Feasibility Evaluation of a Combined Cytogenetic Protocol at CRCN-NE for Triage and Absorbed Dose Estimation in Radiological Emergencies

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The expanding application of ionizing radiation across key societal sectors increases the likelihood of accidental overexposure, making biological dosimetry via cytogenetic assays essential for understanding radiation-induced biological impacts [1]. The cytokinesis-block micronucleus (CBMN) assay provides a rapid and straightforward methodology suitable for mass-casualty emergency triage; however, because micronuclei can also be induced by other genotoxic agents, integration with the gold-standard dicentric chromosome assay (DCA) is required for higher specificity and precise absorbed dose estimation [2]. In large-scale radiological accidents, rapid laboratory feedback is crucial to minimize clinical uncertainty and prioritize medical response [3]. This study evaluates the feasibility of a combined DCA/CBMN protocol implemented at the Biological Dosimetry Laboratory of CRCN-NE/CNEN for emergency triage and dose assessment. Peripheral blood samples (4 mL each) were collected from a healthy, 54-year-old non-smoking female volunteer with no prior exposure history, selected according to established screening criteria [4]. Two samples served as non-irradiated controls, while four samples were irradiated using a ^{60}Co source (Gammacell 220, DEN/UFPE) at absorbed dose rates of 0.911 kGy/h and 0.871 kGy/h to deliver absorbed doses of 2 Gy and 3 Gy (exposure times of 3.387 s and 7.339 s), respectively. Lymphocyte cultures were incubated for 58 hours in RPMI 1640 medium supplemented with fetal bovine serum, phytohemagglutinin, and antibiotics at 37 °C [3]. Cytochalasin B was added at 24 hours to arrest cytokinesis, and colchicine was added at 55 hours to arrest metaphases. Cell harvesting at 58 hours included hypotonic treatment with warm KCl (37°C, 20 min) and fixation with methanol:acetic acid (3:1) [1]. A total of 3,323 binucleated (BN) cells were analyzed (2,168 control and 1,155 irradiated). Control samples exhibited a baseline micronucleus (MN) background frequency of 40 MN per 1,000 BN cells, whereas irradiated samples demonstrated a dose-dependent increase in MN frequency with overdispersion conforming to Poisson expectations [2]. The 3 Gy dose was accurately estimated, whereas the 2 Gy dose was underestimated, likely due to delivery uncertainties associated with extremely short exposure times using the ^{60}Co source. In triage scenarios, scoring 200 BN cells proved sufficient to identify exposed individuals and prioritize clinical care. However, microscopic scoring revealed sub-optimal metaphase quality (excessive chromosome overlaps and incomplete spreads with fewer than 46 chromosomes), hindering accurate dicentric scoring. Methodological adjustments, including extending colchicine treatment (0,1 µg/ml) to at least 4 hours and hypotonic KCl treatment to 30 minutes, are recommended to optimize cell proliferation

and chromosome spreading [5]. Overall, the combined protocol demonstrates strong potential for rapid emergency triage and reliable dose estimation while optimizing laboratory processing time and costs.

References

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