2.0. Materials

The materials used for the present study are listed below:

2.1. Acetic acid: Merck (Cat. # 100063)

2.2. Antibiotics and Antimycotic solution: Sigma (Cat. # A5955)

2.3. Antibodies for γ-H2AX: Merck (Cat. # 05-636, AP124F)

2.4. Antifade DAPI: Vysis. (Cat. # 32-0804830)


The lyophilized form (15 units) was dissolved in 5ml sterile Milli Q water to get a concentration of 2.5mg/ml as 15 units of the vial contain 12.5mg of BLM.

2.6. Bovine serum albumin (BSA): Sigma (Cat. # A-9418)

2.7. Carnoy’s fixative:

Methanol and acetic acid were mixed in a 5:1 ratio respectively to obtain Carnoy’s fixative.

2.8. Centromeric probe: Star Fish (Cat. # 1695-F-02)

From the stock probe (10µl), 1µl was taken and diluted in 15µl of hybridization buffer.

2.9. Cell freezing media: Sigma (Cat. # C6295)

2.10. Colchicine: HiMedia (Cat. # RM342-10G)

The lyophilized form (5mg) was dissolved in 5ml sterile Milli Q water to get a concentration of 1mg/ml of colchicine.

2.11. Cytochalasin-B (Cyto-B): Sigma (Cat. # C6762)

Stock: Cyto-B was dissolved in 5ml DMSO and filtered through a 0.2µ syringe filter. The prepared cyto-B (1mg/ml) was stored at below -20°C in dark.

Working solution: One ml of the above stock solution was dissolved in 10ml of RPMI-1640 media to get a concentration of 100µg/ml; 600µl of working solution was added to the 10ml culture to obtain a final concentration of 6µg/ml.
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2.12. Dimethyl sulphoxide (DMSO): Sigma (Cat. # D2650)

2.13. Di-sodium hydrogen phosphate (Na$_2$HPO$_4$): HiMedia (Cat. # RM 1154)

2.14. Dulbecco’s Modified Eagles Medium (DMEM): Gibco (Cat. # 12800-017)

2.15. Ethanol: Changsu Yangyuan Chemicals (Cat. # XK-13-201-00185)

2.16. Ethanol (70%):

About 70ml of ethanol was diluted in 30ml of Milli Q water and stored at 4°C.

2.17. Ethanol (80%)

About 80ml of ethanol was diluted in 20ml of Milli Q water and stored at room temperature.

2.18. Ethanol (90%)

About 90ml of ethanol was diluted in 10ml of Milli Q water and stored at room temperature.

2.19. Ethanol (100%)

Absolute ethanol was transferred to centrifuge tube and stored at room temperature.

2.20. Fetal bovine serum (FBS): Gibco (Cat. # 10270-016)

2.21. Giemsa stain: Sigma (Cat. # G5637)

Stock: To the 2.5g giemsa powder 135 mL of analytical grade glycerol was added into a conical flask (500ml) and dissolved completely at 60°C for 3hr. It was allowed to cool down to room temperature and 210 mL of analytical grade methanol was added. Mixed thoroughly for overnight, filtered using a Whatmann paper and stored at 4-8°C. Working solution: A 4ml of giemsa stock, 4ml of di-sodium hydrogen phosphate and 4ml of sodium di-hydrogen phosphate buffers, and 38ml of distilled water was mixed well to make a final volume of 50ml of Giemsa working stain (8%).

2.22. Hydrochloric acid (HCl): Fischer (Cat. # H/1150/25)

To prepare 1N HCl, 8.985mL of HCl (12N) was mixed with water up to 100ml.
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2.23. Hypotonic solution (0.075M Potassium Chloride):
About 560mg of potassium chloride was dissolved in 100ml of Milli Q water to obtain 0.075M hypotonic solution.

2.24. Isopropyl alcohol: Fischer (Cat. # P/7/490)

2.25. Minimum Essential Medium (MEM): Sigma (Cat. # M0643)

2.26. Mitomycin C (MMC): Sigma (Cat. # M4287)
The lyophilized form (5mg) was dissolved in 5ml sterile Milli Q water to get a concentration of 1mg/ml of MMC.

2.27. Paraformaldehyde: HiMedia (Cat. # RM 845)

2.28. Phosphate buffer saline (PBS) for 500ml:
Sodium chloride (137mM) – 4.00gm
Potassium Chloride (2.68mM) – 0.1gm
Di-sodium hydrogen phosphate (8mM) – 0.568gm
Potassium dihydrogen phosphate (1.47mM) – 0.1gm
The above mentioned chemicals were dissolved in 500ml of Milli Q water and pH was adjusted to 7.2. Then the buffer was autoclaved and stored at room temperature or 4°C.

2.29. Phytohemagglutinin (PHA): Gibco (Cat. # 10576-015)

2.30. Potassium chloride: Rankeem (Cat. # P0240)

2.31. Potassium dihydrogen orthophosphate: HiMedia (Cat. # RM 1188)

2.32. Propidium iodide (PI): Sigma (Cat. # P4170)

2.33. RNase: Sigma (Cat. # R6513)

2.34. Roswell Park Memorial Institute 1640 (RPMI-1640): Gibco (Cat. # 23400-013)

2.35. Sodium bicarbonate: Rankeem (Cat. # S0680)

2.36. Sodium chloride: HiMedia (Cat. # RM 853)

2.37. Sodium dihydrogen phosphate (NaH₂PO₄): HiMedia (Cat. # RM1255)
2.38. Sodium hydroxide: Qualigens (Cat. # 15895)

Add 4g of sodium hydroxide in 100ml of water to get 1N.

2.39. Sodium phosphate buffer/ Sorenson buffer preparation for 100ml:

Disodium hydrogen phosphate – 1.49g

Sodium dihydrogen phosphate – 1.56g

2.40. Sodium saline citrate (SSC): HiMedia (Cat. # ML030-500ML)

2.41. 0.4X SSC/0.3% IGEPAL

To prepare the 0.4X SSC/0.3% IGEPAL, 10ml of 20X SSC (pH 5.3) and 1.5ml of IGEPAL was added with 475ml Milli Q water, mixed thoroughly until IGEPAL dissolve. Measured and adjusted pH to 7.0-7.5 with sodium hydroxide, and made up to 500ml.

2.42. 0.2X SSC/0.1% IGEPAL

To prepare the 0.2X SSC/0.1% IGEPAL, 5ml of 20X SSC (pH 5.3) and 0.5ml of IGEPAL was added with 475ml Milli Q water, mixed thoroughly until IGEPAL dissolve. Measured and adjusted pH to 7.0-7.5 with sodium hydroxide, and made up to 500ml.

2.43. Triton X-100: HiMedia (Cat. # RM 845)

2.44. Trypsin EDTA (Ethylene diamine tetra acetic acid): Gibco (Cat. # 25200-072)
2.42. Model system

The following cell lines were used in the current study:

**CCRF-CEM (Human acute lymphoblastic leukemia)**
- **Designation**: CCRF-CEM
- **Cell Type**: Lymphoblast
- **Cell subtype**: Peripheral blood
- **Genus Species**: *Homo sapiens*
- **Common Name**: Human
- **ATM status**: Wild type

**HL-60 (Human acute promyelocytic leukemia)**
- **Designation**: HL-60
- **Cell Type**: Promyeloblast
- **Cell subtype**: Peripheral blood
- **Genus Species**: *Homo sapiens*
- **Common Name**: Human
- **ATM status**: Wild type

**AG1522 (Normal diploid human skin fibroblasts)**
- **Designation**: AG1522
- **Cell Type**: Fibroblast
- **Cell subtype**: Skin
- **Genus Species**: *Homo sapiens*
- **Common Name**: Human
- **ATM status**: Wild type
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HLF (Normal diploid human lung fibroblasts)

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<tr>
<td>Cell Type</td>
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<tr>
<td>Cell subtype</td>
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<tr>
<td>Genus Species</td>
<td><em>Homo sapiens</em></td>
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<tr>
<td>Common Name</td>
<td>Human</td>
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<tr>
<td>ATM status</td>
<td>Wild type</td>
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L6 (Normal rat myoblasts)

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<tr>
<td>Cell subtype</td>
<td>Skeletal muscle</td>
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<td><em>Rattus norvegicus</em></td>
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GM4405 (Human fibroblasts)

<table>
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<th>Designation</th>
<th>GM4405</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Type</td>
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<tr>
<td>Cell subtype</td>
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2.1. Methods

2.1.1. Institutional Ethics Committee (IEC)

All necessary human ethics clearance was obtained prior to undertaking this study (Ref no: IEC-N1/09/OCT/12/26). A copy of IEC letter is given in annexure I.

2.1.2. Study population

Heparinized peripheral blood was collected from healthy male and female volunteers (n=8) aged between 21 and 46yrs with informed consent with no known history of smoking and exposures to radiations. About 10ml of peripheral blood (PBL) was collected from eight healthy volunteers and aliquoted in 1ml cryo vials for the experiments. The format of consent form is given in annexure II.

2.1.3. Media preparation

The lyophilized DMEM, MEM and RPMI-1640 medium was added to 980ml of sterile Milli Q water with gentle stirring. To this, 3.7, 2.2 and 2.0g of sodium bicarbonate were added respective media and the pH was adjusted to 7.2 using 1N NaOH (Sodium hydroxide) and 1N HCL (Hydrochloric acid). To prevent the fungi and bacterial contamination 1ml of 100X antibiotic and antimycotic solution was added and the volume was made up to one liter and stirred until the contents completely dissolved. Finally, the medium was filtered using 0.22µm membrane filter and stored at 4°C.

2.1.4. Procurement and maintenance of cell lines

CCRF-CEM, HL-60 and L6 cells were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The AG1522, HLF and GM4405 cells were obtained as a kind gift from Dr. Edouard Azzam, NJMS/UH cancer centre, New Jersy, USA. Upon arrival, the CCRF-CEM (Figure 2.1a) and HL-60 (Figure 2.1b) cells were cultured in RPMI-1640 medium, L6 cells (Figure 2.1c) were cultured in DMEM medium and AG1522 (Figure 2.1d) HLF (Figure 2.1e) and GM4405 (Figure 2.1f) cells were cultured in MEM medium added

Figure 2.1. Cell lines used in the study

a) CCRF-CEM cells  
b) HL-60 cells  
c) L6 cells  
d) AG1522 cells  
e) HLF cells  
f) GM4405 cells

The cells were viewed under 10X magnification
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with 20 or 12.5% FBS and maintained at 37°C in humidified incubator in an atmosphere of 5% CO₂. The CCRF-CEM [p12-p16] and HL-60 [p9-p14] cells were grown in their respective media as suspension culture in T25 culture flasks. Once the cells reached confluency, the medium was removed by centrifugation. Then the cells were resuspended in fresh medium and split as 1:3 ratio. The AG1522 [p10-p15], HLF [p11-p14], GM4405 [p9-p14] and L6 [p30-p34] cells were grown in their respective media as monolayer cultures in T25 culture flasks. Once the monolayers reached confluency, the medium was removed. The cells were washed with 3ml of PBS and incubated with trypsin EDTA (0.025%) for 3-5mins. Then the cells were harvested by gentle tapping, resuspended in fresh medium and split as 1:3 ratio. The cells were expanded, frozen and stored in liquid nitrogen in the laboratory for further experiments.

2.1.5. Freezing of cells

After expansion the cells were frozen at early passages which can be utilized for future experiments. Approximately 1X 10⁵ were suspended in 1ml of cell freezing medium (65% Medium + 30% Serum + 5% DMSO) and transferred to the labeled cryovial. Then the cryovial was transferred into cryo can filled with iso propyl alcohol, maintained at 4°C for 3 to 4hrs and then incubated at 0°C overnight and then for 16 to 20hrs incubated at -80°C. Finally the cryovial were taken out of the cryocan and transferred into liquid nitrogen (-196°C) and stored.

2.1.6. Thawing of cells

The frozen cells are thawed and used for further experiments whenever needed. The cryovial was removed and thawed quickly within 3mins at 37°C water bath. To 1ml of cell suspension, 10ml of medium with serum was added and centrifuged to remove the DMSO. Then the cells were re-suspended in fresh medium and seeded into T25 flask and expanded.
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2.1.7. In vitro irradiation of PBL

Irradiation of PBL was performed using two different radiation sources namely a high energy linear accelerator (LINEAC), (PRIMAS, Siemens, Germany) teletherapy unit at Dr. Kamakshi Memorial Hospital, Chennai, and $^{60}$Co teletherapy unit, Institute of Nuclear Medicine and Allied Science (INMAS) (Bhabhatron II, Panacea Med. Tech. Pvt. Ltd., Bangalore, India). The aliquoted whole blood samples were exposed to 0.1, 0.25, 0.5, 1, 2, 3, and 4Gy doses of 6MeV X-rays at a dose rate of 3 Gy/min (Figure 2.2) and 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4 and 5Gy for $^{60}$Co-γ rays at a dose rate of 0.76Gy/min (Figure 2.3).

2.1.8. BLM exposure

Confluent, growth arrested (~90%, G0/G1) cells (AG1522, HLF, GM4405 and L6) in PD (Petri dish)-60 were exposed to different concentrations of BLM (0, 40 and 80µg/ml) for 3hrs at 37°C [1]. The cells were washed with PBS and supplemented with fresh 3ml of medium and incubated at 37°C for 2hrs and 24hrs; the cells were trypsinized and sub-cultured for the different end points (CBMN assay, γ-H2AX assay and cell cycle). In parallel 2ml of PBL also exposed to BLM (40 and 80µg/ml).

2.1.9. MMC exposure

About 2ml of PBL was exposed to two different concentrations of MMC (2 and 4µg/ml) for 3hrs at 37°C [2].
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Figure 2.2. Lineac X-ray tele therapy unit used to irradiate the PBL

Figure 2.3. Bhabhatron II $^{60}$Co γ-ray tele therapy unit used to irradiate the PBL to γ-radiation
2.1.10. Colchicine exposure

About 2ml of PBL was exposed to two different concentrations of colchicine (0.05 and 0.1µg/ml) for 24hrs at 37°C [3].

2.1.11. CBMN assay

The exposed and unexposed blood samples were processed for culture and harvesting as described earlier [4] with modifications. Blood cultures were initiated using 1ml whole blood in 80% RPMI-1640, supplemented with 20% FBS, 20µg/ml PHA and standard antibiotics (Penicillin and Streptomycin at final concentrations of 100IU/mL and 100mg/mL respectively) at 37°C in a 5% CO2 incubator. At 44hrs post incubation, Cyto-B (6µg/ml) was added aseptically and incubation was continued till 72hrs. The cells were harvested by treating with chilled hypotonic solution (0.075M KCl) and fixing with Carnoy’s fixative (5:1 methanol and acetic acid). Finally, the cells were washed twice with fixative and dropped on to clean glass slides. The slides were air-dried, stained with 8% giemsa in phosphate buffer (pH 6.8) and PI (1µg/ml) and coded; one thousand binucleated cells were scored (40 X magnifications in Axio Imager M2, Zeiss) (Figure 2.4). A copy of scoring sheet is given in annexure III.

The BLM exposed cell lines, trypsinized after 2 and 24hrs post incubation, the cells were seeded in PD-60 and cyto-B (6µg/ml) was added at 24hrs and fixed at 48hrs and stained with DAPI (Figure 2.5) analysed using an epifluorescent inverted microscope (Nikon, TiU, Japan). The adopted scoring criteria were described earlier [5] and mentioned below.
Figure 2.4. Distribution of MN in the binucleated cells of PBL exposed to γ-radiation

Figure 2.5. Binucleated cells with or without MN stained with DAPI in L6 cells and nucleo-plasmic bridge in GM4405 cells exposed in vitro to BLM

2.1.12. MN scoring criteria

Criteria for scoring BN cells and MN are follows:

- The cells should be binucleated and should have intact nuclear membranes and be situated within the same cytoplasmic boundary (Figure 2.6a).
- The two nuclei in a binucleated cell should be approximately equal in size, staining pattern and intensity (Figure 2.6b).
- A cell with two overlapping nuclei can be scored only if the nuclear boundaries of each nucleus are distinguishable (Figure 2.6c).
- The diameter of MN in human lymphocytes usually varies between 1/16th and 1/3rd of the mean diameter of the main nuclei, which corresponds to 1/256th and 1/9th of the area of one of the main nuclei in a BN cell, respectively (Figure 2.6d).
- MN is non-refractile and they can therefore be readily distinguished from artifact such as staining particles (Figure 2.6e).
- MN is not linked or connected to the main nuclei (Figure 2.6f).
- MN may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary (Figure 2.6g).
- MN usually has the same staining intensity as the main nuclei but occasionally staining may be more intense (Figure 2.6h).
Figure 2.6. Diagrammatic representation for scoring criteria of binucleated cells and MN for the exposed cells

A.  B.  C.  D.  E.  F.  G.  H.
2.1.13. Automated MN scoring by Metafer

The Metafer 4 (Version 3.9) software (MetaSystems, Germany) installed on a Dell computer (Dell precision T3600) and a high-resolution, monochrome megapixel charge coupled device (CCD) camera, combined with a microscope (Axio Imager Z2, Zeiss) (Figure 2.7); with the appropriate filter and a motorized scanning stage was employed to score automatically; the system can scan eight slides at a time. The criteria described by Willems et al., [6] was adopted to identify and classify the BN cells and the MN; briefly, the system identify BN cells as two similar nuclei, close to each other; then the classifier determine the BN cell based on similarity of the daughter nuclei shape, size and distance between the two nuclei. When a BN cell is identified, the system will apply a second set of classifier to define the MN; size, shape and the circular area around the daughter nuclei in which the system searches for MN (Figure 2.8).


From the aberration yields, the distribution pattern in cells was ascertained by the standard ‘u’ test as described in the IAEA publication [7]. Curve fitting was done with the help of the statistical software program known as “Poly Fit” developed by National Radiation Protection Board (NRPB), UK [8]. The essence of this program is to fit the data points by a weighted least squares method, thereby taking into account the scoring effort for each point.

2.1.15. Calculation of nuclear division index (NDI)

The NDI provides a measure of the proliferative status of the viable cell fraction. It was calculated by scoring 500 viable cells and then grouped into cells with 1, 2, 3 or 4 nuclei, (Figure 2.9) using the formula:

\[ \text{NDI} = \frac{M_1 + 2M_2 + 3M_3 + 4M_4}{N} \]

Where, M1–M4 represent the number of cells with 1–4 nuclei and N is the total number of viable cells scored (excluding necrotic and apoptotic cells) [5].
2.1.16. Fluorescence in situ hybridization (FISH) assay

The pan-centromeric probe was employed for centromeric detection in the MN of exposed samples. In brief, prepared slides were treated with a series of ethanol concentrations (80%, 90% and 100%) for dehydration and air dried. A diluted probe (1µl of probe in 15µl of hybridization buffer) was added on the slides and sealed under glass cover slips, placed in a moistened hybridizing chamber (HyBriteTM, Vysis) which was programmed for 73°C for 5 mins denaturation and 37°C for 16-18hr hybridization. The slides were then washed with 0.4XSSC/0.3% IGEPAL at 72°C±1°C for 15-20 seconds and 2XSSC/0.1% IGEPAL at room temperature for 5-10 seconds. Cover slips were applied with antifade mountant containing DAPI (Vysis Inc., Downers Grove, USA) and stored at -20°C for an hour to enhance the probe signal. Those were analyzed (Figure 2.10) using a fluorescence microscope (100X) with FITC (Excitation-Emission: 490-525-Green) and DAPI (Excitation-Emission: 358-461-Blue) filters and scored as described earlier [9].

2.1.17. DC assay

In parallel, for the selected doses (0.1, 0.25, 1 and 5Gy), blood cultures were initiated using 1ml whole blood in 80% RPMI-1640, supplemented with 20% FBS, 40µg/ml PHA and standard antibiotics (Penicillin and Streptomycin at final concentrations of 100IU/mL and 100mg/mL respectively) at 37°C in a 5% CO₂ incubator. At 24hr post incubation, colchicine (0.02µg/ml) was added aseptically and incubated for another 24hr. The cells were harvested by treating with pre-warmed hypotonic solution (0.075M KCl) and fixing with Carnoy’s fixative (3:1 methanol and acetic acid). Finally, the cells were washed twice with fixative and dropped on to clean glass slides. The slides were air-dried, stained with 4% giemsa in phosphate buffer (pH 6.8) and coded; one thousand cells were scored (100 X magnifications in Primostar, Zeiss) (Figure 2.11).
Figure 2.9. Cytokinesis arrested binucleated cells obtained from PBL stained in Giemsa showed cells with multiple nuclei.

Figure 2.10. Binucleated cells and MN with or without centromeric signals in PBL exposed *in vitro* to different chemicals.
2.1.18. \( \gamma \)-H2AX analysis by flow cytometry

After exposure to BLM, approximately 1x10^6 cells were fixed in 2% paraformaldehyde and ice-cold methanol, permeabilized using Triton X-100 (1%), blocked in 1% bovine serum albumin/ Triton X-100, and incubated with the primary antibodies (Merck, 1:400) at room temperature for 90mins followed by incubation in secondary antibody, labeled with FITC (Merck, 1:200). Flow cytometry was performed using C6 Accuri; around 10000 events were scored in dot plot (Figure 2.12a). The resulting histogram (Figure 2.12b) is characterized as the mean fluorescence and the relative fluorescence intensity (RFI) was calculated by taking the ratio of the histograms representing the exposed cells to that of control cells [10].

2.1.19. Cell cycle analysis

Approximately 3x10^6 cells were seeded in PD60 and kept for 24hrs at 37˚C after respective post incubation time. After 24hr the cells were trypsinized and fixed with 70% ethanol. The fixed cells were washed with PBS and treated with RNase (200µg), at 37˚C for 30mins. Then washed with PBS and added PI and analysed using C6 Accuri (BD) [11]. A representative histogram obtained for DNA content measurement of the exposed cells are given in figure 2.13.

2.1.20. MN analysis by flow cytometry

Immediately after allowing the cells to repair (2hr), one ml of the irradiated blood was transferred into a culture flask which contains 8ml of RPMI 1640 medium, 2ml of FBS and 200µl of PHA and incubated at 37˚C for 48hr. At the end of 48hrs the cultured content were transferred into centrifuge tubes pre-layered with ficoll histopaque and centrifuged at 2000rpm for 20mins. Then the buffy coat layer alone was taken and transferred into centrifuge tubes and washed twice with PBS. The supernatant was discarded and the cell pellets were disturbed gently. Finally, the cells were fixed with 70% pre-chilled ethanol while
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Figure 2.11. Metaphase chromosomes showing normal and dicentric chromosome obtained from PBL exposed in vitro to ionizing radiation

Figure 2.12. γ-H2AX analysis using flow cytometry in GM4405 cells exposed in vitro to BLM

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Figure 2.13. Cell cycle analysis in HLF cells exposed in vitro to BLM

M4-G0/G1 phase; M5-S phase; M6-G2/M phase

Figure 2.14. MN flow cytometry analysis in PBL exposed in vitro to ionizing radiation

MN-Micronucleus; N-Nucleus

vortexing and incubated at 4°C for overnight. After overnight incubation, the fixed samples were centrifuged at 1000rpm for 10mins. The supernatant was discarded and the cell pellets were disturbed gently. 3ml of PBS (to remove ethanol toxicity) was added and centrifuged at 1000rpm at 10mins. The supernatant was discarded and to the cell pellet 1ml of PBS and add 300µl of RNase (200µg) was added and incubate for 30mins at 37°C. The settings were applied as described earlier [12] and the pellet was resuspended with 20µl of PI and analyzed using flow cytometry (Figure 2.14). In parallel the exposed cell lines (CCRF-CEM and HL-60) were also processed for flow cytometry after 24hr post irradiation with the same parameter settings used for PBL.

2.1.2. Statistical analysis

One way ANOVA (Origin 7 software) was used to compare the MN frequencies obtained from the mean value of three experiments at the different time intervals. The observed results are significant at p<0.05. The distribution of aberrations in cells was studied by the method adapted by Savage [13]. This was done by the standard ‘u’ test using the formula:

\[ u = \frac{d - (N - 1)}{\sqrt{(\text{var} \ d)}} \]

Where, N is the total number of cells scored, d is the coefficient of dispersion (N-1) \( \sigma^2/y \), Y is the mean number of observed aberrations, \( \sigma^2/y \) is the relative variance and Var d is the variance of d given by \( 2(N - 1)(1-1/NY) \). This method makes use of the fact that the variance (\( \sigma \)) equals the mean (Y) i.e., the variance divided by mean is equal to 1, so that u=0. Student ‘t’ test was used to compare the mean of MN and RFI obtained for the different cell lines.

Mean absolute difference (MAD) using Microsoft office Excel 2007, compared the mean MN frequencies between the scoring methods.
The MN frequency and the associated error were calculated using the formula:

\[
\text{Frequency} = \frac{\text{Total MN}}{\text{Number of cells scored}}
\]

\[
\text{SE} = \frac{\sqrt{\text{Total aberrations}}}{\text{Number of cells scored}}
\]

Bonferroni corrections were used to compare the intercept, curvature and slope [14]. Significance levels of \( p<0.05 \) (*), \( p<0.001 \) (**), and \( p<0.0001 \) (***) were indicated in the test bars compared to their respective control bars.
2.2. References


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