Triage biodosimetry using centromeric / telomeric PNA probes and giemsa staining to score dicentrics or excess fragments in non-stimulated lymphocyte prematurely condensed chromosomes

Ioanna Karachristou¹, Maria Karakosta¹, Antonio Pantelias¹, Vasiliki I. Hatzi¹, Pantelis Karaiosk², Panagiotis Dimitriou², Gabriel Pantelias¹, Georgia I. Terzoudi¹

¹ National Centre for Scientific Research "Demokritos", Energy & Safety, Institute of Nuclear & Radiological Sciences & Technology, Laboratory of Health Physics, Radiobiology & Cytogenetics, Athens, Greece
² University of Athens, Medical School, Medical Physics Laboratory, Greece

Study Goal: In this work, we assess the applicability of cell fusion mediated premature chromosome condensation (PCC) methodology in combination with fluorescence in situ hybridization (FISH), using simultaneous centromeric/telomeric (CEN/TEL) peptide nucleic acid (PNA)-probes, or Giemsa staining. This enables the analysis of radiation-induced chromosomal aberrations directly in non-stimulated G₀-lymphocytes, without the 2-day culturing needed for the conventional methodology of analysis at metaphase.

Abstract: The frequency of dicentric chromosomes in human peripheral blood lymphocytes at metaphase is considered as the “gold-standard” method for biological dosimetry and presently it is the most widely used for dose assessment. Yet it needs lymphocyte stimulation and a 2-day culture, failing the requirement of rapid dose estimation, which is a high priority in radiation emergency medicine and triage biodosimetry. The cell fusion mediated premature chromosome condensation (PCC) methodology, enables the analysis of radiation-induced chromosomal aberrations directly in non-stimulated G₀-lymphocytes, without the 2-day culture delay. Despite its advantages, quantification of an exposure by means of the PCC-method is not currently widely used, mainly because Giemsa-staining of G₀-lymphocyte chromosomes facilitates the analysis of fragment and ring chromosomes, but not of dicentrics. To overcome this shortcoming, the PCC-method is combined with fluorescence in situ hybridization (FISH), using CEN/TEL PNA-probes. This new approach enables an accurate analysis of dicentrics and centric rings, which are formed within 8h post irradiation and, therefore, will be present in the blood sample by the time it arrives for dose estimation. For triage biodosimetry, a dose response curve for up to 10Gy was constructed and compared to that obtained using conventional metaphase analysis with Giemsa or CEN/TEL PNA-probes. Since FISH is labor intensive, a simple PCC-method scoring Giemsa-stained fragments in excess of 46 was also assessed as an even more rapid approach for triage biodosimetry. First, we studied the rejoining kinetics of fragments and constructed a dose-response curve for 24h repair time. Then, its applicability was assessed for four different doses and compared with the PCC-method using CEN/TEL PNA-probes, through the evaluation of speed of analysis and minimum number of cells required for dose estimation and categorization of exposed individuals.

Conclusion: FISH analysis of dicentrics in G₀-lymphocyte PCCs by means of CEN/TEL PNA probes is shown to be a reliable, sensitive, accurate, and faster approach for the estimation of absorbed doses, when compared with the analysis of dicentrics at metaphase. The PCC assay based simply on Giemsa stained
excess PCC fragments is also advantageous, and can potentially become a reliable and quick approach for triage biodosimetry, since dose estimates can be obtained within only 2 hours after blood sampling.

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