2021 International Nuclear Atlantic Conference – INAC 2021
Virtual meeting, Brazil, November 29 – December 2, 2021

ISBN: 978-65-594-1256-3

Protocol for Triage Dose Assessment in Case of Large-Scale Radiological Emergency: preliminar results

J.C.F. Lima[1,2], A.O. Torreão[1,2], S. Hwang[1], M.E. Mendes[1,3] and F.F. Lima[1]

[1] *Centro Regional de Ciências Nucleares do Nordeste (CRCN-NE/CNEN)*
Av. Prof. Luís Freire, 200, Cidade Universitária
Recife, PE, Brasil 50730-120
fabiana.farias@cnen.gov.br

[2]*Universidade Federal de Pernambuco*
(UFPE)
Centro de Ciências da Saúde
Av. da Engenharia, s/n, Cidade
Universitária, Recife, PE, Brasil, 52171-011
julianne.caroline1@gmail.com

[3]*Centro Universitário Brasileiro (UNIBRA)*
R. Padre Inglês, 257, Boa Vista, Recife, PE, 50050-230
mariespositomendes@gmail.com

1. Introduction

The widespread use of ionizing radiation for medical, industrial, agricultural, research and military purposes increases the risk of overexposure of radiation workers and individuals in the general population. Biological Dosimetry, based on the study of chromosomal alterations, mainly the dicentric assay, has become routine in the evaluation of accidental doses [1, 2, 3].

Chromosomal analyzes in human peripheral lymphocytes provide a means of estimating whole-body absorbed doses after actual or suspected overexposure to ionizing radiation through the use of pre-established dose-response calibration curves from standard in vitro experiments. However, 50% of the dicentrics can be lost during the first division post-irradiation, so their frequency may be underestimated in any quantitative analysis that does not exclusively use metaphases in its first division cycle [2].

In cases of large-scale radiological accidents, individuals suspected of exposure need a quick assessment that can be capable of estimating the absorbed dose to which they were exposed so that medical intervention can be carried out immediately. In this context, it is necessary to use screening methods so that individuals exposed to higher doses have priority in medical intervention due to the high risk of severe biological effects and death. Thus, the cytokinesis block micronucleus assay emerged as an alternative because it is a technique with a fast analysis, which makes it suitable for screening procedures in emergency situations. Despite this, micronuclei are not specific for ionizing radiation, varying according to age, sex, lifestyle and also appearing due to exposure to other genotoxic and mutagenic agents. Thus, after screening, the dicentric assay must be applied for a more accurate absorbed dose estimate, in view of its high specificity, making it the 'gold standard' for biological dosimetry [2].

Thus, a combined assay that encompasses micronucleus and dicentric techniques and that still allows for the distinction of different phases of the cell cycle, is highly promising for the evaluation of accidental doses in large-scale emergencies [4]. The aim of this work was to standardize and implement the combined protocol of dicentrics and micronuclei in the Laboratory of Biological Dosimetry of CRCN-NE/CNEN.

2. Methodology

For this purpose, blood samples (10 mL) were collected from a voluntary non-smoking woman

with informed consent (ethics approval no. 269.483). Each sample was irradiated with Cobalt60 irradiator (Gammacell 220 ® - MDS Nordion, Ottawa, Canada) with average energies of 1.25 MeV at Departamento de Energia Nuclear (DEN-UFPE, Recife, Brazil) The dose rate was 0.048 Gy/min with uncertainty of 2% at the point of irradiation. The blood sample tubes were wrapped in 4 mm of dense material, following IAEA recommendations [2]. The blood samples were irradiated with 0.50 and 0.75 Gy, and after incubated for 2 h at 37 ºC.

For dicentric assay, heparinized whole blood (0.5 ml) were culture for 48h in 4 mL of RPMI-1640 (Sigma) medium supplemented with 0.2 mL of phytohaemagglutinin (Sigma), and 1 mL of fetal bovine serum (Biological Industries). In addition, 0.1 mL of 0.0016% colchicine (Sigma) was added 46 hours after culture started. At the end of 48 h, the supernatant was removed, and the cell pellet homogenized in 8 mL of 0.075M KCl, and placed at 37º C for 20 min, after the supernatant was removed and cells fixed in 7 mL Carnoy's fixative solution (3:1 methanol: glacial acetic acid mixture). Finally, chromosomal preparations were stained with a 5% Giemsa stain in pH 6.8 buffer for 6 min. We also followed the IAEA recommendation that only complete metaphases be recorded, i.e. those with 46 centromeres and if the cell contains unstable aberrations, then it should balance. Therefore, if a spread containing a dicentric should also have an acentric fragment, yet still count to 46 pieces [2].

For cytokinesis block micronucleus assay, all samples were cultivated with 4 ml of RPMI 1640 medium (Gibco), 0.5 ml of blood supplemented with 25% heat inactivated fetal calf serum (Gibco) and phytohemagglutinin (Gibco) and incubated at 37 °C. After 24 h post PHA stimulation, 20 µl cytochalasin-B (Cyt-B - Sigma) was added to the culture, a final concentration of 6 µg/ml. The lymphocytes were harvested between 68–72 h post PHA stimulation. The cells were hypotonically treated with 7 ml of cold (4 °C) 0.075 M KCl to lyse red blood cells, and centrifuged immediately at 180 g for 10 min. The supernatant was removed and replaced with 5 ml freshly made fixative consisting of methanol: acetic acid (10:1) diluted 1:1 with Ringer's solution (4.5 g NaCl, 0.21 g KCl, 0.12 g CaCl2 in 500 ml H2O). The fixative was added whilst agitating the cells to prevent clumps forming. The cells are then centrifuged again at 180 g for 10 min. For identification of MN, the cells should be binucleated (BN), and the two nuclei in a BN cell should have intact nuclear membranes and be situated within the same size and cytoplasmic boundary. Moreover, MN needs to be morphologically identical but smaller than the main nuclei [5].

For combined protocol of dicentrics and micronuclei, samples were cultivated for 48h and 72h. Thus, in the 48-hour culture, 0.02 ml of cytochalasin B (Sigma) was added after 24 hours and 0.1 ml of 0.0016% Colchicine (Biological Industries) after 45 hours. At the end of 48 hours, the culture was continued following the parameters for the dicentric assay. Within 72 hours, 0.1 ml of 0.0016% Colchicine (Biological Industries) was added after 69 hours, following the same parameters for dicentric assay at the end of 72 hours. This protocol was applied based on the studies by Testa et al. (2019). After the fixation process, slides were made from the cell precipitate resuspended in 0.5 – 0.75 ml of fixative solution. The pellet cell was dropped at two points on each slide and these were allowed to dry at room temperature for 24 h. In the combined technique analysis, metaphases were analyzed exclusively in M1 (46 chromosomes), M2 (92 chromosomes) and with binucleated cells simultaneously (Figure 1). The analysis criteria were the same used in the dicentric and MN analyzes separately and that are recommended by the IAEA [2].

The data obtained from the analysis of dicentric, micronucleus and dicentric plus micronucleus slides were submitted to statistical tests to assess compliance with the Poisson model, using the dispersion index and the u test [2]. Absorbed doses were estimated by Dose Estimate software [6].

3. Results and Discussion

In the dicentric assay, 282 metaphase cells were counted (50 for the 0Gy, 113 for 0.50Gy and 119 for 0.75Gy). It was observed no aberrations in the control group. However, for the irradiated samples, yields were different and increased with the absorbed dose. For the MN assay, 1500 BN cells were analyzed where it was a group of 500 cells for each dose. The yield of MN at the control sample was lower (0.004 MN/cell) than irradiated samples that presented yields of 0.048 MN/cell and 0.080 MN/cell for 0,50Gy and 0.75Gy, respectively. All samples (dicentric and MN) statistically followed the Poisson distribution, since u values are within the range of ±1.96 at 95%confidence limit.

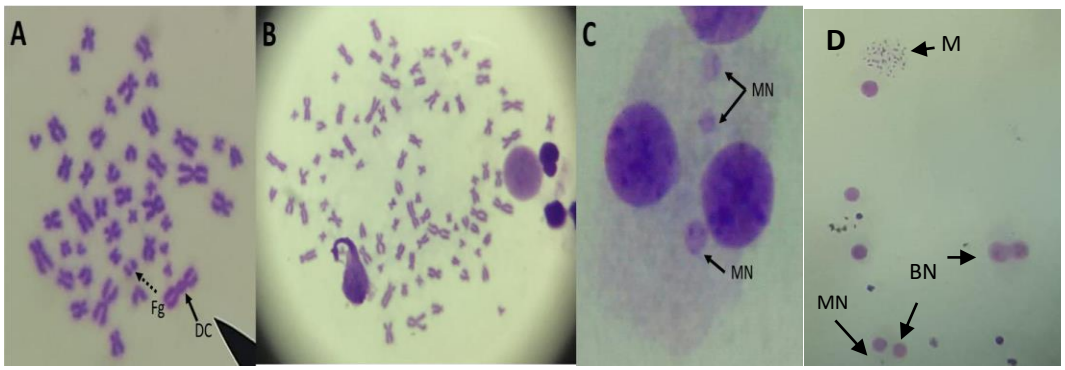

Figure 1: (A) Metaphasic cells in M1 with a dicentric (DC) associated with the acentric fragment (Fg); (B) Metaphasic cells in M2 (it has only 88 chromosomes); (C) A binucleated cell in the presence of three micronuclei; and (D) Image with metaphasic cell (M) and binucleated cells (BN), one of them with a micronuclei (MN).

In the combined DC+MN protocol, two different times of culture were analyzed: 48h and 72h. Table I shows the frequencies of chromosomal alterations in relation to the different times of culture.

Table I: Frequencies of chromosomal alterations in relation to different culture times.

Absorbed Dose (Gy)	Time of culture	M1	M2	BN	BN/M1	DC	YDC	MN	YMN
0*	48h	95	0	34	0,358	0	0,000	0	0,000
0*	72h	98	1	54	0,551	0	0,000	1	0,019
0,5	48h	84	0	21	0,250	2	0,024	0	0,000
0,5	72h	113	2	128	1,133	2	0,018	8	0,063
0,75	48h	65	0	47	0,723	2	0,031	3	0,064
0,75	72h	67	2	71	1,060	2	0,030	5	0,070

*blood control; M1. Cell in first metaphase; M2. Cell in second metaphase; BN. Binuclear cell; DC. Number of dicentrics; YDC. Yield of dicentrics; MN. Number of micronucleus; YMN. Yield of micronucleus.

It can be observed that micronucleus and dicentric frequencies were higher in the irradiated samples compared to the control, being higher for the dose of 0.75 Gy for both 48h and 72h. It was possible to find a total of 5 cells in M2, all within the 72h culture time. All samples (dicentric and MN) statistically followed the Poisson distribution, since u values are within the range of ±1.96 at 95%confidence limit as expected for low LET radiation [2].

Absorbed dose estimates were made using the dose-response curves of dicentric and micronucleus for gamma beam already existing in the Laboratory of Biological Dosimetry of CRCN-NE. The combined protocol in the screening mode showed a very similar behavior in the dose estimation to that found with the use of the two isolated techniques. The values of both the aberrations frequency and the estimated dose were consistent with the respective standard protocols for each technique. Regarding the 48-hour culture, it was possible to estimate absorbed doses of 0.629 Gy and 0.731 Gy for absorbed doses of 0.50 Gy and 0.75 Gy, respectively. With the 72h culture, estimated doses of 0.526 Gy and 0.718 Gy were obtained for these absorbed doses.

The total culture time using this protocol for the 48 hour cultures is shorter compared to the standard CBMN assay (72h) and equal to the standard dicentric assay. Despite this culture time (48h) presenting a low number of binucleated cells, it is still possible to estimate the dose, however further analysis needs to be done.

This protocol has advantages such as saving time, using a single culture for DC and CBMN assays, allowing for savings in reagents, identification of metaphases in M1 and M2 through a simple staining with Giemsa, excluding the need for FPG. Disadvantages related to the use of this protocol are due to additional validation through robust calibration curves with a panel ranging from very low to extremely high doses. In addition, there is a need for intercomparison exercises involving different biological dosimetry laboratories to assess the reproducibility of this assay [4].

4. Conclusions

The use of a single protocol for screening and estimating the absorbed dose in cases of suspected overexposure to ionizing radiation proved to be a promising alternative that should be considered in future analyses. From a combination of techniques, it was possible to perform the dicentric and micronucleus analysis in a single slide.

The combined protocol also made it possible to analyze metaphases exclusively in first cell cycle (M1) based on the amount of chromosomes between M1 and M2 on the same slide.

Absorbed doses estimated by combined protocol were consistent with those that were found using separately dicentric and micronucleus assay.

Acknowledgements

The LaTeX format has been prepared by Professor Helio Pedro Amaral Souto, Instituto Politécnico (IPRJ), Universidade do Estado do Rio de Janeiro (UERJ), Brazil.

References

[1] H. Thierens, A. Vral, "The micronucleus assay in radiation accidents", *Annali dell'Istituto Superiore di Sanita*, Italy (2009).

[2] IAEA - International Atomic Energy Agency, *Cytogenetic analysis for radiation dose assessment: a manual*, International Atomic Energy Agency, Vienna (2011).

[3] M.E.Mendes et al, "Comparative study of micronucleus assays and dicentric plus ring chromosomes for dose assessment in particular cases of partial-body exposure", *International Journal of Radiation Biology*, vol. 95(8), pp. 1058-1071 (2019).

[4] A. Testa, V. Palma, C. Patrono, "a Novel Biological Dosimetry Assay As a Potential Tool for Triage Dose Assessment in Case of Large-Scale Radiological Emergency, *Radiation Protection Dosimetry*, pp. 1-3 (2019).

[5] M. Fenech, "The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations", *Environ Health Perspect*, vol 101, pp. 101-107 (1993).

[6] E.A. Ainsbury, D.C. Lloyd, "Dose estimation software for radiation biodosimetry", *Health Physics*, vol. 98, pp. 290-295 (2010).