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Mean Frequency and Relative Fluorescence Intensity Measurement of γ-H2AX Foci Dose Response in PBL Exposed to γ-Irradiation: An Inter- and Intra-Laboratory Comparison and its Relevance for Radiation Triage

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• **Abstract**

Measurement of γ-H2AX protein changes in the peripheral blood lymphocytes (PBL) of individuals exposed to ionizing radiation is a simple, sensitive, and rapid assay for radiation triage and early marker of dose estimation. The qualitative and quantitative measurements of the protein changes were examined using flow cytometry and microscopy. Whole blood and isolated lymphocytes were exposed in vitro between 0.1 and 5 Gy doses of ^{60}Co γ-radiation at a dose rate of 1 Gy/min. Radiation induced γ-H2AX foci frequency ($n = 3$) and relative fluorescence intensity ($n = 7$) in PBL was measured at 0.5 and 2 hrs postexposure. The observed dose response for γ-H2AX foci frequency at both time points, for whole blood and isolated lymphocytes did not show any significant ($P > 0.05$) differences. However, when compared with γ-H2AX foci frequency scored manually (microscopy), the semiautomated analysis (captured images) showed a better correlation ($r^2 = 0.918$) than that obtained with automated (Metafer) scoring ($r^2 = 0.690$). It is noteworthy to mention that, the γ-H2AX foci frequency quantified using microscopy showed a dose dependent increase up to 2 Gy and the relative fluorescence intensity (RFI) measured with flow cytometry revealed an increase up to 5 Gy in the PBL exposed in vitro. Moreover, a better correlation was observed between the γ-H2AX foci frequency obtained by manual scoring and RFI ($r^2 = 0.910$). Kinetic studies showed that the γ-H2AX foci remain more or less unchanged up to 4 hrs and reduces gradually over 48 hrs of postexposure at 37°C. Further, inter and intra-laboratory comparisons showed consistency in the scoring of γ-H2AX foci frequency by manual and semiautomated scoring. The overall results suggest that measurement of γ-H2AX (microscopy and flow cytometry) should be employed within 4 to 6 hrs for a reliable dosimetry either by sharing the work load between the laboratories or investing more manpower; however, triage can be possible even up to 48 hrs of postirradiation.

• **Key terms**

γ-H2AX foci; dose response curve; γ-irradiation; biodosimetry; inter and intra-laboratory comparison

Introduction

Biomarkers plays an important role to substantiate the suspected over exposure of occupational workers and to assess absorbed dose in the absence of physical devices during radiation accident as well as medical management of exposed individuals (1). Of the various changes that occurs in the irradiated cell of an exposed indi-