Feasibility on use of gel electrophoresis-based quantification of DNA double strand break

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Background and Objective

- ✓ A well-developed and reliable method of biological dosimetry is required to quantify the amount of dose received during any radiation accidents [1].
- ✓ This study, proposes to use gel electrophoresis based biodosimetric method for quantifying the DSB in DNA for irradiated of human lymphocytes.

Methods

Collection of Blood and storage:

- ✓ 5 ml of blood from 2 healthy individuals were collected under aseptic condition by venipuncture and stored in a heparin container to prevent clotting.

Sample Irradiation:

- ✓ The collected blood samples were irradiated in Equinox 80 (cobalt 60) machine for the doses of 0, 5, 10, 20, 25 and 30 Gy respectively.

Lymphocyte separation and lysis:

- ✓ The irradiated blood samples were mixed with histopaque 1077 solution followed by centrifugation at 3000 rpm for 30 minutes. The buffy coat of lymphocytes was aspirated into 1.5 ml centrifuge tube followed by 15 minutes of lysis using 20 µl of proteinase k at 55^0 C.

Electrophoresis, image capture and analysis:

- ✓ The samples were loaded into wells of 1.2 % normal melting point agarose gel and were immersed in alkaline solution of pH >13.
- ✓ The lymphocyte samples were subjected to electrophoresis for 3 hours and mixed with 50 µl of ethidium bromide. Later, gel was gently removed and the samples were exposed to UV transilluminator (figure 1).
- ✓ The captured images were analyzed using Fiji software for measuring the sheared DNA length (figure 2).

Results and Discussion

- ✓ It was observed that the technique had a saturation effect for doses below 20 Gy but was sensitive to doses above it.
- ✓ The dose vs sheared DNA length graph was plotted while using 1.2 % gel after subtraction of the control (0 Gy) pixel value (figure 3) which infers a good correlation between the sheared DNA verses the doses ranging between 20 to 30 Gy.
- ✓ The same procedure was performed for 1.5 % gel where the migration of DNA fragments was restricted and hence was found unsuitable for biological dose measurements (figure 4).

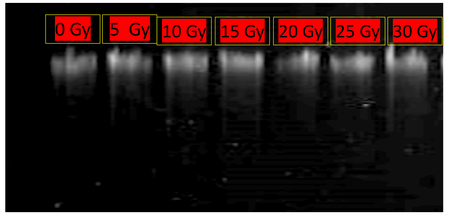

Figure 1: Image captured under UV transilluminator for 1.2 % gel.

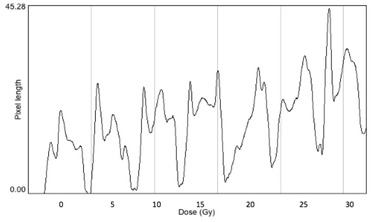

Figure 2. Area profile data using Fiji software

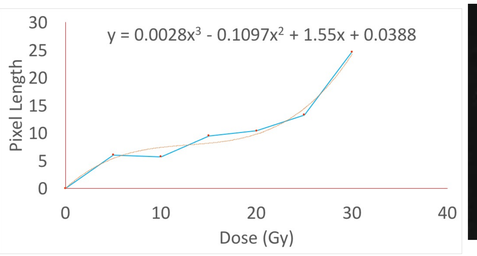

Figure 3. Dose vs migrated DNA fragment length (pixel)

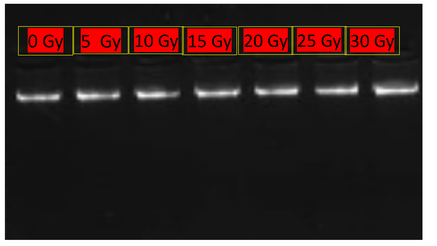

Figure 4: Image captured under UV transilluminator for 1.5 % gel.

Conclusions

- ✓ Dicentric chromosomal assay which is considered to be the gold standard technique in biological dosimetry has a demerit due to its incapability to measure doses above 6 Gy[2]. The gel electrophoresis based biodosimetric technique has proven to be a suitable method for estimating doses greater than 20 Gy.
- ✓ Therefore, this technique could be used to measure doses for radiation accidents where it might involve very high dose over exposures and applications involving high dose irradiations for T lymphocyte suppression such as blood irradiation.

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References

1. Van Moore A. Radiological and nuclear terrorism: are you prepared? Journal of the American College of Radiology. 2004 Jan;1(1):54–8.
2. Lindholm C, Stricklin D, Jaworska A, Koivistoinen A, Paile W, Arvidsson E, et al. Premature Chromosome Condensation (PCC) Assay for Dose Assessment in Mass Casualty Accidents. Radiation Research. 2010;173(1):71–8.