Contribution ID: **72** Type: **Poster**

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

Introduction

The need for hospitals to be prepared for the act of clinical mistreatment, radiation accidents and radiological terrorism is of high priority. 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 DCA lab is quite high.
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 and materials.
5 ml of blood from the 2 healthy individuals were collected 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 Gy respectively. The irradiated blood samples were subjected to Ficoll solution followed by centrifugation at 3000 rpm for 30 minutes. The buffy coat of lymphocytes was aspirated into 1.5 ml centrifuge tube. Later the cells were subjected for 15 minutes of lysis using 20 µl of proteinase k at 550 Celsius. Soon after the lysis procedure the samples were subjected for 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 which was used as a running buffer. 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 and the captured images were analysed using ImageJ software for measuring the sheared DNA length.

Results and discussion.
It is observed that up to 10 Gy there is an increase in response with the given dose. After 10Gy the dosimeter reaches to its saturation as shown in figure 1. This could be a result of complete DNA damage of lymphocytes in the given sample. From the above results it is evident that the gel electrophoresis method is a simple, rapid and cost-effective method for of quantifying DNA double strand break thereby serving as a suitable micro-biodosimeter.

Country or Int. Organization

India

Affiliation

Christian Medical College

Primary author: Mr RAJ, Jose (Christian Medical College)

Co-authors: Dr SINGH, Rabi (Christian Medical College); Dr SANTHOSH, Timothy (Christian Medical College)

Presenters: Mr RAJ, Jose (Christian Medical College); Dr SINGH, Rabi (Christian Medical College); Dr SANTHOSH, Timothy (Christian Medical College)

Session Classification: Paper Session 4: Radiobiology