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

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BACKGROUND AND OBJECTIVE

The need for hospitals to be prepared for the act of clinical mistreatment, radiation accidents and radiological terrorism is of high priority(1). In order to quantify the amount of dose received during any radiation accidents, a well-developed and reliable method of dosimetry is required. At present, physical dosimeters are well established to quantify radiation doses. Whereas, on the other hand estimation of biological doses is also of key importance as these physical dosimeters do not correlate directly with the biological changes that conceivably happen after irradiation. At present, dicentric chromosomal assay (DCA) is considered to be the gold standard biodosimetric method due to its high robustness and reproducibility. Whereas, the major shortcoming of DCA is that the time taken for quantification of biological dose is 4 days and the cost for establishing a dicentric chromosomal assay lab is quite expensive.

This study reveals the use of gel electrophoresis based biodosimetric method for quantifying the double stranded break in DNA that happens upon irradiation of human lymphocytes for gamma rays. The time taken for completing the entire procedure to dose reporting takes 8 hours and the cost for establishing this biodosimeter is relatively low.

METHODS

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

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. After the lysis procedure the samples were subjected to gel electrophoresis.

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

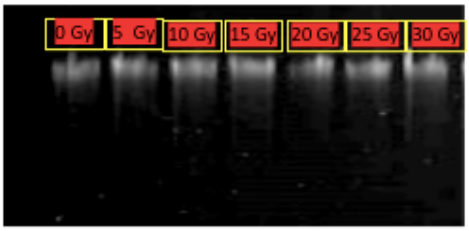

Figure 1: Image captured under UV transilluminator for 1.2 % gel.

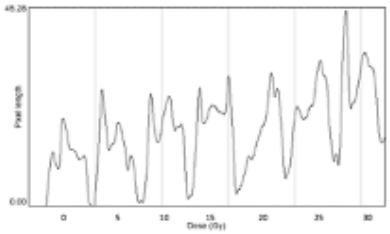

Figure 2. Area profile data using Fiji software

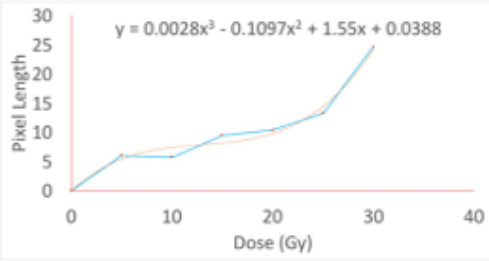

Figure 3. Dose vs migrated DNA fragment length (pixel)

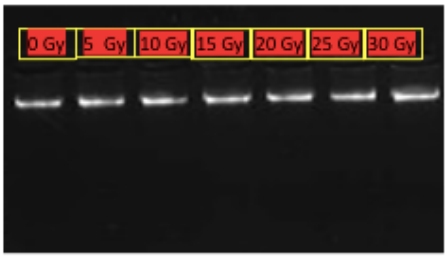

Figure 4: Image captured under UV transilluminator for 1.5 % gel.

RESULTS AND DISCUSSIONS

It was found that the technique was sensitive for doses above 20 Gy. Below 20 Gy the technique had a saturation effect therefore it is not suitable for measuring the doses below 20 Gy.The dose vs sheared DNA length graph was plotted while using 1.2 % gel after subtraction of the control (0 Gy) pixel value as shown in figure 3 which proves that there is a good correlation between the sheared DNA verses the doses ranging between 20 to 30 Gy.The same procedure were performed for 1.5 % gel where the migration of DNA fragments were restricted and were found to be not suitable for biological dose measurements (figure 4).

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(3). 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 doses.

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