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Biological Dosimetry Following X-ray Irradiation

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Abstract: Control radiation dose-response curves are necessary in evaluating the absorbed radiation dose of similar radiation quality following radiation accidents or in monitoring the health of both the public and radiation workers. Each biological dosimetry laboratory should establish its own control dose-response data. In this study our aim was to establish control curves for 200 kVp X-rays in our laboratory in order to estimate absorbed radiation doses following exposures.

Blood samples from healthy individuals with no radiation working history were irradiated in heparinized tubes at 10 different doses 0.10-5.00 Gy. Cells from irradiated whole blood were incubated in culture containing phytohemagglutinin for lymphocyte propagation. Dividing cells were blocked at metaphase, fixed, stained, and unstable chromosome aberrations were scored.

Yields of dicentrics and excess acentrics following different radiation doses were used to establish control curves. The data were fitted to the linear-quadratic (LQ) equation. The parameters of the LQ equations were used in the Q_{dr} method to estimate an absorbed radiation dose in five people working with medical X-ray radiation for a relatively long time. Estimations showed that four workers received doses below the permissible levels but one showed an indication of overexposure.

Key Words: Biological dosimetry, X-ray, chromosome aberrations.

Introduction

Biological dosimetry, following an investigation of using chromosome aberrations from metaphase blocked cells (1,2), has gained an acceleration for evaluating the absorbed radiation dose in circulating lymphocytes of radiation victims or radiation workers (3-6). The maximum permissible whole-body dose to radiation worker is 50 mSv per year, or 30 mSv per quarter, or to a member of the public is about 10 times less than these values (7). Any violations of these limits give an alert for the safety of both radiation workers and normal individuals. Therefore there is a need to define any overexposed individuals in order to apply any immediate medical care.

Radiation dose estimations from blood lymphocytes provide a valuable tool especially in acute and homogenous irradiations. Distribution of unstable chromosome aberrations, particularly dicentrics, among cells follow the Poisson statistics. However, estimations based on past exposures and non-uniform irradiations are difficult due to lymphocyte life-time and dilution of damaged cells from undamaged parts of the body respectively (7). The Q_{dr} method of Sasaki (3) relating aberrations (dicentrics and centric rings) only in damaged cells to a radiation dose overcomes the problems of dependency on time after exposure and inhomogenous irradiation.

Differences exist in the background frequencies of chromosome aberrations in different populations. These differences are due to biological diversity, geographical situation, atmospheric pollution, the extent of environmental clastogenic chemicals, or the use of medical drugs alone or in combinations (8,9). Due to the variations of the background frequencies of aberrations, each biological dosimetry laboratory should establish its own control dose-response curves for any different LET radiations available (2). Therefore, we intend to establish in this paper a control curve for 200 kVp X-rays and to apply this curve to estimate if there is any absorbed radiation dose over the permissible levels in five radiation workers.

Materials and Methods

Control dose-response curves

Blood samples were taken from non-smoking healthy donors (3 male and 1 female) with no radiation working history to establish control dose-response curves. Their ages ranged from 28 to 42 years. In addition, blood samples from 5 radiation workers were taken to estimate an absorbed radiation dose from the control dose-response curves. The microculture method of Moorhead et al. (10) was used for blood lymphocytes with small modifications as follows: for each donor, 0.5ml of irradiated whole blood was added in culture containing 4 ml of RPMI-1640 with glutamine (Sigma) supplemented with 1ml newborn calf serum, 100 µg/ml streptomycin, 100 IU/ml penicilin and 15 µg/ml phytohemagglutinin, and incubated at 37° for 45 h. After adding 1 µg/ml colcemid solution, cells were incubated for another 3 h. Fixation, staining and chromosome preparations were performed according to standard procedures (2) with minor modifications.

Fifty percent of dicentric chromosome aberrations are lost following the first post-irradiation division in proliferating cells (1), leading to underestimation of radiation doses. Therefore, dose estimates should mainly be based on the first cycle metaphases. Total culturing time of lymphocytes in our study gave an M_2/M_1 ratio of 7.8% after fluorescence plus Giemsa staining (11). According to the IAEA bulletin (2), this indicated that chromosome aberration analysis was carried out exclusively on the first cycle metaphases.

Irradiation

Irradiation was performed using a Stabilipan-II Deep Therapy Machine (Siemens) at 200 kVp and 10 mA with a 0.5 mm Cu filter. Heparinized tubes containing 4.5 ml of donors' blood were irradiated homogeneously at 10 different doses between 0.10 and 5.00 Gy at 37° and one was left for control in establishing dose-response curves.

Scoring chromosome aberrations

Unstable chromosome aberrations of asymmetrical exchange types were scored for both donors and workers. Control dose-response curves were established from dicentric and excess acentric yields following the irradiation of donors' blood at different radiation doses. Homogeneous low LET radiations produce random ionizations in cells leading to random distribution of chromosome aberrations in low frequencies, especially at low doses, and this follows Poisson distribution (12,13). Overdispersion of aberrations is observed in non-uniform irradiations. The magnitude of overdispersion is related to the heterogeneity of irradiation (14). In order to test the homogeneity of irradiation, the dispersion index (σ^2/Y, the ratio of variance to dicentric yield) was calculated at each radiation dose. If the dispersion index equates to 1 it can be presumed that dicentrics are distributed according to Poisson. In addition, the U-test (15,16) was used to acquire statistical evidence of whether the ratio σ^2/Y differs significantly from 1. The magnitude of test quantity U, which approximates to a unit normal deviate and which is between the values of –1.96 and 1.96, relates to Poisson distribution.

A weighted least square regression analysis was used to fit the dicentric data to the linear-quadratic model, $Y = \alpha D + \beta D^2$, by minimizing the residual sum of squares (weights were chosen as Poisson estimates).

Results

A total of 10440 cells were analyzed. Metaphase cells were scored by direct viewing down the microscope. Chromosome pieces less than 46 were left out of the analysis. Aberrations were observed following an administration of irradiation. Figure 1 shows dicentric and acentric chromosome aberrations after 4.00 Gy irradiation.

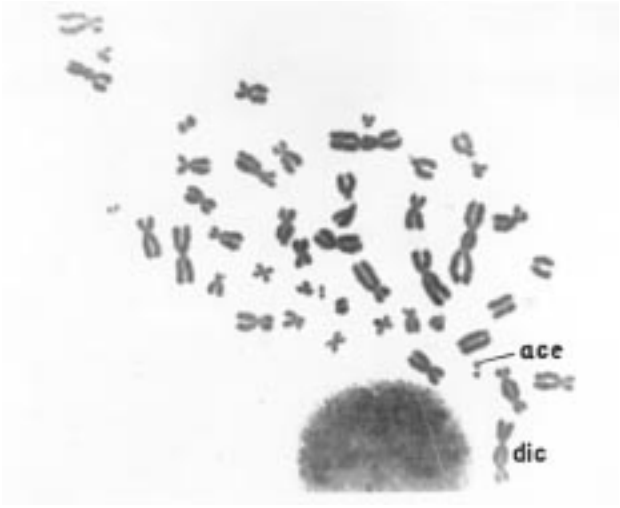

Figure 1. Metaphase chromosomes containing dicentric (dic) and acentric (ace) chromosome aberrations following 4.00 Gy irradiation.

All chromosome aberrations at each radiation dose were recorded (Table 1). Increases in the amounts of aberrations with an increasing dose were absorbed. Tricentrics and centric rings were not found at low doses of radiation. The acentric fragments associated with dicentrics, tricentrics or rings which were direct consequence of irradiation were not included in the number of excess acentrics.

Only the dicentric and acentric aberration yields were used to make control dose-response curves, because other aberration types (tricentrics and centric rings) occurred in lower amounts. The intercellular distribution of dicentric chromosomes at different radiation doses is shown in Table 2. The number of dicentrics increased with increasing radiation dose. The yield of dicentrics at 0.00 Gy dose, which relates to the natural background, was 0.44×10^{-3}. Increases in dose resulted in high numbers of dicentric distribution in cells. In order to test the homogeneity of irradiation the dispersion index (σ^2/Y) and the magnitude of statistical test quantity U are also given in Table 2. The dispersion index centers around 1 for almost all doses. Calculation of the U-test showed that only the distribution at 4.00 Gy deviates a little with a value of -2.14, outside the values between -1.96 and 1.96.

Table 1. Distribution of chromosome aberrations for different doses of 200 kVp X-rays.

Dose (Gy)	Metaphases Scored	Number of dicentrics	Number of tricentrics	Number of centric rings	Number of excess acentrics
0.00	2260	1			2
0.10	1408	14			19
0.25	1361	28			16
0.50	1548	45		1	50
0.75	888	64		4	77
1.00	449	51			40
1.50	979	196		3	136
2.00	1074	317	2	10	279
3.00	591	364	3	6	247
4.00	168	196	2	6	89
5.00	114	206		10	118

Table 2. Intercellular distribution of dicentric chromosomes for different doses of 200kVp X-rays.

Dose (Gy)	Metaphases scored	Number of Dicentrics	Distribution							σ^2/Y	U
			0	1	2	3	4	5	6		
0.00	2260	1	2259	1						1.00	0.00
0.10	1408	14	1394	14						0.99	-0.25
0.25	1361	28	1333	28						0.98	-0.53
0.50	1548	45	1503	45						0.97	-0.80
0.75	888	64	824	62	1					0.96	-0.84
1.00	449	51	398	47	2					0.97	-0.50
1.50	979	196	783	155	19	1				1.03	0.56
2.00	1074	321	753	231	39	4				1.02	0.46
3.00	591	370	221	198	65	14				0.95	-0.79
4.00	168	210		70	42	12	1		1	0.77	-2.14
5.00	114	206		33	39	20	5	3		0.74	-1.94

The control curve of the dicentric yield as a function of radiation dose is shown in Figure 2. The dose-effect relationship was expressed with the linear-quadratic model, $Y=\alpha D+ \beta D^2$. In this equation α represents the linear component, where chromosome aberrations are the result of single-track events and it is mostly responsible for aberrations at low doses. β represents the 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 with their standard errors were $3.75x10^{-2}\pm 0.96x10^{2}$ and $6.41x10^{-2}\pm 0.34x10^{-2}$ respectively. The α/β ratio was 0.59 Gy, representing the dose at which both track events are responsible for the aberrations equally. This shows that there is also a contribution of the β-component at low radiation doses of the dose-effect curve.

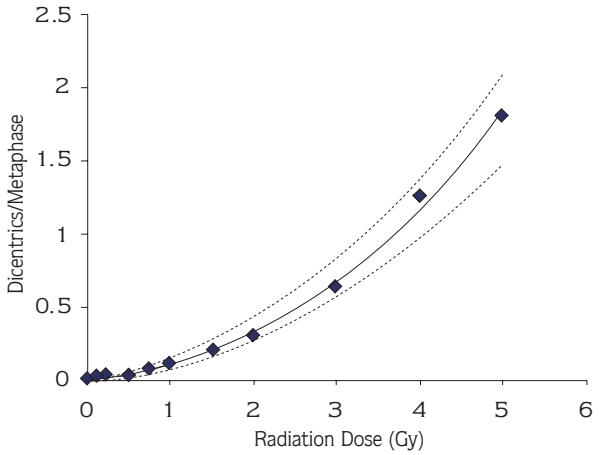

Figure 2. 200 kVp X-ray induced linear-quadratic dose response curve for dicentric chromosomes. Dotted lines represent 95% confidence intervals.

The intercellular distribution of acentric fragments not associated with dicentrics, tricentrics or rings at different radiation doses is given in Table 3. The number of excess acentrics increased with increasing radiation dose. The background level of excess acentrics was $0.88x10^{-3}$. At high doses of radiation, higher numbers of acentric distribution in cells were observed. When the Poisson statistics were applied it was shown that the dispersion index (σ^2/Y) was not close to 1 for most of the radiation dose points.

The U-test also confirmed this by showing most of the figures deviating out of the significance range for Poisson distribution.

The dose-response curve of the excess acentric yield was fitted to the linear-quadratic model in Figure 3. The values of α and β with their standard errors were $6.34x10^{-2} \pm 1.36x10^{-2}$ and $2.43x10^{-2} \pm 0.43x10^{-2}$ respectively and the value of the $^{\alpha}/_{\beta}$ ratio was 2.6 Gy. Single-track events are responsible for the aberrations up to quite high doses.

In order to apply our control dose-response curves to estimate an absorbed radiation dose, 5 radiation workers

Table 3. Intercellular distribution of excess acentrics for different doses of 200 kVp X-rays.

Dose (Gy)	Metaphases scored	Number of excess acentrics	Distribution						σ^2/Y	U
			0	1	2	3	4	5		
0.00	2260	2	2258	2					1.00	-0.02
0.10	1480	19	1390	17	1				1.09	2.60
0.25	1361	16	1347	14	1				1.11	3.07
0.50	1548	50	1503	42	2				1.13	3.61
0.75	888	77	832	68	3	1			1.07	1.49
1.00	449	40	412	32	4				1.11	1.72
1.50	979	136	868	109	12	1			1.08	1.84
2.00	1074	279	886	169	46	6			1.20	4.64
3.00	591	247	499	141	42	6	1		1.12	2.04
4.00	168	89	150	40	16	4		1	1.33	3.05
5.00	114	118	106	29	18	12	3	1	1.37	2.77

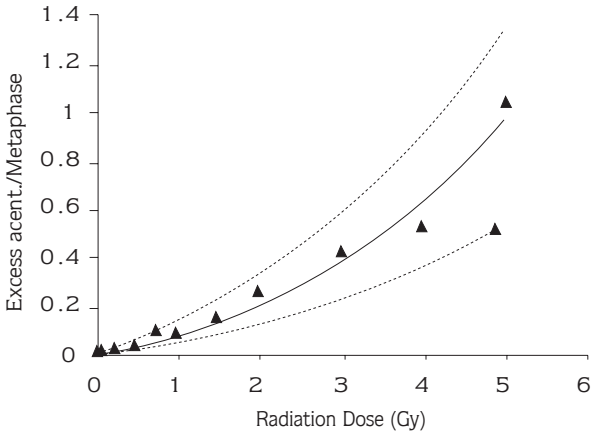

Figure 3. Linear-quadratic dose-response curve for excess acentric chromosomes. Dotted lines represent 95% confidence intervals.

with a reasonably long radiation working history were chosen. Dicentrics and accentric fragments from their peripheral blood lymphocytes were scored while no centric rings were observed (Table 4). Radiation dose estimations were performed using the Q_{dr} method of Sasaki (3). The Q_{dr} value was taken as the ratio of the number of dicentrics and rings to the cells containing dicentrics, rings and/or acentric fragments for each radiation worker; $Q_{dr} = Y_{dr} / 1 - \exp-(Y_{dr} + Y_{ace})$, Y_{dr} is the dose response relationship for dicentrics and centric rings, and Y_{ace} is the dose response relationship for excess acentrics that were obtained from control dose-response curves. The equation of dicentric yield (Y_d) was used instead of Y_{dr} as no rings were observed in workers. Dose estimations from calculating Q_{dr} are shown in Table 4.

The 152 mSv estimated radiation dose with the 840 mSv of the upper level of 95% confidence interval for worker number 1 was considerably larger than the absorbed dose estimates of the other workers.

Discussion

Dicentrics are two-break chromosome aberrations and are therefore especially specific to radiation and their background frequency is low. Therefore, they can exclusively be used in estimating absorbed acute whole-body radiation doses (4,5,8). But radiation accidents do not always occur in ideal situations. Instead radiation is absorbed partially as acute exposure or a long time passes following an absorption. In addition, an individual can be exposed to low doses of radiation chronically as in the cases of radiation workers. These handicaps in radiation dose estimations are overcome by various methods (2). Of these, the Q_{dr} method of Sasaki (3), which takes only the damaged cells into consideration, provides an invaluable tool in estimations excluding the possible conflicts that can be raised consequent to partial body, non-uniform or past exposures. The ratio of the number of dicentrics + rings to the damaged cells containing dicentrics, rings and/or acentric fragments is related to the LQ parameters of the control dose-response curves in the method.

We have established in this paper control dose-response curves of dicentric aberrations and excess acentrics for 200 kVp X-rays. Ten different radiation doses were used from 0.10Gy to 5.00Gy. There were 4 dose points at low doses between the control and 1.00 Gy dose range at which most of the possible radiation accidents occur (17). The yields of both dicentrics (Table 2) and acentrics (Table 3) increased with increasing radiation dose. Lower amounts of metaphases were observed as radiation increases. This was the result of interphase death of lymphocytes bringing fewer cells for metaphase analysis. The yield of dicentrics at 0.00 Gy dose, which relates to the natural background, was 0.44×10^{-3}.

Table 4. Analysis of chromosome aberrations in medical-radiation workers.

Workers	Sex	Age	Metaphases Scored	Number of dicentrics	Number of excess acentrics	Estimated Dose (mSv)
1	M	39	612	5	7	152
2	M	42	1202	3	20	<0.1
3	F	30	795	2	21	<0.1
4	M	40	578	4	17	<0.1
5	F	28	948	4	16	<0.1

The homogeneity of irradiation was confirmed in this study by showing the distribution of dicentrics following different radiation doses to be Poisson except at 4.00 Gy where there was a little deviation (Table 2). The linear-quadratic parameters, α and β, giving the relationship between the yield of dicentric aberrations and radiation were $3.75x10^{-2}$ and $6.41x10^{-2}$ respectively. These values were comparable with those in the literature (18,19).

Deviations from the Poisson for most of the dose points were observed in the distribution of excess acentrics (Table 3). Formation of excess acentrics is not specific to radiation as they may occur as a result of an interaction with some other clastogenic agents. Therefore this type of aberration is not used in radiation dose estimations alone. We used the LQ parameters of the dose-response curve of acentric in calculating the Q_{dr} equation.

Radiation dose estimations using the Q_{dr} equation for medical radiation workers (3 male and 2 female) are given in Table 4. Their age ranged from 28 to 42. At the time of sampling the employment history was 5-25 years. Centric rings were not observed in any of them. There were variations in the numbers of dicentrics and excess acentrics. The estimated doses were less than 0.1mSv, which is below the permissible dose levels for four radiation workers, but for worker number 1 it was 152 mSv with the lower and upper 95% confidence levels of 0 mSv and 840 mSv respectively. This person had worked with X-ray irradiation for about 20 years in medical diagnosis centers. Considering the UNSCEAR report 1988 (20), which states 400 mSv as the recommended sum of doses over total employment time, absorption of 152 mSv stays under the permissible dose level for this radiation worker. But considering the upper level of the 95 % confidence interval an indication of overexposure of a life-time can not be excluded.

In conclusion, we established the control dose-response curves of chromosome aberrations for 200 kVp X-rays in our biological dosimetry laboratory. This will enable us to estimate a magnitude of an absorbed radiation dose in any overexposed individual and to discuss the results with any other investigating laboratories for the benefit of a person.

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