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Determination of dose-response relationship in cultured human lymphocytes for biological dosimetry

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ABSTRACT

Background: Lymphocyte-dicentric assay is the most generally accepted method for biological dosimetry of overexposed individuals. In this study, the frequency of unstable chromosome aberration in blood lymphocytes was used to estimate radiation dose received by individuals. Evaluation of dose using a calibration curve produced elsewhere may have a significant uncertainty; therefore, experiments were performed to produce a dose-response curve using an established protocol of international atomic energy agency.

Materials and Methods: Lymphocytes in whole peripheral blood obtained from healthy individuals, were exposed to various doses of gamma radiation (0.25 – 4 Gy). Then after 1 hour of incubation in 37 ^{o}C, were cultured in complete RPMI-1640 medium. 500 mitoses were analysed for the presence or absence of unstable chromosomal aberrations for each radiation dose after the standard metaphase preparation and staining slides.

Results and Conclusion: Intercellular distribution of dicentric chromosomes at each radiation dose has been used to contrast a dose-response curve. It seems that dose-effect relationship follows with the linear-quadratic model. There is a good agreement between our dose-response curves with similar published studies by other laboratories. *Iran. J. Radiat. Res., 2004; 2 (2): 85-88*

Keywords: *Biological dosimetry, calibration curve, dicentric chromosome, human lymphocytes.*

INTRODUCTION

Application of the lymphocyte-dicentric assay for biological dosimetry has made significant contributions in both accidental and occupational overexposures and this method plays an important role in diagnosis and prognosis of overexposed individuals (Voisin *et al.* 2001). By this assay, the frequency of unstable chromosomal aberrations (dicentric and centric ring) in lymphocyte is used to estimate radiation doses received by individuals (IAEA 1986, 2001).

The advantages of dicentric assay have made this biological dosimeter the most generally accepted method for dose estimation. This biological dosimeter is the most comprehensively investigated system (Muller and Streffer 1991). Dicentrics are considered relatively radiation specific; only a few chemicals are known to interfere with this assay. Low background levels (about 1 dicentric in 2000 cells), high sensitivity (a threshold dose of 0.05 Gy), and known dose dependency up to 4Gy (for low-LET radiation) make this assay quite robust (Greenstock and Trivedi 1994). Effects of radiation quality and dose rate are also well characterised (Edwards 1997). The influence of the interval between radiation exposure and analysis for a broad dose range is not critical for, at least, the first 2 weeks after exposure (IAEA 2001).

Published reports show that differences exist in the measured yield of dicentrics per Gy among several laboratories (Lloyd 1987), and evaluation of dose using a calibration curve, produced by another laboratory may introduce

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substantial uncertainty; therefore, it is advised that each laboratory establishes its own dose-response curves for the induction of dicentrics by different radiation types over a range of doses and dose rates (IAEA 2001). Since most accidental overexposures are to X or gamma radiation sources, in order to reduce the uncertainty in dose assessment, this paper presents the experiments carried out at cytogenetic laboratory of Novin medical radiation institute to prepare lymphocyte dicentric calibration curve for gamma radiation using a protocol established by the international atomic energy agency (IAEA 2001).

MATERIALS AND METHODS

Heparinized blood samples were obtained from healthy male (mean age 26±4) and female (mean age 28±7) donors with no drug or radiation exposure last one month prior to sampling. After dividing blood samples in microtubes, samples were fixed in a plastic water tank in vertical position. The tank was filled with water up to the level of blood to produce a homogenous environment and reduce the scatter effect. Samples were irradiated by a gamma rays from a Co-60 source (Teratron 78 ^{o}C, Canada) in 37 ^{o}C with total absorbed doses ranging from 0.25 to 4 Gy with a dose rate of 0.8 cGy/min. Source surface distance (SSD) and field size were fixed to 80cm and 20×20cm^2 respectively. After irradiation, the samples were incubated for 1 hour in 37 ^{o}C and then 0.4 ml of whole blood from each vial was cultured in 4.5 ml RPMI-1640 (Sigma) medium supplemented with 15% inactivated foetal calf serum (Gibco), antibiotics (Penicillin, 100 iu/ml and Streptomycin, 100 µg/ml), L-glutamine and 0.1 ml of phytohemaglutinin (PHA) (Gibco-BRL) at a final concentration of 5 µg/ml as mitogen to each culture tube.

Forty-six hours after culture initiation 0.2 µg/ml colcemid (Gibco) was added to each culture tube for 2 hours to arrest cells at metaphase. Cells were harvested and exposed to hypotonic solution (KCl, 0.075 M) for 10 minutes, then fixed in Carnoy's fixative (3:1 v/v methanol: Glacial acetic acid). Slides were prepared using air drying technique and stained in 5% Giemsa solution (Merck). 500 mitoses were analysed for the presence or absence of chromosomal aberrations for each radiation dose.

RESULTS AND DISCUSSION

A total of 34300 cells were analyzed from 16 donors. In scoring aberrations, only metaphase spreads with 46 chromosomes were scored and those with less than 46 chromosomes were ignored. Intercellular distribution of dicentric chromosomes at each radiation dose is given in table 1. As shown in table 1, increasing radiation dose,

Table 1. Distribution of dicentric chromosomes in human lymphocytes exposed *in vitro* to different doses of ^{60}Co gamma radiation.

Dose (Gy)	Sample size	Metaphase scored	Mean ± 2SD (Dicentric/Cell)	Mean Excess Acentric fragments /Cell
0	16	5900	0.000125 ± 0.001	0.004
0.25	16	5900	0.007875 ± 0.007262	0.011
0.5	16	5900	0.017938 ± 0.021534	0.016
0.75	16	5900	0.037 ± 0.034718	0.038
1	10	5900	0.062875 ± 0.044157	0.055
2	10	2000	0.2207 ± 0.067112	0.132
3	9	1800	0.437778 ± 0.181598	0.324
4	5	1000	0.794 ± 0.07823	0.472

the number of dicentrics increased. The yield of dicentrics at 0 Gy doses which relates to the spontaneous chromosome aberration was 0.17×10^{-3}. Increases in dose resulted in higher frequency of dicentrics in irradiated lymphocytes. Performing of U-test analysis showed that the data fitted in Poisson distribution as the values ranged between -1.96 and 1.96; therefore, the irradiations were homogeneous. The frequency of dicentric was used to construct dose-response curves to estimate radiation absorbed doses; to do so, eight different radiation doses were used (0.25 Gy to 4.00 Gy). There are 3 dose points at low doses between control and 1.00 Gy dose range at which most of the possible radiation accidents occur (Zoetelief and Broerse 1990). Dose-response curve of the yield of dicentric aberrations as a function of radiation dose is shown in figure 1. The relationships between chromosome aberrations and radiation (figure 1) were best expressed with the linear quadratic equation. Dose effect relationship was expressed with the linear-quadratic model, $y= \alpha D + \beta D^2$. In this linear-quadratic equation, α represents linear component where chromosome aberrations are the result of single-track events and it is mostly responsible for aberrations at low doses. β represents quadratic component where chromosome aberrations are the result of two-track events and it is mostly responsible for aberrations at high doses. The values of α and β were 0.012 and 0.0461 respectively. The number of excess acentrics was increased with increasing radiation dose (table 1). At high doses of radiation, higher numbers of acentric distribution in cells were observed. Acentric fragments associated with dicentrics, tricentrics, tetracentrics or rings were not included in the number of excess acentrics. The yields of both dicentrics and acentrics were increased with increasing the radiation dose. Formations of excess acentrics are not specific to radiation since they may occur as a result of exposure to other clastogenic agents. Therefore, these types of aberrations were not used in radiation dose estimations alone. Less metaphase were observed as radiation dose increased which was due to the interphase death of lymphocytes bringing fewer cells for metaphase analysis. Scoring unstable chromosome aberrations which defines the morphological cytogenetic changes

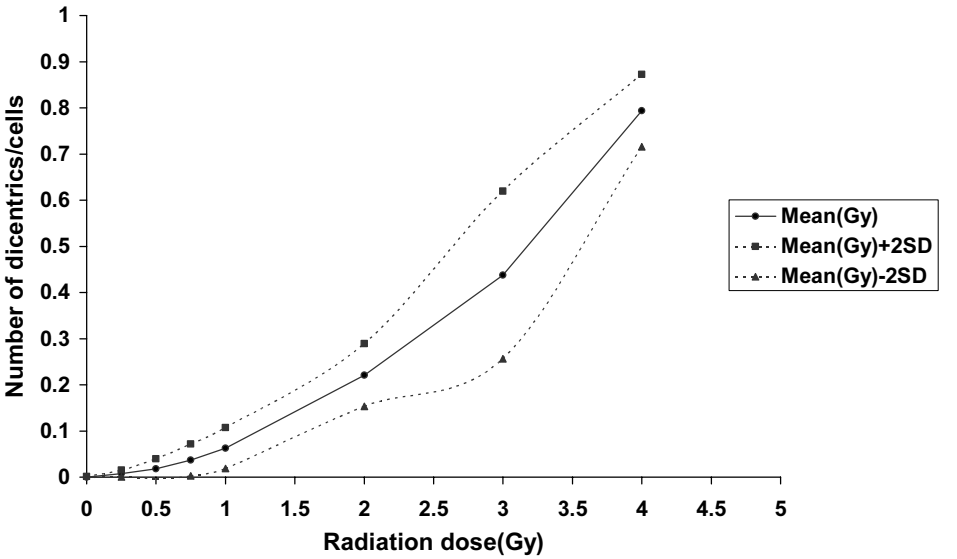

Figure 1. Dose-response calibration curve for the induction of dicentrics in human lymphocytes following *in vitro* exposure to various doses of ^{60}Co gamma rays. The mean number of dicentrics per cell as a function of radiation dose was fitted to a linear-quadratic $Y= 0.012D + 0.0461D^2$ equation. These results represent the pooled mean from several independent experiments.

is a widely used method for biological dosimetry and worthwhile over to scoring stable aberrations (Ramalho *et al.* 1998, Balakrishnan and Rao 1999). Besides the inter-laboratory variations in dose-response curves, aberration yields, and dose estimates for simulated accidents which noted by Lloyd *et al.* (1987), comparison of our dose-response curve with similar published studies from other laboratories represent a good general agreement (Edwards 1997, Lloyd *et al.* 1987, Bauchinger *et al.* 1984). Discrepancies related to dose-response curves and aberration yields may be overcome by adopting centromere painting with a pancentromeric DNA-hybridization probe for aberration analysis (Schmid *et al.* 1995, Roy *et al.* 1996).

In conclusion the established dose-response curve of chromosome aberrations for Co-60 gamma irradiation in our laboratory enable us to estimate a magnitude of an absorbed dose in any accidental or occupational radiation exposures in the range of 0.25 - 4 Gy.

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