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DOSE-RESPONSE CALIBRATION CURVE FOR MICRONUCLEUS ASSAY: PRELIMINARY STUDY

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ABSTRACT

The biological dosimetry was developed to assess or estimate the absorbed dose by individuals that are involved or suspected of involvement in an ionizing radiation exposure event. The absorbed dose estimate is based on the analysis of sensitive and specific biomarkers to radiation and that better reflect the biological damage caused by it. The micronucleus (MN) assay is the most commonly used technique in radiation exposure events involving many people, because it can be applied as a triage method in the determination of exposed individuals significantly or not to ionizing radiation. The international atomic energy agency recommends that any laboratory intending to carry out biological dosimetry should establish its own dose–response data for MN assay due to differences between laboratories in response to MN dose, including the use of different protocols and scoring criteria. This study aimed to start construction of the dose-response calibration curve for MN in the Brazilian Northeast region. As such, blood samples were irradiated with three different absorbed doses (0.5 Gy, 0.75 Gy and 1.0 Gy) using a cobalt-60 source located at the Department of Nuclear Energy (DEN/UFPE). The lymphocyte culture, fixation procedure, staining and analysis of slides were according to the protocols set out in the manual of the IAEA. We observed that with the increasing of the absorbed dose, there is an increase in the presence of MN, but it is still necessary the study of other doses for the construction of a more statistically robust calibration curve.

1. INTRODUCTION

The *in vitro* cytokinesis-block micronucleus (CBMN) assay was developed by Fenech and Morley in 1985, with this method is possible quantify chromosome breakage and loss in nucleated cells. The Micronuclei (MN) results during exposure to various clastogenic agents and is the result of non- or misrepaired DNA double strand breaks. MN could be formed by small acentric chromosome fragments or can also contain whole chromosomes that lag behind at anaphase during nuclear division and by consequence are not incorporated in the main nuclei [1].

Many studies of MN frequency showed a reliable biomarker in biomonitoring studies among human populations, such as in human populations therapeutically, occupationally, and accidentally or environmentally exposed to ionizing radiation. Most of these studies reported significantly higher MN rates in exposed populations than in controls [2].

The CBMN assay, that can be used as an alternative method for scoring dicentric chromosomes, is done in lymphocytes and converted into absorbed dose using the dose-

response calibration curves. Each point of calibration curve represents an average dose absorbed by the irradiated lymphocytes, being approximated to a whole body dose considering that lymphocytes are widely distributed in the body and furniture. Once generated and established the calibration curve, it is possible to estimate the dose absorbed by the individual organism exposed to ionizing radiation [3,4].

This study aimed to start construction of the dose-response calibration curve for MN in the Brazilian Northeast region.

2. MATERIALS AND METHODS

2.1 In vitro irradiation and cell cultured

Blood samples (5 ml) were collected from one healthy voluntary. Each sample was irradiated with gamma rays (Gamma cell 220 irradiator), with air kerma rate range from 0.04 Gy/min with uncertainty of 2% at the point of irradiation, and three different absorbed doses: 0.50, 0.75 and 1 Gy were used.

2.2 Cell cultured and cytogenetic analysis

Before cellular culture, the samples remained in the incubator at 37 °C for two hours, time required for the performance of cellular repair mechanism. The protocol for cell culture and cytological preparation for chromosome analysis were obtained from the procedures adopted by the IAEA (2011) [3].

The criteria for selecting binucleated and for scoring micronuclei were based on IAEA manual (figure 1), and at least 1000 viable cells were counted.

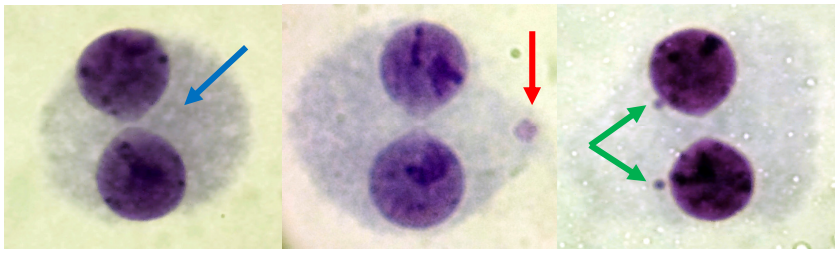

Figure 1. Binuclear cell without MN (blue arrow), binuclear cell with one MN (red arrow) and binuclear cell with two MN´s (green arrows).

2.3 Data Analysis

It was determine the mean and variance of each sample for calculate those dispersions indexes. All samples were tested for analyze your conformity with Poisson distribution, for that it was used the *u* test of Papworth [5, 3].

It was used the Dose Estimate [6] for build the calibration curve. It was obtained through a linear-quadratic equation, $Y = C + \alpha D + \beta D^2$, where: C indicate the background of MN, α and β are linear and quadratic coefficients, respectively, Y is the means of MN and D corresponds to absorbed dose [7, 3].

3. RESULTS AND DISCUSSION

In this work was analyzed more than 4000 viable binuclear cells and the number of micronuclei (MN) scored, their frequencies and cell distributions at four different absorbed doses are presented in Table 1.

Table 1. Distributions of MN with dispersion indexes and *u* values.

				Cell distribution				
Dose (Gy)	**Cells scored**	**MN**	**y**	**0**	**1**	**2**	**σ^2/y**	**u**
0	1005	5	0.005	1000	5	-	0.996	- 0.100
0.50	1007	29	0.029	978	29	-	0.972	- 0.635
0.75	1002	53	0.053	950	51	1	0,986	- 0.320
1.00	1197	113	0.094	1092	97	8	1.050	1.180

The background of MN´s frequency (0Gy) was in conformity with literature, because it was reported frequency of 0 to 40 per 1000 binuclear cells [3]. It is possible to observe the relationship with absorbed dose and frequencies of MN, and it was confirmed that had an increase of MN´s frequencies associated with elevation of absorbed doses. With absorbed doses more elevated, we observed binuclear cells with more than one MN.

All doses points were test for Poisson distribution with dispersion index and *u* values were calculated. It possible observed that *u* values were not significant, because all *u* values were in the range of ± 1.96 in the 95% confidence limits, this demonstrates that *in vitro* experiment follows the model of Poisson distribution. However, the 1 Gy point presented overdispersion´s tendency. Analyzing others works, it was observed the same tendency of overdispersion of cell distribution of MN even at doses below 1.0 Gy, and this probably come to the point that it had on significant dispersion [8, 10]. This overdispersion appearance may be associated with many factors that influencing the formation and origin of MN, and still difficult determinate exactly if the MN is formatted just for fragments acentric, dicentric or both aberrations.

The fitted calibration curve not obtained good results with only four absorbed doses, because the chi-square value is not relevant (table 2). Other studies demonstrated good results with yours calibrations curves, because they analyzed more than 1000 binuclear cells and over an voluntary, thus they increased the confidence of yours results [8,9].

Table 2. Calibration curves and statistics data.

	C ± SE	α (Gy^{-1}) ± SE	β (Gy^{-2}) ± SE	X^2	*df*	*p value*
Calibration curve	0.0050 ± 0.0022	0.0036 ± 0.0229	0.0845 ± 0.0278	0.15	1	0.0

4. CONCLUSIONS

To confirm our results and finish the curve construction, it is necessary to use other doses, different voluntaries and analyze a larger number of cells in order to obtain more robust results.

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