Developing Biological Dosimetry Laboratory for the Assessment of Radiation Overexposure in Saudi Arabia

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Abstract

In cases of individual radiation overexposure or radiological accidents, it is important to provide suitable dose assessment, medical triage, diagnoses and treatment to victims. Cytogenetic bio-dosimetry, based on scoring of dicentric chromosomal aberrations, is a proven, ISO and IAEA standardized biotechnology technique for calculating medically relevant radiation doses. The aim of this project is to set up a national biodosimetry laboratory and establish a national standard dose-response calibration curve, pre-required to estimate doses received in case of accidental radiation overexposure. Peripheral blood lymphocytes were collected from healthy Saudi volunteers and irradiated with different doses (0, 0.5, 1, 3 and 5 Gy) of 320 KeV X-Rays. Then, stained cytogenetic slides were prepared from cultivated lymphocytes according to the IAEA protocol. The Metafer system (MetaSystem, Germany) was used for automatic metaphase finding and assisted scoring of dicentric chromosomes. Results were fit to the linear-quadratic dose-effect model. The preliminary dose-response calibration curve for the induction of dicentric chromosomal aberrations in Saudi Arabia was comparable to those described in other population (Wilkins et al. Radiation Research 169, 2008).

Currently, more volunteers and experiments are being conducted; gamma rays and neutrons will be used for irradiation. The laboratory will also seek accreditation from IAEA and WHO, and cooperation with international biodosimetry network. It is expected that the various activities of the biological dosimetry laboratory will add depth to information for decision-makers and public health officials who assess the magnitude of public, medical, occupational and accidental radiation exposures in addition to providing a platform for advanced education, research and development in Kingdom of Saudi Arabia and neighbouring countries

Key Words: Biodosimetry, Overexposure, Dicentric chromosomes, Dose-response calibration curve.

1. Introduction

In a radiation emergency scenario, timely assessment of the amount of exposure will help guide the actions of emergency officials and health care personnel (IAEA 2005; WHO 2003). Therefore, in cases of individual radiation overexposure or radiological accidents, it is important to provide suitable dose assessment, medical triage, diagnoses and treatment to victims (Alexander *et al.* 2007; Blakely *et al.* 2005; IAEA 2005; Turai *et al.* 2004). Cytogenetic abnormalities are one of the most striking and consistent effect of ionizing radiation on living organisms. When the energy associated with ionizing radiation is transferred to molecules in cells, the DNA that embeds the genetic materials is damaged in proportion to the type and amount of energy that is absorbed. In human lymphocytes, this leads to the appearance of structurally abnormal chromosomes when cells attempt to divide following radiation exposure. Between the different types of chromosomal aberrations induced, dicentric chromosomes appear to be more specific to radiation exposure with a background level practically equal to zero. Hence, the number of dicentrics is quantified and compared to a calibration dose-response curve, established in vitro, in order to derive an estimate of possible dose received. This strategy is valid because lymphocytes express the damage regardless of whether they are irradiated in vivo or in vitro.

The cytogenetic dicentric assay became the internationally recommended method for biological dosimetry by ISO (International Organization for Standardization, ISO 19238, 2004) and International Atomic Energy Agency (IAEA Technical Report Series No. 405, 2001) (IAEA 2001; ISO 2004). It uses the genetic effect of ionizing radiation on human body and relies on the frequency of dicentric chromosomal aberrations found in metaphases from cultured human peripheral blood lymphocyte. In this report we describe the current status of establishing a cytogenetic biodosimetry capability and produce the in-house dose-response calibration curve that will be used to estimate the dose received in accidental radiation exposure.

2. Materials and Methods

The dicentric assay is the internationally recommended method for biological dosimetry for which the methodology is standardized and described in full in the IAEA Technical Report Series No. 405, and the ISO 19238 and also the practical step-by-step procedures described by the DRDC (Defense Research and Development Canada) Ottawa Working Standard for Biological

Dosimetry, Technical Report DRDC Ottawa TR 2005-106 (IAEA 2001; ISO 2004; Segura *et al.* 2005). This assay relies on the frequency of dicentric chromosome aberrations found in metaphases from cultured human peripheral blood lymphocyte samples. These blood samples are cultured either as whole blood or isolated lymphocytes and culture conditions are controlled to ensure an adequate mitotic index and a predominance of first division metaphases. Metaphase spreads are prepared for analysis by standard methods. Stained microscope slides are methodically scanned to identify dicentric and ring aberrations. The frequency of dicentrics observed in an appropriate number of scored metaphases is converted to an estimate of radiation dose by reference to calibration data produced in the same biodosimetry laboratory.

The methodology followed is essentially for the establishment of a dose-response calibration curve, which is an essential step before being able to assess clinical samples for doses accidentally received, a healthy Saudi individual, unexposed to radiation, who had voluntarily consented to give 2 X 10 ml blood. This is necessary to carry out multiple replicates and sufficiently cover the dose range between 0.25 and 5 Gy, necessary to construct an accurate dose-response curve. Peripheral blood lymphocytes were collected from the healthy Saudi volunteer and irradiated with different doses (0, 0.5, 1, 3 and 5 Gy) of 320 KeV X-Rays. Then, stained cytogenetic slides were prepared from cultivated, phytohemagultinn (PHA) stimulated lymphocytes according to the IAEA protocol. The Metafer system (MetaSystem, Germany) was used for automatic metaphase finding and assisted scoring of dicentric chromosomes.

3. Results and Discussions

This study describes the development of biodosimetry laboratory capacity to assess health consequences of radiological and nuclear accidents in Saudi Arabia. The aim is to enhance the Nation's casualty management capabilities by providing rapid diagnostic techniques for triage and medical management purposes as part of a national emergency response plan. Here we describe the current status of the pre-required establishment of the national dose-response calibration curve to estimate the radiation dose received in case of individual or mass overexposure events.

3.1. Dosimetry of Blood Irradiation Using Radiochromic Film

For biodosimetry, it is important to precisely measure the radiation doses received by blood samples used to construct the dose-response calibration curve using dicentric chromosomal aberration assay. For this we adopted the EBT-2 GAFCHROMIC^{TM} film calibration system. Briefly, it was found that this radiochromic film dosimetry method can provide accurate measurement of absorbed doses with an overall uncertainty level of 3.30% for doses larger than 25 cGy, 2.00% for doses larger than 50 cGy, and as low as 1.5% for doses larger than 100 cGy. The relative dose errors between delivered and calculated doses were all within ±2.00%. Now, after the calibration of the EBT-2 GAFCHROMIC^{TM} film, the system can be used to measure radiation doses received by blood samples to accurately construct the relationship between dicentric yields and doses received which is the basis of standard calibration curve for individuals in Saudi Arabia.

3.2. Construction of a preliminary calibration curve for cytogenetic biodosimetry in Saudi Arabia

Experiments were conducted on volunteer blood to measure the yield of dicentric chromosomal aberrations following 0, 0.5, 1, 3 and 5 Gy X-rays. Blood samples irradiation and preparation for cytogenetic analysis for dicentric chromosomes were carried out as per the experimental protocol described. Figure 1 show representative example of metaphase with dicentric chromosomes captured by the Metafer system that can also be automated for dicentric scoring (Flegal *et al.* 2012). A recent study has also demonstrated that automatic detection of dicentrics is a credible alternative for recent and acute cases of whole- and partial-body accidental exposures to ionizing radiation (Vaurijoux *et al.* 2011). The results obtained in our study are summarized in Table 1.

Table 1: Inter-cellular distribution of dicentric chromosomal aberrations after X-rays irradiation of blood in a Saudi volunteer.

Dose (Gy)	***N. metaphases***	***N. dicentrics***	***D0****	***D1****	***D2****	***D3****	***D4****	***D5****	***D6****	***Y***
0	1229	11	1218	11	0	0	0	0	0	0.00895
0.5	1160	22	1138	22	0	0	0	0	0	0.01897
1	662	52	611	50	1	0	0	0	0	0.07855
3	502	219	324	142	31	5	0	0	0	0.43625
5	496	422	271	104	68	34	15	4	0	0.85081

N. metaphases: number of cells in metaphase assessed. N. dicentrics: total number of dicentrics found in the cells assessed. * Number of metaphases with 0, 1, 2, 3, 4, 5, 6 dicentrics, respectively. Y: yield of dicentrics, i.e. the number of dicentrics per cell (metaphase).

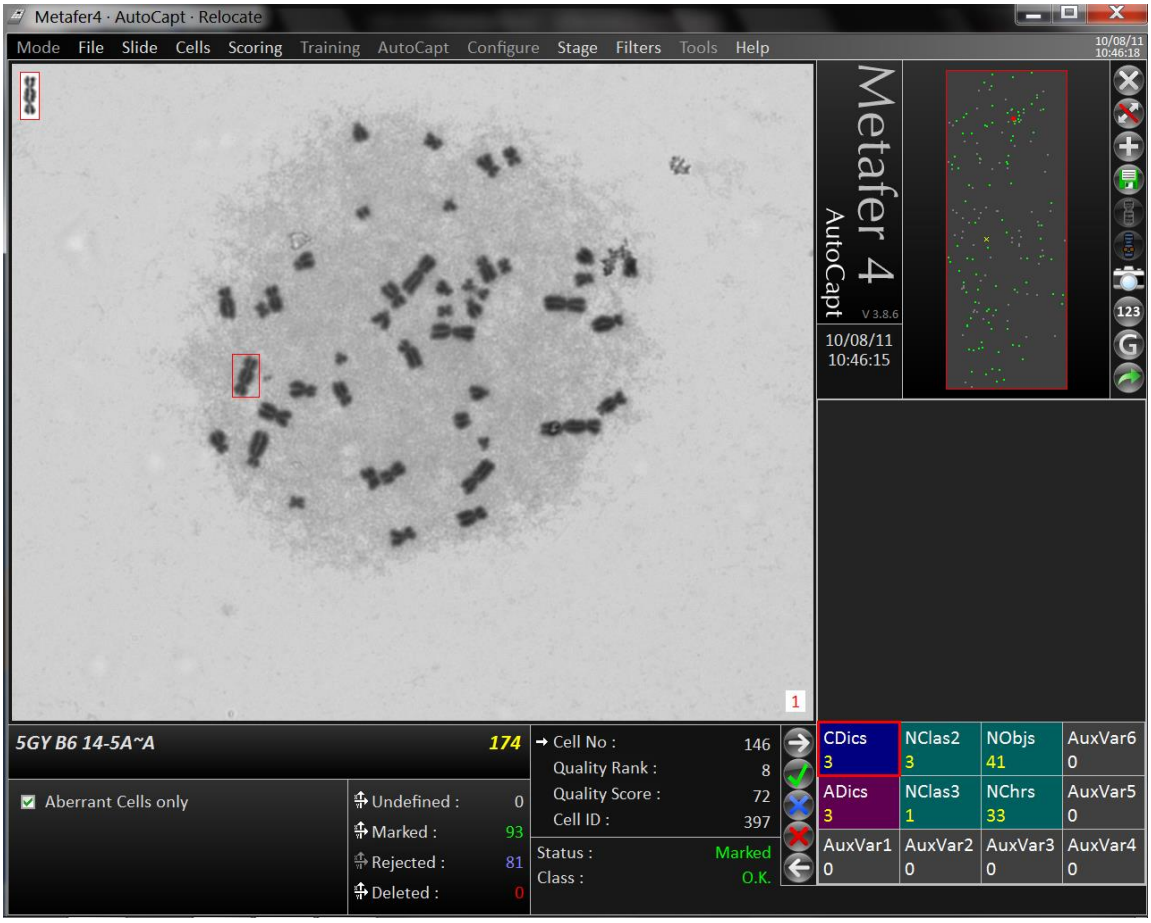

Figure 1 show representative example of metaphase with dicentric chromosomes captured by the Metafer system.

The dicentrics chromosomal data were fit to the linear-quadratic dose-effect model: $Y = C + \alpha D + \beta D^2$; where Y is the yield of dicentrics, D is the dose, C is the control (background frequency), α is the linear coefficient and β is the dose squared coefficient. The following equation was derived (± Standard Error):

$$Y_{Dic} = - 0.015 (\pm 0.024) + 0.098 (\pm 0.029) \times D + 0.015 (\pm 0.006) \times D^2$$

The resulting preliminary dose-response calibration curve for the induction of dicentric chromosomal aberrations in Saudi Arabia is shown in Figure 2. The preliminary results showed that the dose-response calibration curve for dicentric chromosomal aberrations in Saudi Arabia is comparable to those described in other population (Wilkins *et al.* 2008). Currently, more experiments are being conducted; gamma rays and neutrons will be also used for irradiation.

More detailed analysis, description and comparison with published data will be reported upon completion of the study.

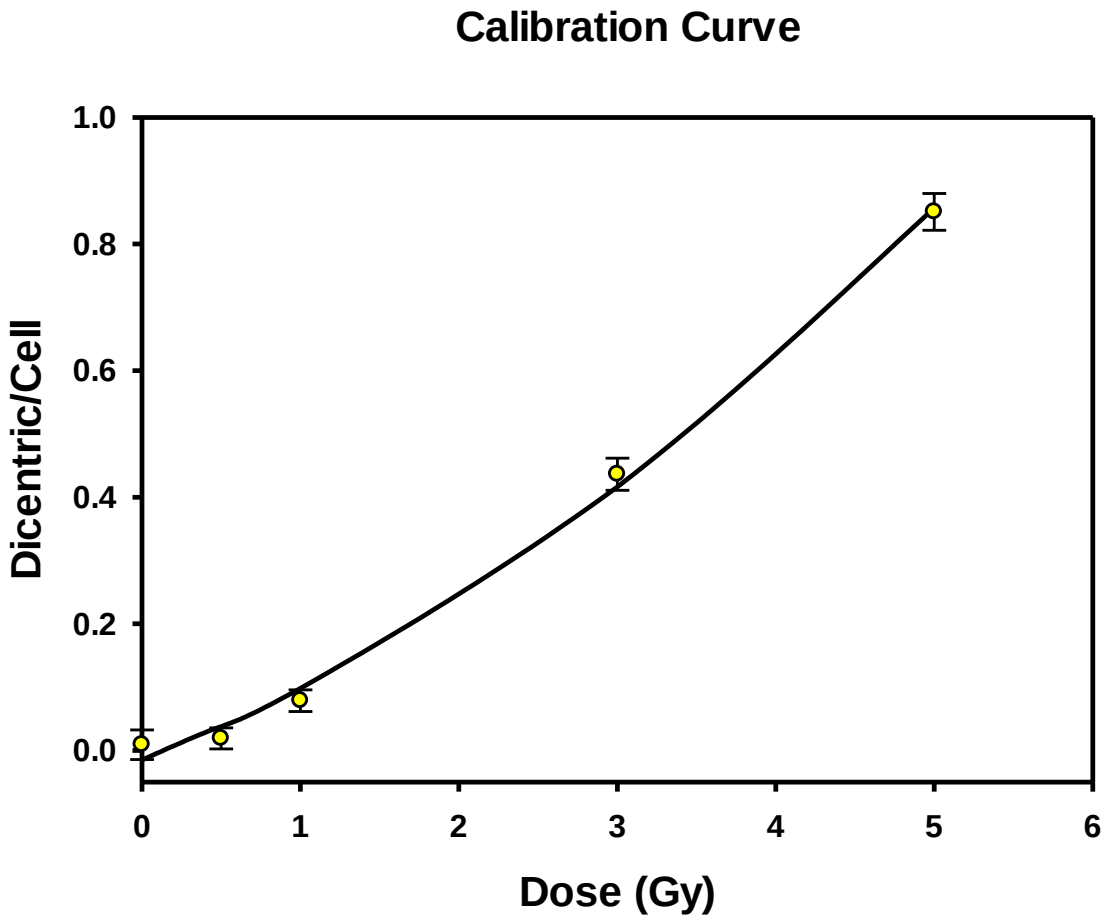

Figure 2: Linear-quadratic standard calibration curve (solid line) for dicentrics induced in human lymphocytes by X-rays exposure (320 KeV, maximum photon energy is 1.3 Gy/min). Data points are the yield of dicentrics per metaphase analyzed. Error bars are the standard error of the yield.

4. Conclusions

The dose-response calibration curve for dicentric chromosomal aberrations in human lymphocytes from blood irradiated by 320 KeV X-ray machine in a dose range of 0.00-5.00 Gy has been established in Saudi Arabia and comparable to those described in other population obtained by international laboratories. The activities of the biological dosimetry laboratory will provide information for decision-makers and public health officials who assess the magnitude of public, medical, occupational and accidental radiation exposures.

The formation of the biological dosimetry laboratory will facilitate the networking of national, regional and international cytogenetic experts and links service laboratories with medical emergency response professionals. It will also provide emergency response officials with tools to distinguish affected individuals from the worried well, and alleviate public concerns about the health effects of possible radiological exposures. Within the future research component of this project, the laboratory will examine other methods as possible high-throughput biological dosimeters or indicators of exposure such as assessing radiation-induced micronuclei in interphase leukocytes, spectral karyotyping (SKY) of human chromosomes, electron paramagnetic resonance, molecular biology methodologies to investigate and identify novel biomarkers of radiation exposure.

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