

A comparison between Two Cytogenetic Methods for Identification of Chromosome Aberration in Irradiated Blood Cells

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Abstract

Background: Understanding the genomic alteration induced by ionizing radiation still remains as a methodological challenge in the field of genetics. The energy released from this type of radiation can potentially cause structural and numerical alterations in lymphocytes, which in turn converts them into abnormal tumor cells. Chromosomal abnormalities associated with specific type of hematological malignancies are determinant factors in the evaluation of radiation dose and its potential in harming the body. Nonetheless, early detection of chromosomal aberration (CA) is crucial in the prognosis and selection of a therapy for the people exposed to irradiations.

The aim of this study was to explore a swift and accurate genetic test that identifies CAs in radiologists exposed to X-rays. In addition, synergistic effect of other clastogens in irradiated workers was also evaluated.

Materials and Methods: Thirty-four heparinized blood samples were obtained from radiology workers exposed to X-rays. Blood samples were cultured in RPMI 1640 and F-10 Medias with and without PHA stimulation. Lymphocytes were harvested, separated and arrested at metaphase and their chromosomes were analyzed by solid and G-Banding techniques. Lymphocytic CA was also analyzed through whole chromosome painting using fluorescence in situ hybridization (FISH).

Results: Of the 37 blood samples from workers, 60% had various structural aberrations in which both the frequency and type of CAs were intensified among tobacco smokers.

Conclusion: The results did not show any significant differences between the genders but other carcinogens like smoking can significantly increase the rate of CAs.

Keywords: Chromosome aberration; X-ray radiation; G-banding; Painting FISH

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Introduction

Aberrations in chromosome structures have always been regarded as an underlying factors in determining the extension, duration and dose of ionizing radiations such as gamma, alpha, UV and X-rays (1). Chromosomal aberrations (CA) induced by irradiations and other clastogens are also involved in quantitative alteration of chromosomes. Both numerical and

structural changes in cell genomes can ultimately modify the expression and behavior of genes through mutation. Mutated genes in nucleus can activate proto-oncogenes, deactivate tumor suppressor genes or malfunction the DNA repair system (2). In all these events, hampering the mitotic process as well as apoptosis in cell lead to tumorigenesis and life-

threatening metastasis (3, 4). Structural rearrangement induced by irradiation can also change the gonadal cells which ultimately influences the fertility and conceptus formation (5-7).

Conventional and routine cytogenetic techniques are the best tools used to identify the biological effects and to measure absorbed dose in people who have been directly or indirectly exposed to irradiation through their profession or nuclear accident. Chromosome banding techniques such as negative staining and G-banding are among the common cytogenetic methods used to detect breakage, various translocations and deletion in chromosomes. Previous studies have illustrated formation of tumor in various organs induced by various doses of X-rays (3, 8, 9). The late effects of radiation for individuals occupationally exposed to radiation are very important. Literature has reported higher frequencies of chromosomal aberrations, stable and unstable, in people exposed to different doses of radiation, especially in radiation workers compared to controls (3, 8, 11, 12).

For several decades, chromosome aberration analysis in human peripheral blood lymphocytes has been successfully used to examine people working in ionizing radiation zone. Radiation-induced aberrations can be observed in human lymphocytes within a few hours of exposure. Their frequency is strictly related to the radiation dose and quality (13-16), and they can be detected in blood samples taken long after exposure. Moreover, due to cumulative action of radiation, it is very important to get insight into the biological response of an organism to the given exposure conditions.

The frequencies of CA are correlated to the extent of genomic instability. Genomic instability is a well-characterized hallmark of the cancer cell. Unstable (dicentric, acentric fragments, and rings) and stable (translocation) aberrations are the two major classes of CAs induced by irradiation of cells in the G0 or G1 stage of the cell cycle (17, 18). Peripheral blood lymphocytes from radiation workers were chronically exposed to ionizing radiation in comparison to matched non-exposed individuals. Chromosomal aberrations have been correlated with genetic changes that can trigger the development of cancer (19). Therefore, a biological dosimeter based on CA frequencies makes possible to estimate the cancer risk.

In this study, conventional cytogenetic analysis along with whole chromosomal painting FISH technique were performed to measure complex CAs in a group of individuals occupationally exposed to sparsely and densely ionizing radiation many years earlier. Each genetic profile was also evaluated for tobacco exposure as a second carcinogen. The FISH refers to

the use of labeled nucleic acid sequence probes for the visualization of specific DNA or RNA sequences on mitotic chromosome preparations or in interphase cells. It plays an increasingly important role in a variety of research areas, including cytogenetic, prenatal diagnosis, tumor biology, gene amplification and gene mapping (20). The study was approved by Allied health department of IMMS and by each individual prior to the experiment.

Materials and methods

A questionnaire consisted of 15 questions was given to each worker exposed to X-ray radiation. The questions inquired information such as name, gender, height, duration of exposure, type and dose of radiation, health status, and drug and tobacco consumption. Then, 3-5 ml of heparinized blood was collected from exposed subjects to study various genomic parameters in lymphocytes.

In vitro culture of irradiated lymphocytes

Since the CAs are correlated with various blood tumor, blood samples were cultured in two types of media such as 90% RPMI 1640 and F-10 media (Sigma Chemical Co, St Louis, MO), 10% heat inactivated Fetal bovine serum (HI-FBS), 10 mmol/L HEPES, 100 u/ml penicillin, 100 µg/ml Streptomycin, PH adjusted to 7.2-7.3 with 7.5% Sodium bicarbonate. Blood samples were divided into two groups of which one group was stimulated with phytohemagglutinin (PHA) and the other group was stimulated without PHA. Short- (24 hrs.) and long-term cultures (48-72 hrs.) were performed at 37 °C in humidified at 6% CO₂ atmosphere.

Harvesting and metaphase cell preparation

The cell content of cultured flask was transferred in to a conical tube and centrifuged at 800-1000 RPM for 8 minutes. Pellets in the residual supernatant were suspended and treated with prewarmed hypotonic KCL (37 °C, 0.075 mol/L). After the removal of KCL solution, pellets were fixed and washed with freshly prepared fixative (1 part acetic acid to 3 parts absolute methanol). Lymphocytes were washed several times with fixative and then were dropped on slide to prepare metaphases.

Chromosomal Banding of metaphases

All prepared slides were aged 1-3 days at room temperature and then dried in oven (55-60 °C) for 3-4 hours. Slides were then stained with trypsin – Giemsa staining (G-banding) in one group and with solid staining without trypsin in the other group.

Analysis and cytogenetic profiles

Banded slides were analyzed under the microscope

for various CAs and a genetic profile was prepared for each individual worker.

Whole chromosomal painting FISH

Fresh solids of metaphase were treated with pepsin solution and then washed with PBS, followed by 2XSSC. After washing; slides were serially dehydrated in 70%, 80% and 100% ethanol. For hybridization, cells were treated with the commercially available 24-color paint mixture spectra Vision assay (Vysis Inc., Downers Grove, IL) at 78°C hotplate. Cells were left to hybridize for 36-48 h at 37°C before being washed in 0.4-SSC-0.3% IGEPAL (Sigma) (71°C for 1.5 min) and 2-SSC-0.1% IGEPAL (room temperature for 10 s). Cells were counterstained using DAPI (4, 6-diamidino phenylindole) III (42 ng/ml in antifade mounting solution), sealed, and stored in the dark at -20°C. Metaphase chromosomes were visualized by using a 6-position Zeiss fluorescent microscope containing individual filter sets for each component fluorescent of the Spectra Vision probe mixture plus DAPI (Spectrum Gold, Spectrum Far-red, Spectrum Aqua, Spectrum Red, and Spectrum Green).

A cell was classified as being apparently normal if all 46 chromosomes were observed by this process, and subsequently compared with appropriate cytogenetic metaphase profile. This study aimed to study chromosomal aberration induced by X-rays in radiologists of various hospitals using a cytogenetic profile through banding technique and reconfirmation of the analysis by whole chromosomal painting FISH. Nevertheless, our study examines other carcinogenic factors that synergistically contribute to ill effect of X-ray on lymphocyte chromosomes. In radiation analysis, dose rate, total doses scheme fractionation, volume of affected tissue, duration of exposure and radio sensitivity should all be considered as modifying agents for intensity of damage on chromosome.

Statistical analysis

The comparison of different CAs between workers and control was done using t-test. Pearson correlation

test was also used to evaluate the effective dose on CAs of workers and control. The control group included the persons who did not work as radiologist and were selected randomly from healthy persons. All the analysis was performed by SAS 9.1 statistical software (21).

Results

Observed chromosome aberrations in the present study are shown at Figure 1. Of the 37 blood samples from radiology workers, 14 (38%) had 10% or more various CAs in their lymphocytes and 8 had only 4-8 % and the rest had no structural aberration.

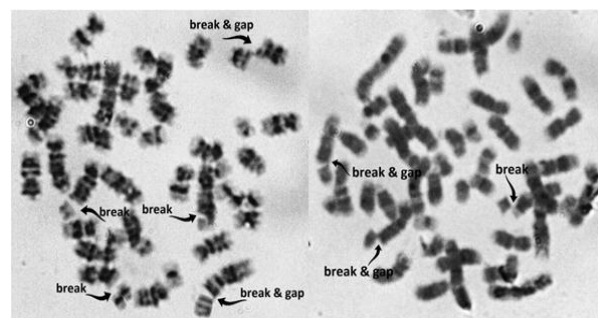


Figure 1. Observed chromosome aberration in the present study.

The frequency of CAs was increased by 15-18 % (Tables 1 & 2).

Table 1. Frequency of chromosome aberrations in studied samples.

Worker	translocation	chtg	chtb	chte	dic	ace
14		3%	4%	1.5%	1.5%	2%
8		2%	3.5%	-	1%	0.07

Chtg=gap in chromatid, Chtb=break in chromatid, Chte=exchange in chromatid, Dic=dicentric chrom

Overall, the rate of gaps and breaks were significantly higher than other types of CAs on workers' lymphocytes (Table 2). Eighty-three percent of workers with 10% CAs and 68% of those with 4-8% CAs were smokers.

Table 2. Description statistics and overall frequency of various CAs in worker blood samples.

Group	Sample size	No. cell scored	Total aberrant cell	Chtg Total Mean \pm S.D	Chtb Total Mean \pm S.D	Chg Total Mean \pm S.D	Chb Total Mean \pm S.D	F Total Mean \pm S.D	R Total Mean \pm S.D
control	7	700	273	81 11.5 \pm 0.97	54 7.7 \pm 1.7	54 7.7 \pm 0.75	54 7.7 \pm 0.75	5 0.71 \pm 0.75	2 0.29 \pm 0.48
x-ray	15	1500	683	21 13.8 \pm 2.8	180 12 \pm 3.04	127 8.4 \pm 1.9	144 9.6 \pm 1.6	15 1 \pm 1.1	6 0.4 \pm 0.5

Chtg=gap in chromatid, Chtb=break in chromatid, Chg: chromosome gap, Chb: chromosome break, F: fragment, R: rearrangement

Although cytogenetically 10% CA was considered to be the cut-off point for clinical significance we

included everybody with CAs regardless of the rate of their aberrations.

The mean frequency of CAs for both male and female workers was compared to assess the

difference (Table 3).

Table 3. P-value obtained from independent t-test for gender effect on CAs of workers and control

Parameter	Chromosome aberration						F	R
	Trans	chtg	chtb	chrg	chrb			
Gender (worker)	-	0.8	0.25	0.8	1	1	0.5	
Gender (control)	-	0.11	0.25	0.75	0.57	0.84	-	

Chtg=gap in chromatid, Chtb=break in chromatid, Chte=exchange in chromatid, Dic=dicentric chrom

The rate of structural aberration among two genders did not differ significantly. Our result indicated a

direct correlation between the rate of CAs on chromosome and the dose of irradiation (Table 4).

Table 4. P-value obtained from Pearson correlation test for effective dose on CAs of workers and control

Parameter	(P-value)							
	trans	chtg	chtb	chrg	chrb	F	R	
Exposed worker	Age	-	0.50	0.45	0.40	0.45	0.40	0.20
	Duration of exposure	-	0.90	0.45	0.70	0.56	0.45	0.20
	Effective dose per year	-	0.56	0.45	0.40	0.09	0.0	0.0
Control	Age	-	0.62	0.80	0.25	0.60	0.40	0.0
	Duration of exposure	-	-	-	-	-	-	-
	Effective dose per year	-	-	-	-	-	-	-

Chtg=gap in chromatid, Chtb=break in chromatid, Chte=exchange in chromatid, Dic=dicentric chrom

Discussion

Although various studies on X-ray exposed populations have clearly demonstrated a significant genotoxic effect on lymphocytes, proper genetic technique with prompt identification of aberration remains to be a great diagnostic challenge. The CAs induced on irradiated chromosome include structural damage such as translocation, gap in chromatid, break in chromatid, exchange in chromatid and dicentric chromosome. To measure the CA rate on cytogenetic profile, whole chromosome painting (FISH) method was used on lymphocytes obtained from the radiologists. Over 20,000 metaphases were analyzed for CA using painting probes. The results did not show a significant correlation for the frequency of translation affecting the lymphocytes ($P \geq 0.01$), but a good agreement on dicentric chromosome frequency of dicentric and acentric chromosomes was found between painting FISH and conventional cytogenetic analyses ($P \geq 0.1$).

Nevertheless, the frequencies of FISH translocation were used along with the dicentric rate to estimate the effective genotoxic dose of radiation. Furthermore, the whole chromosome painting FISH may be used as a complementary method not only to reconfirm the conventional metaphase analysis but also to evaluate the therapeutic effect of drugs on the chromosomes

(22). Our preliminary data suggests that smoking tobacco can significantly increase the genotoxicity of ionizing radiation and generates more CAs in the normal cells which in turn will increase the risk of tumor.

No relation was found between the age and gender, and frequency of CAs on the chromosomes, but some studies have indicated that the rate of CAs can be increased at the higher age (3, 10). Moreover, the duration of exposure to radiation did not have any effect on the CAs. This could be due to instability of CAs induced by ionizing radiation. Individual immune status of workers may also play a role in fending off many CAs on their chromosome. G-banding was also used as a complementary staining method to solid banding to identify every type of CAs that might occur on chromosome and also to detect various translocations that cannot be vividly observed by solid staining. Overall, our study revealed that conventional banding methods had more sensitivity and specificity in revealing the structural and numerical abnormalities than the whole chromosomal painting FISH, because FISH probe cannot identify dicentric or gaps on chromosome. The main goal of present study was to explore a swift and reliable method to identify CAs in people exposed to ionizing radiation. Moreover, the conventional

cytogenetic profile using banding techniques was compared to new molecular whole chromosomal painting FISH. Micronucleus (MN) is another cytogenetic assay that is commonly used to assess the DNA damage as well as biodosimetry (23). Our results like the other studies (1, 2, 8, 9) indicated higher rate of CAs in those exposed to ionizing radiation. The frequencies of both CAs and MNs in lymphocytes are used as the specific biologic parameters to assess the damage on DNA, to make a prognosis of the incoming tumor and to evaluate environmental radiation, and therapeutic response in patients (24, 25).

The MN is also used as an indication of multiple chromosomal fragments and explains the occurrence of radiation-induced DNA damage. More ionizing radiation causes qualitative aberration on cell chromosomes and vital enzymes (26, 27).

Conclusion

Our overall results suggest that both G and solid banding are much better than FISH method in revealing various structural aberrations on chromosome. Whole chromosome banding FISH can be used as a reliable complementary technique to conventional banding methods for assessing numerical aberration on chromosomes. The obtained data for CAs also did not show any significant differences between the genders but other carcinogens like smoking can significantly increase the rate of CAs.

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Author contributions

SM designed the projects and performed the experiment, ZG, FH, and SS performed the metaphase preparation and chromosome banding, AF performed the FISH and wrote the paper and MM selected the radiologists and consulted the filling the questionnaire.

Conflict of interest

It is declared that there is no conflict of interest.

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