Original Article

Evaluation of Deoxyribonucleic Acid Damage Using Neutral Comet Assay for High Radiation Doses: A Feasibility Study

Jose Solomon Raj, J. Suganthy[1], B. S. Timothy Peace, Selvamani Backianathan, I. Rabi Raja Singh, Reka Karuppusami[2], Christhunesa Soundararajan Christudass[3], Raja Solomon[3]

Departments of Radiotherapy, [1]Anatomy, [2]Biostatistics and [3]Neurochemistry, Christian Medical College, Vellore, Tamil Nadu, India

Abstract

Purpose: This study aims to investigate the use of the neutral comet assay to assess deoxyribonucleic acid (DNA) damage in lymphocytes exposed to high doses of radiation. **Materials and Methods:** The research was conducted by obtaining informed consent, after which blood samples were taken from seven healthy individuals and this study was approved by the institutional ethics committee. At first, for the determination of dose-effect curves, samples obtained from the first five individuals were irradiated for doses ranging from 0 to 35 Gy after which they were processed under neutral comet assay. In order to verify the determined dose-effect curves, a test dose of 15 Gy was delivered to the samples obtained from the sixth and seventh individuals. The amount of DNA damage from the obtained comet assay images was analyzed using four comet assay parameters namely % tail DNA, tail length, tail moment (TM), and Olive TM (OTM). The most suitable comet assay parameter was evaluated based on the obtained dose-effect curves. Furthermore, the distribution of individual cells for each dose point was evaluated for all the four comet assay parameters to find the optimal parameter. **Results:** From our results, it was found that from 0 to 25 Gy all the four comet assay parameters fit well into a linear quadratic curve and above 25 Gy saturation was observed. Based on the individual cell distribution data, it was found that % tail DNA could be an optimal choice to evaluate DNA damage while using neutral comet assay for high-dose ionizing radiation. **Conclusion:** The neutral comet assay could be a potential tool to assess DNA damage from high doses of ionizing radiation greater than 5 Gy.

Keywords: Comet assay, comet assay parameters, deoxyribonucleic acid damage

Received on: 25-02-2023 **Review completed on: 09-05-2023** **Accepted on: 10-05-2023** **Published on: 29-06-2023**

Introduction

Several assays such as gamma H2AX assay, neutral elution, comet assay, and dicentric chromosomal assay (DCA) are being used to quantify the radiation-induced deoxyribonucleic acid (DNA) damage.[1-3] Of these techniques, the neutral elution technique requires the use of radioisotopes which is hazardous.[3] DCA, which is a cytogenetic method and widely considered the gold-standard technique, is limited in the high dose region by the saturation that is observed above 5 Gy due to mitotic death.[4,5] Similarly, the gamma H2AX bio-marker saturates at doses above 6 Gy, respectively.[6]

Balakrishnan *et al*. have proposed the use of premature chromosomal condensation (PCC) assay for rapid estimation for high-dose accidental exposures (6.2–24.5 Gy) in which they observed a saturation above 18 Gy.[7] Moreover, few other authors have also reported on the saturation that occurs around 18–20 Gy while using the PCC assay.[8,9]

Access this article online

Quick Response Code:

Website: www.jmp.org.in

DOI: 10.4103/jmp.jmp_25_23

Due to these limitations, the use of these biological assays for the evaluation of DNA damage in case of accidental exposures and for high radiation dose applications such as blood irradiation poses a challenge. Blood products are generally irradiated at a dose of 25 Gy before transfusion to prevent graft-versus-host disease.[10] The neutral comet assay can quantify the DNA damage for radiation doses >5 Gy.[11] Single-cell gel electrophoresis or comet assay is a method of evaluating DNA damage at the individual cell level and was first developed by Ostling and Johanson.[12] The comet assay works on the principle of visualization of the damaged DNA loops

Address for correspondence: Mr. Jose Solomon Raj, Department of Radiotherapy, Christian Medical College, Vellore, Tamil Nadu, India.
E-mail: josesarc@gmail.com

How to cite this article: Raj JS, Suganthy J, Peace BS, Backianathan S, Singh IR, Karuppusami R, *et al*. Evaluation of deoxyribonucleic acid damage using neutral comet assay for high radiation doses: A feasibility study. J Med Phys 2023;48:146-53.

that are suspended in agarose and migrate toward the positive electrode during electrophoresis resulting in the formation of a comet-like appearance consisting of a head and a tail.[13] While the former consists of the undamaged DNA, the latter consists of the damaged DNA that has migrated from the head, thus indicating the level of damage induced by ionizing radiation.[14] In order to evaluate the DNA damage from the acquired comet image, parameters such as % tail DNA, tail length (TL), tail moment (TM), and Olive TM (OTM) are used.[15,16] While % tail DNA represents the percentage of damaged DNA in the tail region, TL is the length of the comet tail, TM is the product of % tail DNA and TL, and OTM developed by Olive *et al.*[13] refers to product of the % tail DNA and the distance between the head and the density-weighted centroid of the comet tail. Dusinska and Collins stated that % tail DNA is a suitable parameter to evaluate DNA damage as it has good correlation with genotoxic agents.[17] TL is a sensitive parameter which is used to detect the degree of DNA damage.[18] TM which has a combination of % tail DNA and TL is considered a suitable parameter to evaluate DNA damage.[19] Since OTM can detect the variation of DNA distribution in the comet tail it can be a useful parameter to portray the heterogeneity within a cell population.[20]

This study investigates the potential use of neutral comet assay to quantify the DNA damage in human lymphocytes that have been exposed to high-dose radiation as well as for irradiated blood used for transfusion. The dose-effect curves were determined by correlating radiation dose and comet parameters for doses ranging from 0 to 35 Gy and the individual cell response was analyzed. In light of the fact that various studies have recommended different comet assay parameters for the quantification of DNA damage,[16,19,20] this study aims to find out the appropriate comet parameter for the evaluation of DNA damage due to high radiation doses.

Materials and Methods

Sample collection

Blood samples (10 ml) were collected from seven healthy individuals by venipuncture method under aseptic conditions and stored in ethylenediamine tetra-acetic acid (EDTA) tubes. Out of the seven 4 were male and 3 were female aged between 22 and 41 years. Informed consent was obtained from these participants who were involved in the study before blood collection. The study was approved by the institutional ethics committee, and the data of the participant were anonymized. The blood samples from the first five individuals were used for the determination of dose-effect curves and sixth and seventh individuals were used for test dose verification.

In-house phantom and planning

An in-house developed cylindrical water phantom of 12 cm diameter and 17 cm height with 4 mm thick perspex was used in this study for irradiation. This phantom was designed to accommodate an EDTA tube of dimension 1 cm diameter and 10 cm length at the center [Figure 1]. Computed tomographic (CT) images of this phantom were acquired using SOMATOM definition as open CT simulator (Siemens Medical Solutions, Germany) and images were transferred to Eclipse™ treatment planning system (TPS) (version 13.7, Varian Medical Systems, Palo Alto, CA). To prevent the irradiation of blood samples during CT which could result in DNA damage, water was used to simulate the blood sample. The treatment plans were generated on the CT slices of the phantom using eclipse TPS for a field size of 20 cm × 20 cm with 1.25 MeV photon beam from a telecobalt unit. The volume of the blood sample in the EDTA tube was considered as target for irradiation and the treatment time for different doses was calculated.

Irradiation set-up

Blood samples from the first five healthy individuals were collected and each sample was divided into five subsamples of 2 ml each and irradiated to different doses viz., 0 (control), 5, 15, 25, and 35 Gy respectively in Equinox™ 80 telecobalt unit (TeamBest®, Ontario, Canada) at a dose-rate of 1.07 Gy/min. Samples were irradiated at a source-to-surface distance of 80 cm with a field size of 20 cm × 20 cm and the irradiation setup is shown in Figure 2.

Neutral comet assay

The neutral comet assay was prepared for each sample after irradiation as per the procedure given below. The time interval of 45 min between irradiation and start of the processing of the blood sample was maintained throughout the study, since comet assay results may vary due to repair.

Sample preparation

The blood sample was added to Histopaque 1077 (Sigma) at 1:1 ratio and lymphocytes were first isolated using the gradient centrifuge method in which the samples were centrifuged at 1500 rpm for 30 min. After this, the buffy coat that contains the lymphocytes appears in between the Histopaque 1077 and the plasma. The lymphocytes were removed from the buffy coat layer and washed using phosphate-buffered saline and stored in micro vials.

Slide preparation and layering of gel

First layer

Microscopic slides of 7.5 cm length × 2.5 cm width of 0.135 cm thickness were used for the study. Normal melting point agarose (NMPA) of 1% was heated in a microwave oven until the agarose was completely melted. Subsequently, the liquid agarose was gently poured on a microscopic slide and evenly spread out by placing a parafilm tape and another microscopic slide over it, and allowed to set in at 4°C in a refrigerator for 10 min.

Second layer

After solidification of the first layer, the parafilm tape was removed following which 80 µl of 1% low melting point agarose (LMPA) gel was mixed with 20 µl of lymphocytes and coated evenly over the first layer of NMPA and allowed to solidify as mentioned above. The liquid LMPA was maintained

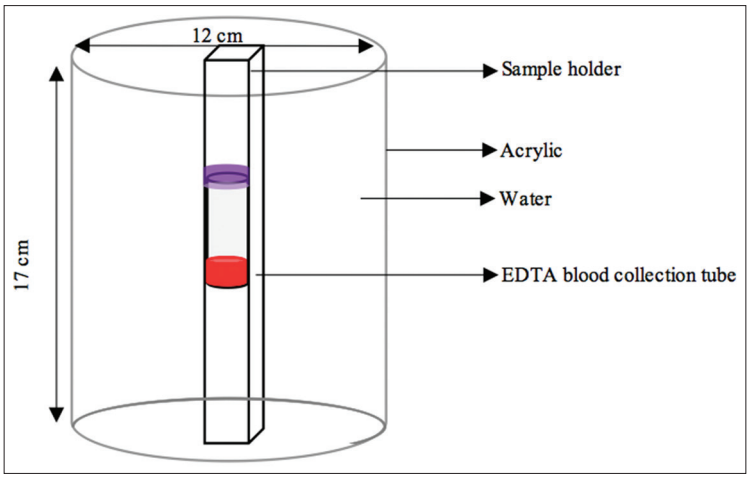

Figure 1: The schematic diagram of the in-house developed cylindrical water phantom used during irradiation of blood samples

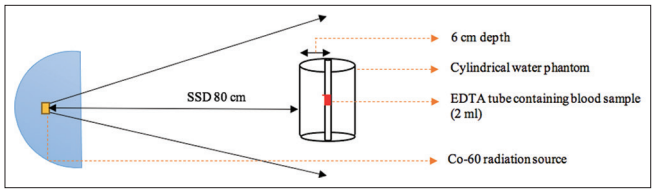

Figure 2: Set-up diagram for blood sample irradiation in telecobalt unit

at a temperature of 39°C while the lymphocytes were being added to prevent heat-induced lymphocyte damage.

Lysis of cells

The lysis solution (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris-HCl, 1% Triton ×100) was prepared by adding 146.1 g of NaCl, 37.2 g of Na_2 EDTA and 1.2 g of Tris-HCl to 700 ml of cold distilled water (4°C) and stirred until completely dissolved after which it was refrigerated for 1 h at 4°C. Subsequently, 12 g of NaOH in the form of pellets was added to the mixture and gently stirred for 30 min following which 10 g of sodium lauroyl sarcosinate was added. The double-distilled water was added to the above-mentioned solution to maintain a pH value of 9.5 that is well below the denaturing condition.[12] 1% Triton ×100 was added to the lysis solution and the prepared slides were immediately immersed in it for 12 h.

Neutral unwinding, electrophoresis, neutralization, and staining

After lysis, the slides were immersed for 45 min in tris-acetate EDTA buffer solution of pH 8.4 for neutral unwinding. Following this, electrophoresis was carried out under neutral condition for 45 min with a potential difference of 25 V and 300 mA.[21] Subsequently, the slides were neutralized with a neutral buffer and stained with 1 ml propidium iodide (2.5 µg/ml). The slides were washed with a neutral buffer to eliminate the excess stain before imaging.

Magnification, image acquisition, and analysis

In order to visualize the comets that were formed during the electrophoresis, the stained slides were magnified to ×20 and the images were acquired in "tiff" format using an inverted fluorescent microscope (Leica DMI6000B, Germany). The recommendation for the minimum number of comets to be evaluated per dose point is 50.[22] In this study, we have evaluated 100 comets for each dose point using the "open comet"[16] plugin that is available online (http://cometbio.org/download.html) for "Fiji,"[23] a commonly available image processing software. Using this plugin, comet parameters such as % tail DNA, TL, TM, and OTM were determined for 100 cells for each dose point. The units for TL, TM, and OTM given by software were in terms of pixels and arbitrary units which were converted into µm.

Dose-effect calibration curves

Dose-effect calibration graphs were plotted for each of the five individuals, relating the dose with the four comet parameters namely % tail DNA, TL, TM, and OTM. To plot the response for each dose point, the average comet parameter value of the 100 cells that were evaluated was used. The mean dose-effect curve was plotted by taking the determined average comet parameter for the five individuals. In addition, the distribution of individual cell response, based on each of the four comet parameters, was plotted for 500 cells for each dose point corresponding to 100 cells analyzed for each of the 5 individuals.

Relative sensitivity factor

To investigate the sensitivity of the four-comet parameters, the relative sensitivity factor (RSF) which is the ratio between irradiated (×Gy) and unirradiated (0 Gy) comet parameters for different doses was calculated. The RSF was calculated using the formula given in Equation (1) and this factor would help determine the comet parameter that was most sensitive to radiation in the dose range of 0–35 Gy.

$$\text{RSF: } Comet\ Parameter_{0\ Gy}^{x\ Gy} = \frac{Comet\ paramter(x\ Gy)}{Comet\ paramter(0\ Gy)} \quad (1)$$

Test dose verification

To verify the dose-effect curves, the blood samples collected from the sixth and seventh individuals were irradiated to a

test dose of 15 Gy at a dose rate of 1.07 Gy/min. Following this, the DNA damage was estimated using the neutral comet assay, and the corresponding dose was determined from the equations obtained using the mean dose-effect curves of the four comet parameters.

Statistical analysis

The statistical analysis was carried out using the statistical package for social sciences (SPSS) software Version 21.0 (Armonk, NY, USA: IBM Corp). For the continuous data, the descriptive statistics such as mean ± standard deviation were computed. The dose (Gy) was correlated with mean values of % tail DNA, TL, TM, and OTM and was plotted. In addition, the R^2 statistics (goodness of fit) for the dose-effect curves were computed.

Results

Dose-effect calibration curves

Figure 3 depicts the mean dose-effect calibration curves for each comet parameter of the first five individuals. It was observed that dose versus % tail DNA, TL, TM, and OTM followed a linear quadratic fit up to 25 Gy beyond which saturation was observed for all the four comet parameters. Third order polynomial curve was fit which could estimate the doses in the range of 0–35 Gy. The corresponding "goodness of fit" (R^2) for % tail DNA, TL, TM, and OTM dose-effect curves were found to be 0.9935, 0.9978, 0.9995, and 0.999, respectively, as shown in Figure 3a-d. It was also noted that the effect of saturation beyond 25 Gy was more pronounced in the TL dose-effect curve when compared to % tail DNA, TM, and OTM dose-effect curves.

The distribution of individual cell response was plotted for all the four comet parameters and is represented in Figure 4a-d which consists of the response of 500 cells per dose point. It was observed that % tail DNA showed a clear demarcation of cell responsible for the doses from 0 to 25 Gy with an overlapping of 35 Gy data points with the upper region of the 25 Gy band as depicted in Figure 4a. An enhanced overlap of datapoints was noted for doses beyond 15 Gy for the cell response measured using TL, whereas for TM and OTM had overlap above 25 Gy as shown in Figure 4b-d. The microscopic appearance of the comet for different doses is shown in Figure 5 where we can observe the relationship between dose and the comet images. Cell concentration is important for better image analysis, as overlapping comet images could affect comet parameter measurements, as shown in Figure 6. We also have observed microscopically that the tail did not get completely separated from the head, indicating that the neutral comet assay can provide the information on the relaxation of super-coiled loops and free ends migration at high doses thus representing the damage.[24]

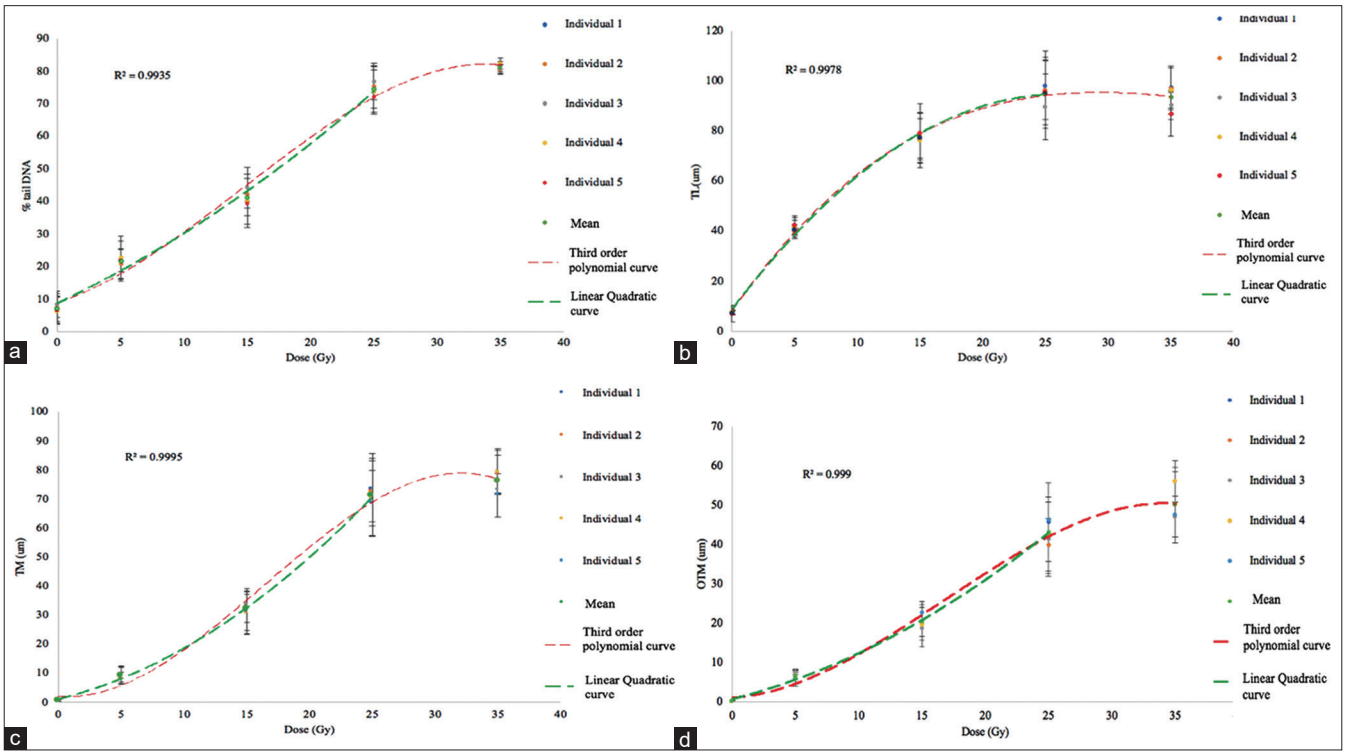

Figure 3: Each individual's response to radiation dose measured using four comet parameters and the mean dose-effect calibration curve of the five individuals (a) Dose (Gy) versus % tail DNA, (b) Dose (Gy) versus TL (μm), (c) Dose (Gy) versus TM (μm), (d) Dose (Gy) versus OTM (μm), ± SD. The green dashed line indicates the mean of five individual's comet parameter with linear quadratic fit up to 25 Gy for which *R*2 statistics were computed and dashed red lines indicates the third order polynomial fit up to 35 Gy. To estimate test dose (15 Gy) the linear quadratic fit was used. DNA: Deoxyribonucleic acid, TL: Tail length, TM: Tail moment, OTM: Olive TM, SD: Standard deviation

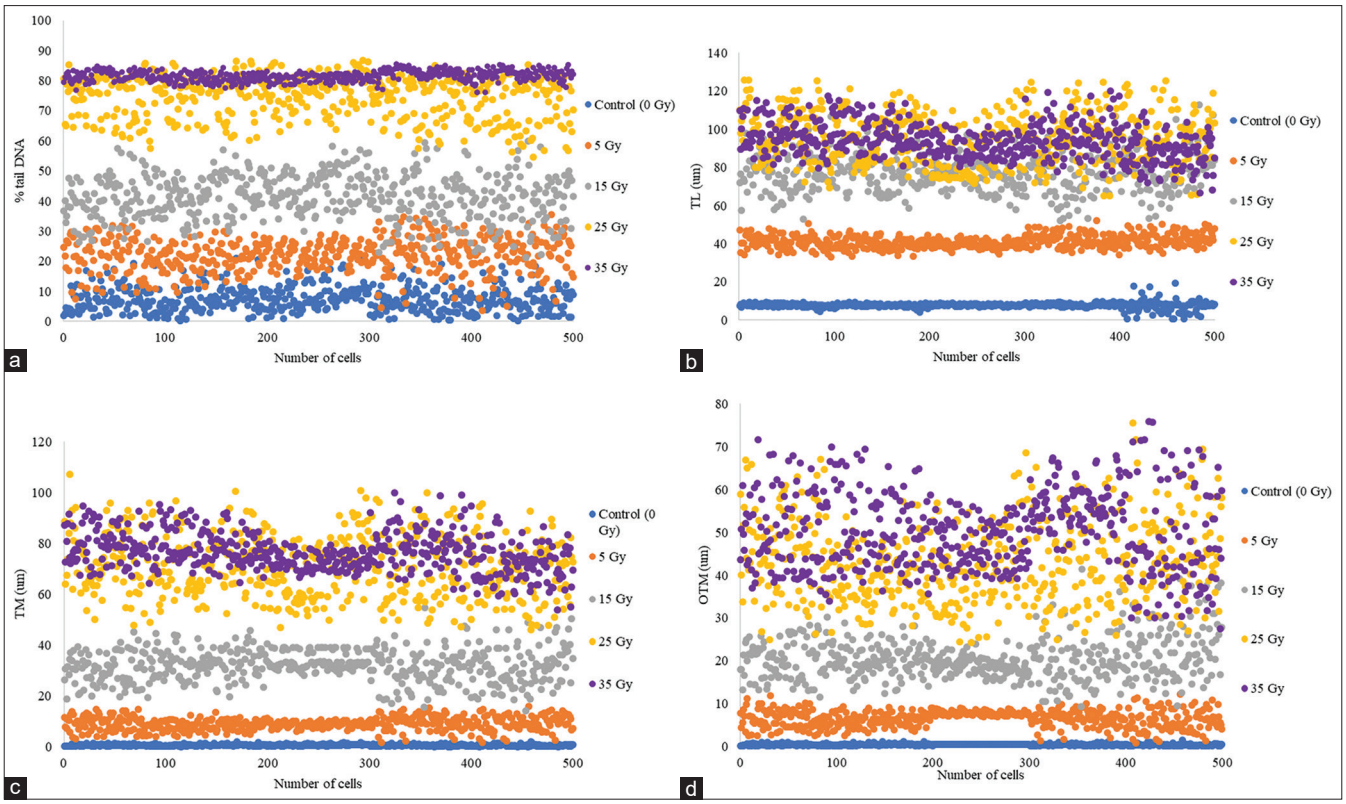

Figure 4: Distribution of individual cells based on comet parameters for control and irradiated cells ([100 cells per dose point] ×5 individuals = 500 cells in total per dose point was plotted in this graph) (a) Distribution of DNA damage measured using % tail DNA, (b) Distribution of DNA damage measured using TL (c) Distribution of DNA damage measured using TM (d) Distribution of DNA damage measured using OTM. DNA: Deoxyribonucleic acid, TL: Tail length, TM: Tail moment, OTM: Olive TM

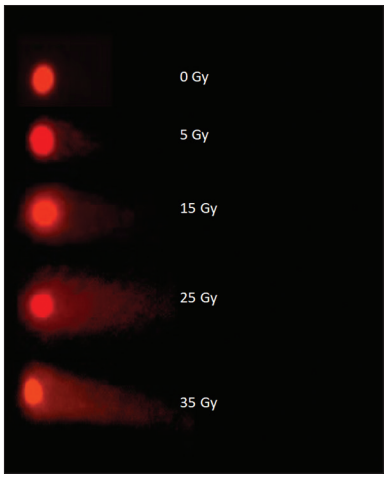

Figure 5: Microscopic appearance of comets for different doses

Table 1: Relative sensitivity factor for the comet parameters

RSF	Dose (Gy)			
	5	15	25	35
Percentage tail DNA $\frac{x\ Gy}{0\ Gy}$	3.02	5.69	10.26	11.22
TL $\frac{x\ Gy}{0\ Gy}$	5.38	10.18	12.48	12.28
TM $\frac{x\ Gy}{0\ Gy}$	16.07	56.9	126.5	136.15
OTM $\frac{x\ Gy}{0\ Gy}$	19.01	59.76	126.78	147.56

RSF: Relative sensitivity factor, TL: Tail length, TM: Tail moment, OTM: Olive TM

Relative sensitivity factor

To analyze the sensitivity of the comet parameters the RSF was determined in different doses as tabulated in Table 1. It was noted that OTM and TM were the most sensitive comet parameters when compared with % tail DNA and TL. The sensitivity of TM and OTM was found to be 5.32 and 6.29 times greater for the RSF calculated for $TM_{0\ Gy}^{5\ Gy}$ and $OTM_{0\ Gy}^{5\ Gy}$ when compared to $\%\ tail\ DNA_{0\ Gy}^{5\ Gy}$.

Test dose verification

The data obtained from the irradiated blood samples taken from sixth and seventh individuals are tabulated in Table 2. Based on the linear-quadratic equation obtained from the mean dose-effect curves, the dose estimated using the mean % tail DNA, TL, TM, and OTM for both the individuals were found to be 14.52, 14.23, 14.90, and 14.32 Gy.

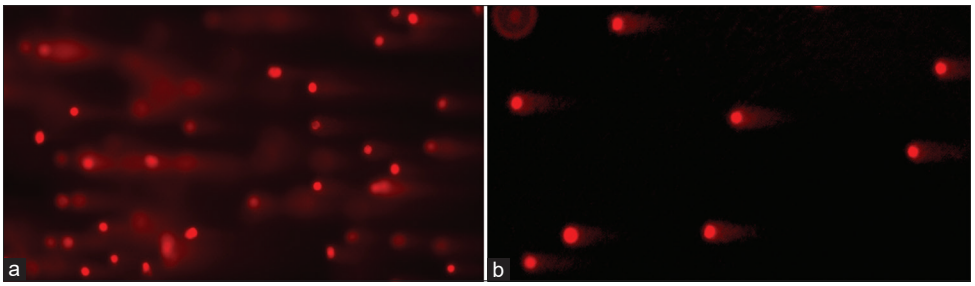

Figure 6: Microscopic images of irradiated lymphocytes (a) Before optimization of the cell concentration (40 μl of lymphocytes mixed with 80 μl of LMPA) (b) After optimization of the cell concentration (20 μl of lymphocytes mixed with 80 μl of LMPA). LMPA: Low melting point agarose

Table 2: Test dose validation results for individual 6 and 7 estimated using the linear quadratic equation obtained from the mean-dose effect curves

Comet parameter	Comet parameter values (average of 100 cells per dose point)		Average comet parameter values of individual 6 and 7	Dose (Gy) estimated using the linear quadratic equation
	Individual 6	Individual 7		
Percentage tail DNA	40.37	43.65	42.01	14.52
TL (μm)	76.26	77.60	76.93	14.23
TM (μm)	30.63	33.71	32.17	14.90
OTM (μm)	20.17	18.97	19.57	14.32

TL: Tail length, TM: Tail moment, OTM: Olive TM

The equations used for estimation of doses are $Y = 0.0304\ X^2 + 1.8401\ X + 8.8932$ obtained from % tail DNA mean dose-effect curve, $Y = -0.1256\ X^2 + 6.568\ X + 8.8951$ from TL mean dose-effect curve, $Y = 0.0689\ X + 1.0617\ X + 1.0481$ from TM mean dose-effect curve and $Y = 0.0349\ X^2 + 0.8149\ X + 0.7471$ from OTM mean dose-effect curve, respectively. The aforementioned linear quadratic equation is analog to the equation defined by the International Atomic Energy Agency ($Y = \beta D^2 + \alpha D + C$) for determining dose using DCA. To find the dose from the linear quadratic equation, International Atomic Energy Agency for DCA has recommended an equation where $Y = \beta D^2 + \alpha D + C$, and $D = [(-\alpha + \sqrt{(\alpha^2 + 4\beta\ (Y\text{-}C))})/2\beta]$ was used to determine the test dose.[25]

Discussion

The neutral comet assay of the irradiated blood samples from seven healthy individuals was carried out. Several research studies have been performed using the blood from healthy individuals to rule out the effect of confounding factors such as tobacco smoking and alcohol consumption in addition to exposed occupational radiation workers who may show increased DNA damage in unirradiated "control" cells.[17,26] Therefore, healthy individuals who were not exposed to any of the above-mentioned confounding factors were chosen for this study.

Dose-effect calibration curves were determined in our laboratory due to the high inter-laboratory variation which is a limitation in the preparation and analysis of comet assay.[27] This study investigates the most suitable comet parameter to evaluate the DNA damage in the high dose range and the findings are discussed in this section. The comet assay parameters such as % tail DNA, TL, TM, and OTM were used to evaluate the DNA damage induced by ionizing radiation dose up to 35 Gy. Based on our observations, it was found that through the test dose verification measured using all the four comet parameters, yielded good results for the given dose of 15 Gy.

The mean dose-effect curves for the four comet assay parameters had a good correlation with dose, where the R^2 values were >0.99 for the linear-quadratic fit up to 25 Gy. This indicates that neutral comet assay is advantageous to evaluate DNA damage in the high dose range as also reported by Haines *et al*.[28] For the TL comet parameter, an onset of the saturation was observed at 15 Gy and complete saturation at 25 Gy which indicates that there was no increase in the length of the comet tail [Figure 3b]. On analyzing the distribution of individual cell response (500 cells per dose point) to different doses, it was observed that there was an overlap of the 25 Gy TL data points with 15 Gy TL data points as depicted in Figure 4b. This infers that the DNA damage measured with TL dose-effect curves could have a higher uncertainty in evaluating doses above 15 Gy as compared to the other three comet parameters analyzed in this study. Although the mean dose-effect curves are an average representation of 500 cells per dose point, the visualization of the dispersion of individual cells can give valuable insight into the amount of overlap between different dose bands. This information could aid in determining the maximum dose point to which the cell response could be evaluated with each comet parameter.

A saturation was observed in the mean dose-effect curves beyond 25 Gy for %tail DNA, TM, and OTM as represented

in Figure 3a, c, and d. Furthermore, from the distribution graph of individual cells response, it was identified that there was no overlap of data points for these three comet parameters [Figure 4a, c, and d], indicating that they could be used to evaluate DNA damage up to a high dose of 25 Gy. Although the analysis of TM mean dose-effect curve and distribution of individual cells does not show any saturation or overlap of data points up to 25 Gy, the estimation of DNA damage with TM comet parameter for dose above 15 Gy could be of concern as TM is the product of % tail DNA and TL that was found to saturate beyond 15 Gy. Thereby, % tail DNA and OTM could be potential parameters to evaluate the DNA damage for high doses up to 25 Gy, similar to the conclusion of Kumaravel and Jha.[29] Notwithstanding the fact that OTM shows good correlation with dose, the OTM values could vary with different imaging software due to the disparities in determining the density-weighted centroid accurately.[20] Therefore, it is to report using the % tail DNA as it could be a suitable tool that could be used for DNA damage evaluation.

To investigate the sensitivity of the comet parameters, a new factor RSF has been introduced. Based on the RSF values up to 35 Gy, it was found that the OTM and TM comet parameters were more sensitive when compared with % tail DNA and TL. Although RSF factor lends insight into the sensitivity of the comet parameters to different doses, it is the saturation and overlap of data points across the dose bands that play a greater role in identifying the suitable comet parameter for DNA damage evaluation. This novel factor could be a promising tool to evaluate the sensitivity to DNA damage in the dose range within the limits of saturation and the absence of overlap.

This study has proven that neutral comet assay with % tail DNA comet parameter could be used as a biological indicator for high doses. This can be potentially used to verify the high dose in the order of 25 Gy that is required to prevent transfusion-associated graft versus host disease by the evaluation of DNA damage of freshly irradiated blood samples.

Since this was a feasibility study it was performed using a small cohort of blood samples from seven individuals that may not represent a larger group, which is also the limitation of this research work. Extensive studies with a larger number of samples may further validate the current research work. A study on the effect of repair using comet assay is in the scope of our future work. Moreover, as the methodology for repair study might differ from the current methodology, we are venturing on generating timeline-based calibration curves as a separate research work.

Conclusion

The use of neutral comet assay as a biological indicator to assess the DNA damage for blood samples exposed to high amount of ionizing radiation was investigated. Mean dose-effect calibration curves were established in our laboratory that showed a good correlation between the absorbed dose and the DNA damage for doses up to 25 Gy using the neutral comet assay. In addition to the mean dose-effect curves, visualization of the distribution of the individual cell response to dose could be paramount in selecting the suitable comet parameter for the evaluation of DNA damage. Our results argue in favor of the use of % tail DNA comet parameter to assess DNA damage for high radiation doses. Earlier higher doses in the order of 10 Gy were considered lethal however due to advancement in recent technology in stem cell transplantation it has been made possible to save lives at higher doses. Therefore, to estimate higher doses during over-exposure and as well verification of blood samples irradiated for a dose of 25 Gy can be verified using which neutral comet assay. Extensive research with a large cohort along with further studies on repair may validate the present work.

Ethical standards

The study was carried out in accordance with the ethical standards of the Institutional Review Board (ethics committee).

Acknowledgement

This research is a part of the Ph.D. thesis work of the first author who is registered under The Tamil Nadu Dr. M. G. R. Medical University, Chennai, India. The authors would like to thank the Centre for Stem Cell Research Imaging Core facility (A unit of in Stem, Bengaluru), Bagayam, India for providing the imaging slots. The authors appreciate the contribution of the volunteers who donated blood for our study.

Financial support and sponsorship

This work was supported by the Christian medical college Vellore fluid research grant (institutional review board number: 11,242).

Conflicts of interest

There are no conflicts of interest.

References

1. Piotrowski I, Dawid A, Kulcenty K, Suchorska WM. Use of biological dosimetry for monitoring medical workers occupationally exposed to ionizing radiation. Radiation 2021;1:95-115.
2. Goutham HV, Mumbrekar KD, Vadhiraja BM, Fernandes DJ, Sharan K, Kanive Parashiva G, *et al.* DNA double-strand break analysis by γ-H2AX foci: A useful method for determining the over reactors to radiation-induced acute reactions among head-and-neck cancer patients. Int J Radiat Oncol Biol Phys 2012;84:e607-12.
3. Goutham VH, Kamalesh MD, Guruprasad PK, Vadhiraja MB, Satyamoorthy K, Rao Bola Satish S. A modified fluorimetric neutral filter elution method for analyzing radiation-induced double strand break and repair. Anal Biochem 2011;414:287-93.
4. Meenakshi C, Venkatachalam P, Chandrasekaran S, Venkatraman B. Construction of dose response curve for 6 MV LINAC x-rays using premature chromosome condensation assay for radiation dosimetry. Appl Radiat Isot 2021;173:109729.
5. Karachristou I, Karakosta M, Pantelias A, Hatzi VI, Karaiskos P, Dimitriou P, *et al.* Triage biodosimetry using centromeric/telomeric PNA probes and Giemsa staining to score dicentrics or excess fragments in non-stimulated lymphocyte prematurely condensed chromosomes. Mutat Res Genet Toxicol Environ Mutagen 2015;793:107-14.
6. Wanotayan R, Wongsanit S, Boonsirichai K, Sukapirom K, Buppaungkul S, Charoenphun P, *et al.* Quantification of histone H2AX phosphorylation in white blood cells induced by *ex vivo* gamma irradiation of whole blood by both flow cytometry and foci counting as

a dose estimation in rapid triage. PLoS One 2022;17:e0265643.
7. Balakrishnan S, Shirsath K, Bhat N, Anjaria K. Biodosimetry for high dose accidental exposures by drug induced premature chromosome condensation (PCC) assay. Mutat Res 2010;699:11-6.
8. Kanda R, Hayata I, Lloyd DC. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. Int J Radiat Biol 1999;75:441-6.
9. Puig R, Barrios L, Pujol M, Caballín MR, Barquinero JF. Suitability of scoring PCC rings and fragments for dose assessment after high-dose exposures to ionizing radiation. Mutat Res 2013;757:1-7.
10. Chapman J, Finney RD, Forman K, Kelsey P, Knowles SM, Napier JA, *et al*. Guidelines on gamma irradiation of blood components for the prevention of transfusion-associated graft-versus-host disease. Transfus Med 1996;6:261-71.
11. Wojewódzka M, Buraczewska I, Kruszewski M. A modified neutral comet assay: Elimination of lysis at high temperature and validation of the assay with anti-single-stranded DNA antibody. Mutat Res 2002;518:9-20.
12. Ostling O, Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Biophys Res Commun 1984;123:291-8.
13. Olive PL, Banáth JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the "comet" assay. Radiat Res 1990;122:86-94.
14. Beedanagari S. Genetic toxicology. In: Comprehensive Medicinal Chemistry III. Oxford: Elsevier; 2017. p. 195-203. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124095472123819. [Last accessed on 2022 Jan 12].
15. Geric M, Gajski G, Orešcanin V, Garaj-Vrhovac V. Seasonal variations as predictive factors of the comet assay parameters: A retrospective study. Mutagenesis 2018;33:53-60.
16. Gyori BM, Venkatachalam G, Thiagarajan PS, Hsu D, Clement MV. OpenComet: An automated tool for comet assay image analysis. Redox Biol 2014;2:457-65.
17. Dusinska M, Collins AR. The comet assay in human biomonitoring: Gene-environment interactions. Mutagenesis 2008;23:191-205.
18. Surniyantoro HN, Darlina, Rahardjo T. Alkaline comet assay as a predictor of DNA damage in medical radiation workers. J Phys Conf Ser 2020;1436:012023.
19. Lu Y, Liu Y, Yang C. Evaluating *in vitro* DNA damage using comet assay. J Vis Exp 2017;128:56450.
20. Kumaravel TS, Vilhar B, Faux SP, Jha AN. Comet assay measurements: A perspective. Cell Biol Toxicol 2009;25:53-64.
21. Giovannelli L, Cozzi A, Guarnieri I, Dolara P, Moroni F. Comet assay as a novel approach for studying DNA damage in focal cerebral ischemia: Differential effects of NMDA receptor antagonists and poly (ADP-ribose) polymerase inhibitors. J Cereb Blood Flow Metab 2002;22:697-704.
22. Møller P, Loft S. Statistical analysis of comet assay results. Front Genet 2014;5:292.
23. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, *et al.* Fiji: An open-source platform for biological-image analysis. Nat Methods 2012;9:676-82.
24. Olive PL, Banáth JP. The comet assay: A method to measure DNA damage in individual cells. Nat Protoc 2006;1:23-9.
25. International Atomic Energy Agency. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies, Emergency Preparedness and Response. Vienna: IAEA; 2011. p. 247.
26. Azqueta A, Ladeira C, Giovannelli L, Boutet-Robinet E, Bonassi S, Neri M, *et al.* Application of the comet assay in human biomonitoring: An hCOMET perspective. Mutat Res Rev Mutat Res 2020;783:108288.
27. Collins AR, Oscoz AA, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, *et al.* The comet assay: Topical issues. Mutagenesis 2008;23:143-51.
28. Haines G, Marples B, Daniel P, Morris I. DNA damage in human and mouse spermatozoa after *in vitro*-irradiation assessed by the comet assay. In: del Mazo J, editor. Reproductive Toxicology. Boston, MA: Springer US; 1998. p. 79-93. Available from: http://link.springer.com/10.1007/978-1-4899-0089-0_10. [Last accessed on 2023 Feb 07].
29. Kumaravel TS, Jha AN. Reliable comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. Mutat Res 2006;605:7-16.