1	0	7	1	0	4	4	3	2	2	1	0
	11	0	2	9	1	1	8	5	3	1	0	0	1
	Total	10	14*	105	8	9**	85	55	41	17	14	11	8

Dic= Dicentric, Chd. Ab.= Chromatid aberration, RG.=Radiographer, Con.=Control,

* Comprising 4 isogaps, 8 breaks, 2 exchanges

** Comprising 3 isogaps, 5 breaks, 1 exchange

The frequency of unstable aberration of the chromosome and chromatid type stained by the conventional Giemsa method were indistinguishable between the two radiographer groups and also the control group. In analysing by the FISH technique there was no significant difference at the 95% level of confidence. The overall conclusion is that any difference that might exist between radiographers and controls has not been detected by this scoring.

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