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Standardisation and Validation of Cytogenetic Markers to Quantify Radiation Absorbed Dose

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ABSTRACT

The amounts of radiation exposure received by radiation workers are monitored generally by physical dosimeters like thermoluminescence dosimeter (TLD) and film badge. However, in practice the over-exposure recorded by physical dosimeters need to be confirmed with biological dosimeters. In addition to confirming the dose recorded by physical dosimeters, biological dosimeters play an important role in estimating the doses received during accidental exposures. Exposure to high levels of radiation induces certain biochemical, biophysical, and immunological changes (biomarkers) in a cell. Measurement of these changes are generally precise but cannot be effectively used to assess the dose, as the level of these changes return to normalcy within hours to months after exposure. Thus, among various biological indicators, cytogenetic indicators are considered practical and reliable for dose estimation. The paper highlights the importance and establishment of biodosimetry facility using genetic markers such as the sensitive dicentric chromosomes, rapid micronucleus assay and stable translocations measured using fluorescence *in situ* hybridisation and GTG banding for retrospective dose estimation. Finally, the development of γH2AX assay, as a potential marker of triage dosimeter, is discussed.

Keywords: Dicentric chromosomes, micronucleus, translocations, γH2AX assay, biological dosimetry, biomarkers

NOMENCLATURE

DC	Dicentric chromosomes
FISH	Fluorescence *in situ* hybridisation
IgG	Immunoglobulin-G
GTG	G-banding using Trypsin and Giemsa
Gy	Gray
MN	Micronucleus
m-FISH	Multiple fluorescence *in situ* hybridisation
NRPB	National Radiation Protection Board
PBS	Phosphate buffered saline
PHA-M	Phytohemagglutinin
RT	Reciprocal translocation
TL	Translocation
TLD	Thermoluminescence dosimeter
WCP	Whole chromosome painting

1. INTRODUCTION

In India, substantial progress has been made to meet the energy demands from nuclear resources and construction of plants is underway. Parallely, it becomes increasingly necessary to have facilities where biodosimetry can be performed in case of unlikely events. During rare incidents like threat of dirty bombs and nuclear terrorism, a possibility of mass exposures to radiation is unavoidable. In such scenarios, quantification of radiation absorbed dose might be helpful in the management of exposed individuals. Globally, genetic markers play an important role in monitoring occupational and accidental exposures to radiation[1,2] and to rule out suspected over exposures[3]. Considerable developments are in progress to use biomarkers as triage dosimeters to screen large populations in a short time.

Exposure to ionising radiation induces a spectrum of changes in a cell, collectively known as radiation biomarkers. This includes changes in the cellular ratio, variation in enzyme levels, altered membrane permeability, mutations, chromosomal alterations, etc. Of the various biomarkers, chromosomal changes are considered reliable for the estimation of dose. Biodosimetry, using chromosomal analysis (cytogenetic markers), is based on the relationship between chromosome aberration frequency and the amount of absorbed dose can be effectively used to monitor and assess the dose after exposure. While breaks formed in chromosomes upon radiation exposures get repaired, in addition to restitution, asymmetrical exchanges like dicentric chromosomes (DC), tricentrics, ring chromosomes and symmetrical exchanges like translocations (TL) or inversions can occur[4], all of which can be related to dose. Generally the techniques used to detect these damages are named after the type of chromosomal aberrations seen or the stains used to visualise the chromosomes. Interestingly, the aberration frequency is measured in lymphocytes of the exposed

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individuals, as it is easy to collect blood and which has a convenient life span to make these biodosimetric studies[5].

2. CHOICE OF CYTOGENETIC END POINTS

Measurement of dicentric chromosomes (DC) in uniformly stained metaphase chromosome preparations is a most widely employed method to quantify the absorbed dose in many accidental and suspected overexposures due to its specificity and sensitivity (10cGy). In addition to the distribution frequency, it is possible to differentiate partial-body exposure to whole-body exposure. Alternatively, scoring of micronucleus (MN) is in practice as a rapid method to estimate the radiation absorbed dose[6] with a sensitivity of 25cGy[7]. Fragments of chromosomes or whole chromosomes, which fail to get incorporated into daughter nuclei during mitosis, either due to spindle poison or lack of centromere, develop into MN. However, both the DC and MN are an unstable type aberration and the cells carrying such aberrations are eliminated from the body as a function of time. Hence, it is of limited use for the assessment of exposures received in the distant past [3,8].

Reciprocal translocations (RT) induced by radiation are a stable type aberration and have been shown to remain in circulation more or less permanently[9-11] and used in cumulative and retrospective dose estimation[12]. Banding and fluorescence *in-situ* hybridisation (FISH) techniques are in practice to score TL and relate to its dose[12-14]. Though the sensitivity of these techniques is equivalent to that of DC (10cGy), these are either laborious or costly and not specific to ionising radiation. Generally, the dose is estimated either by measuring the TL in entire genome using GTG banding[15] or FISH with few painted chromosomes[16,17]. A major concern in the TL scoring with FISH is that, extrapolation of genomic TL with few painted chromosomes to entire genome is based on assumptions[9]. Alternatively, the TL frequency in the entire genome as well as their origin can be analysed through m-FISH without extrapolation.

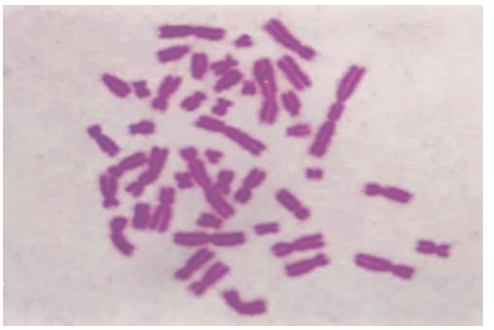
In case of accidental exposures to radiation, there may be a need to screen large populations for medical management. As a progress towards the alternative triage dosimeter, automated analysis of MN and the γH2AX assays were suggested[18]. Because H2AX foci formation was observed within seconds of induction of DNA breaks and the level of phosphorylation increases linearly with the amount of damage, it is now a widely accepted marker of DSBs[19,20].

3. CONVENTIONAL CYTOGENETIC TECHNIQUE TO QUANTIFY ACUTE RADIATION EXPOSURE

Measurement of DC in the blood lymphocytes of exposed individuals is considered as the "gold standard" in biodosimetry[5]. Though the dosimetry techniques are well established, measurement of radiation dose, using biological dosimetry requires very good standardisation. The dose-response curve obtained in one laboratory cannot be readily used for dose estimation in another because of variation in the yield, base-line aberration frequency, chemicals used, etc. Hence, the authors started constructing their own dose response curve towards the development of biodosimetry laboratory. The blood sample was irradiated with different doses (0.5 and 4.0 Gy) either to LINEAC X-ray (1.0 Gy/min) or to γ-radiation (0.75 Gy/min) using Co^{60} *in-vitro*. Irradiation was carried out at 37 °C and samples were maintained at the same temperature for an hour immediately after irradiation to enable repair of chromosome damage.

The blood samples thus irradiated were used for the preparation of metaphase chromosomes as described in IAEA report[5] with modifications for further analysis. About 1 ml of the irradiated sample was added into 80 per cent culture medium (RPMI-1640), supplemented with 20 per cent fetal calf serum, 200mM L-Glutamine, penicillin 100 units/ml and streptomycin 100 mg/ml. Then 200 µl of PHA-M and 10^{-7} M of methotrexate were added to the culture to initiate cell division as well as to arrest the cells at S-phase respectively and incubated at 37 °C for 66 h. Methotrexate block was released by adding excess thymidine (10^{-5} M) at the 66th h of culture as described by Yunis[21]. The cell synchronisation was carried out to increase the mitotic index as large number of cells needs to be analysed at low doses. Colcemid at a final concentration of 0.1µg per culture was added at the 67th h to block the cells at metaphase. These cells were harvested at the 72nd h, given hypotonic treatment, fixed, casted and used for further analysis. To score DC, the metaphase chromosomes were stained with 10 per cent Giemsa and the slides were mounted using DPX. These were then observed under the microscope and the numbers of DC (Fig. 1(a)) were recorded. The distribution of DC in cells was studied by the method described by Papworth and adopted by Savage[22]. A programme known as Poly fit, developed by NRPB, was used to construct the dose response curve[23] for *x*-irradiation (Fig. 2) with 95 per cent confidence limits and the values for α and β coefficients were found (Table 1).

The baseline DC frequency obtained in the study was 0.012 ± 0.0004, which is acceptable to the internationally published values[24,25]. The background chromosome aberration frequency is very important when radiation exposure to be determined is less than 25 cGy. The background frequency of chromosome aberrations is generally not available for comparison while determining accidental exposures. If the exposure is more than 25 cGy and the blood sample is collected soon after exposure, the non-availability of the individual's background chromosome aberration frequency may not pose a problem. On the contrary, if the exposure is lower than 25 cGy, then the reliable estimation of dose will depend heavily on the background frequency[1]. Thus it is necessary to establish the background chromosomal aberration frequency of individuals who work with radiation and radioactive sources. Similarly, α and β coefficients obtained is also comparable to that of reported values[25]. Further, to check the suitability of the dose estimation using DC, the DC were scored from the blood samples collected from workers occupationally exposed to radiation



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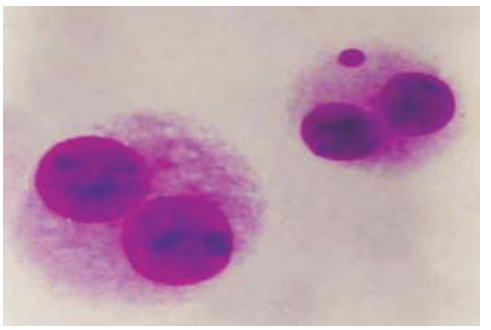

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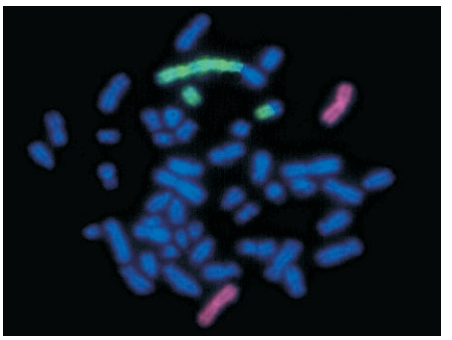

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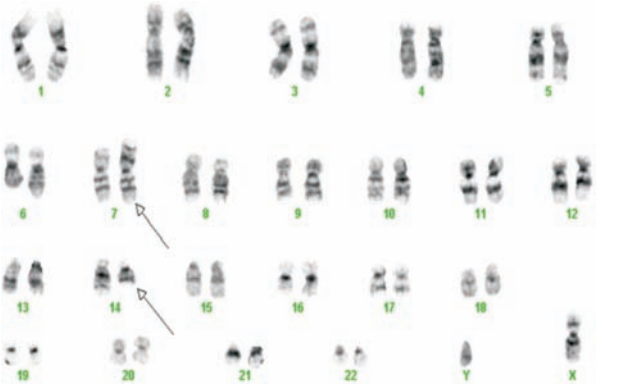

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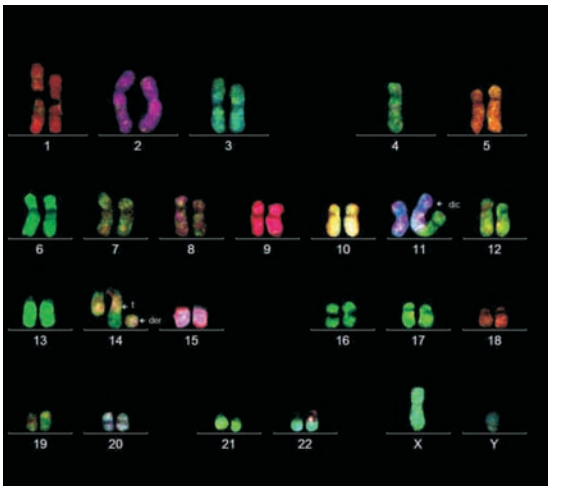

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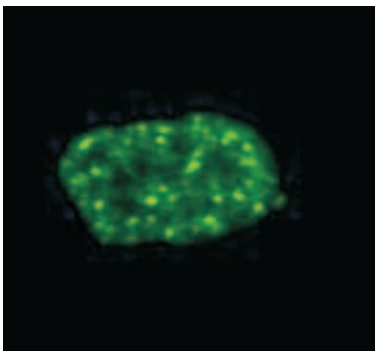

(f)

Figure 1. **Photographs of metaphase chromosomes of blood lymphocyte showing abnormalities after exposures to *in vitro* x-irradiation: (a) DC assay (Giemsa stained), (b) MN assay (Giemsa stained), (c) FISH–Reciprocal translocations, (d) GTG banded chromosomes [t(7:14) indicated by arrow], (e) Metaphase chromosomes after m-FISH [dic(4:11) indicated by arrow)], and (f) H2AX ASSAY.**

and cancer patients who had undergone radiotherapy. Dose estimated using the DC aberration frequency is comparable to physical dosimetry in occupational workers who have received a dose more than that of the minimum detection limit of the assay[26] (10cGy) and calculated equivalent whole-body dose in cancer patients[14].

It has been suggested that the distribution of DC would be helpful to differentiate the whole-body exposure from that of partial-body exposure[5]. Hence, the distribution of DC obtained with different doses was analysed using the Papworth test (Poisson distribution if the *u* value is between ± 1.96). The *u* value obtained at various doses after *in vitro* exposures between –0.11 and +1.31 suggest that *in vitro* exposures to radiation and the DC obtained in blood samples follow Poisson distribution. Similar distribution pattern has been reported for DC by other laboratories[24]. In the DC analysis of the blood samples obtained from cancer patients, where the distribution varies between –0.62 and +22.81, shows a majority of either a under-dispersion or over-dispersion, while *in vitro* exposures

follow Poisson[14]. Thus the results confirmed that the DC analysis would be used to quantify the radiation absorbed dose and nature of exposure. Universally, networking and automated scoring of DC for triage dosimetry are in progress[27].

4. RAPID MICRONUCLEUS ASSAY TO QUANTIFY ACUTE RADIATION EXPOSURE

Amongst existing cytogenetic techniques, while DC remains a widely used technique to estimate the amount of radiation exposure with a sensitivity of 10 cGy, the MN technique is easier to perform and can be rapidly analysed with a lower sensitivity of 25 cGy. MN is formed because of the non-inclusion of acentric fragments and lagging whole chromosome into the main nucleus during division[28]. The blood samples were irradiated as explained in DC analysis, cultured for a period of 72 h. Cytochalasin-B, at a final concentration of 3 µg/ml, was added to each sample at the 44th h to arrest the cells at cytokinesis stage and the cells were harvested after a further incubation of 28 h[6]. Then the slides were prepared, stained, and used to score the MN frequency. Cells with two daughter nuclei, surrounded by cytoplasm and cell membrane were scored for the presence of MN (Fig. 1(b)) according to the modified criteria[29]. The reference dose response curve for MN frequency was constructed from the blood samples exposed to x-irradiation (Fig. 3) and the coefficient are given in Table 1. Similar to DC, the MN dose response curve was used to quantify the absorbed dose in cancer patients and occupational workers[14,26]. While the dose estimation carried out in cancer patients using MN frequency showed a comparable calculated equivalent whole-body dose, in occupational workers, biological dose estimation was not possible due to its lower sensitivity. However, the culture methodology was also modified to increase the sensitivity of the assay as closer to 10 cGy similar to the sensitivity of DC[30]. Though the scoring of MN is easier and faster when compared

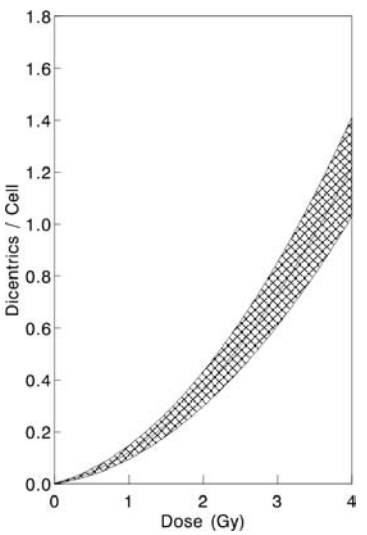

Figure 2. DC dose-response obtained from blood samples exposed *in vitro* to *x*-radiation at a dose-rate of 1.0 Gy/min (The shaded region shows 95 per cent confidence limit).

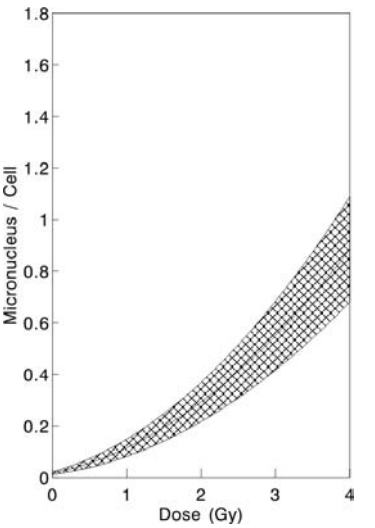

Figure 3. MN dose-response curve obtained with synchronised culture method for 60 Co gamma radiation (0.75 Gy/min). Shaded region indicates 95 per cent confidence limit.

to that of DC, the distribution of MN follows over-dispersion in whole- and partial- body exposures; it cannot be used to differentiate whole-and partial-body exposures.

5. FLUORESCENCE *IN SITU* HYBRIDISATION (FISH) TO QUANTIFY CUMULATIVE AND RETROSPECTIVE DOSE ESTIMATION

Both DC and MN are of unstable type aberrations and the cells carrying such aberrations are eliminated from the body as a function of time. RT, induced by radiation is of stable type aberration and has been shown to remain in circulation more or less permanently[1]. As it has been suggested that measurement of such TL may provide cumulative radiation exposure, the FISH technique were satndardised to score TL. This technique is based on the affinity among nucleotide bases in homologous sequences compared to non-homologous sequences. Using fluorescent labelled DNA probes, one can selectively paint a chromosome which can be seen easily under a fluorescent microscope. During hybridisation, the fluorescent-labelled DNA probes bind to its complementary strand which helps in the detection of rearrangement, if any, which has taken place in these labelled chromosomes. The chromosomes, which are not painted with fluorescent material, are stained with different colour. The fluorescent-labelled chromosome, if undergone TL, will exhibit a bicolour (Fig. 1(c)) one can easily identify.

The slide with metaphase chromosomes prepared, followed by irradiation as described in the conventional DC analysis, was used to measure RT using FISH. The FISH was performed with WCP 4 (Spectrum green) as well as WCP 1 and WCP 3 (Spectrum green and orange respectively) according to the manufacturer's protocol (Vysis, Abbott, USA). The slides were dehydrated in ethanol series (70 per cent, 85 per cent and 100 per cent) each for 2 min. About 10 µl of the probe mixture was applied to the slide, co-denaturation (75 °C for 2 minutes) and hybridisation (37 °C 24 h) was

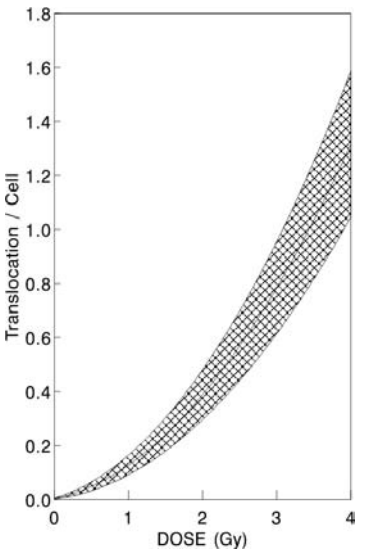

Figure 4. TL dose-response obtained from blood samples exposed *in vitro* to *x*-radiation at a dose-rate of 1.0 Gy/min (The shaded region shows 95 per cent confidence limit).

performed using the HYbrite. After completion of hybridisation, slides were washed to remove the unbound probe, counterstained with DAPI in antifade, observed under the fluorescent microscope and the aberrations were classified using PAINT nomenclature[31]. The genomic TL frequency was determined as described earlier[9] and a dose response curve was constructed.

To compare the TL detected by FISH with painting of chromosomes #1 and #3, the slides prepared for 2 and 4 Gy doses were further processed by GTG banding and m-FISH. For GTG banding, aged slides were exposed to trypsin (1mg/ml, 30 s), stained with 4 per cent giemsa. The metaphases were scored and karyotyped (Fig. 1(d)) using the applied imaging karyotyping system and the damages were classified as per ISCN nomenclature[32]. The m-FISH was carried out with Vysis probes for the entire genome as per manufacturer's instructions (Fig. 1(e)) with Spectral image system.

The background translocation frequency observed in the present study (0.0044/cell) is comparable to that reported by others[33-35] (0.002 to 0.008/ cell). The dose-response curve (Fig. 4) constructed using TL frequency for x-irradiation follow linear quadratic model as reported by others[2,9,16]. The distribution of TL frequencies observed in the present study indicates that this type of aberration follows Poisson distribution, similar to that of DC, indicating that measurement of TL frequency can also differentiate between whole- and partial-body exposures. The almost exact values of α and β coefficients obtained for translocation and DC dose-response (Table 1) indicate the induction of TL and DC is the same at a given dose of radiation[14]. The advantage with measuring TL frequency is that it being a stable type aberration, the exposure received during an accident can be measured even after a long duration[26]. *In-vitro* studies with blood lymphocytes[12], animal experiments[10] and in human population exposed accidentally[11] demonstrated the stability of TL.

Generally, the dose was estimated either by measuring the TL in entire genome using GTG banding[15] or FISH with a few painted chromosomes[16,17]. The concern in the TL scoring with FISH is that, extrapolation of genomic TL with a few painted chromosomes to the entire genome is based on assumptions[9], though literature shows evidence that radiation-induced TL are distributed randomly[36] as well as non-randomly[37]. Hence, the TL frequency was examined in the PBL exposed *in vitro* to γ-radiation, through WCP with two chromosomes, GTG banding as well as m-FISH to validate FISH with selective painting of a few chromosomes for biological dosimetry. Thus, the TL measured using both FISH techniques by painting only two chromosomes namely #1 and #3 and extrapolating it to the entire genome is comparable with GTG banding as well as m-FISH at selective doses (2 Gy and 4 Gy). TL measured by all the three methods did not show any significant difference when it was measured by any of the other methods (Fig. 5). Thus, the obtained result supports the assumption that radiation-induced exchange type aberrations are randomly distributed in the genome. In fact, chromosomal involvement in TL based on DNA content was seen in the descending order with larger chromosomes being more involved when compared to smaller chromosomes with minor variations[9].

6. γH2AX ASSAY–POSSIBLE TRIAGE BIODOSIMETRY

Markers based on the chromosomal abnormalities and /or gene mutations are suitable to quantify the residual damage but not the actual amount of damages induced due to exposure. Ample evidence has been generated that radiation exposures induced a wide spectrum of DNA damages. It has been generally assumed that the double strand breaks (DSB) are considered a lethal event as it can result in the formation of stable chromosomal aberrations and lead to late consequences like genomic instability and carcinogenesis. The H2AX, a variant of histone H2A, is

Table 1. Comparison of α, β coefficients obtained for translocation, DC and MN dose-response curves

End point	$\alpha \pm$ SE	$\beta \pm$ SE	C $\pm$ SE	α/β ratio
Translocation	0.0568 ± 0.0191	0.0678 ± 0.0053	0.0042 ± 0.0019	0.838
DC	0.0596 ± 0.0093	0.0612 ± 0.0037	0.0012 ± 0.0004	0.974
MN	0.0598 ± 0.0110	0.0392 ± 0.0036	0.0166 ± 0.0026	1.526

α: Alpha coefficient; β: Beta coefficient; C: Control value; SE: Standard error

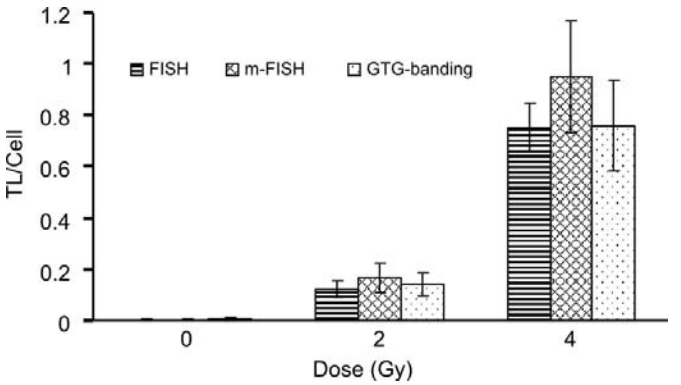

Figure 5. Comparison of TL obtained from blood samples exposed to x-irradiation and detected by WCP, GTG banding and m-FISH (*in vitro* exposure).

rapidly phosphorylated at Ser-139 (termed γH2AX) by members of the phosphatidyl-inositol 3-kinase family and upon DSBs induction, form foci at these sites[19] and promote recruitment of other DNA damage-response proteins[39]. Measuring the γ-H2AX foci after *in situ* immuno-fluorescence or flow cytometry have been in use to quantify the exact amount of double strand breaks produced by ionising and non-ionising radiations and to evaluate the repair kinetics[39].

The γ-H2AX foci were measured microscopically after *in situ* immuno-fluorescence (Fig. 1(f)) in blood lymphocytes exposed to bleomycin and diagnostic X-rays. The lymphocytes were isolated from the whole blood using Ficoll density gradient separator, washed with PBS, cell pellets were re-suspended in the medium and ~6.5 x10^3 cells were exposed to x-irradiation or bleomycin. The cells were then fixed by adding 2 ml of ice-cold methanol followed by 3 ml of 1 per cent BSA-PBST (37 °C for 30 min). Then 1 ml of primary antibody of H2AX (rabbit polyclonal I gG, 1 µg/ml) was added and incubated at 4 °C for 16 h in a humid chamber and the cells were washed with PBS. Finally, secondary antibody (goat polyclonal IgG with FITC conjugate, 2 µg/ml) was added, incubated at room temperature for 1 h in a dark area and cells washed with PBS and counterstained with DAPI were observed under fluorescent microscope using FITC filter. About 100 cells were scored randomly and the number of H2AX foci was recorded using image analysis system (Cytovision, Version 3.2 from Applied Imaging, UK) and the mean frequency of H2AX foci was calculated.

The baseline H2AX foci obtained in the peripheral blood lymphocytes was (0.19 ± 0.038) higher when compared to those of DC (0.001 ± 0.0007), MN (0.005 ± 0.002), and TL (0.0045 ± 0.002). The lower chromosomal aberration frequencies compared to those of H2AX foci in the control lymphocytes may be due to repair of some of the double strand breaks when the cells are stimulated to enter into cell cycle with mitogen where only the mis-repaired breaks appear as residual damages. Quantification of H2AX foci with flow cytometery is a choice and potential candidature for triage dosimetry, which is to screen huge populations exposed to ionising radiations. A detailed status of bidosimetry based on the cytogenetic techniques can be obtained from recent publications[40,41].

7. CONCLUSIONS

Scoring DC is a sensitive (10cGy) method to quantify radiation absorbed dose for acute exposure. Alternatively, MN is the choice for rapid dose estimation with sensitivity of 25cGy. When compared to the universally accepted and accurate method of detecting chromosome translocations, GTG -banding is labour-intensive and time-consuming, especially at low-dose exposures. The FISH method is much faster and demonstrates identical results when scaled to the full genome, providing a practical new biomarker for applications that require the scoring of large number of cells and individuals; however cost is a limiting factor. Flow cytometry analysis of γH2AX is an attractive choice as a triage dosimeter.

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REFERENCES

1. Bender, M.A.; Awa, A.A.; Brooks, A.L.; Evans, H.J.; Groer, P.G.; Littlefield, L.G.; Pereira, C.; Preston, R.J. & Wacholz, B.W. Current status of cytogenetic procedures to detect and quantify previous exposures to radiation. *Mutation Research,* 1988, **196**, 103-59.
2. Bauchinger, M.; Shmid, E. & Braselmann, H. Cytogenetic evaluation of occupational exposure to external γ-rays and internal ^{241}Am contamination. *Mutation Research,* 1997, **395**, 173-78.
3. Straume, T.; Lucas, J.N.; James, D.T.; Bigbee, W.L. & Langlois, R.G. Biodosimetry for a radiation worker using multiple assays. *Health Phy.,* 1992, **62**, 122-30.
4. Brown, J.M.; Evans, J.W. & Kovas, M.S. Mechanism of chromosome exchange formation in human fibroblasts: Insights from chromosome painting. *Environ. Mol. Mutagenesis,* 1993, **22,** 218-24.
5. IAEA Technical Report. Biological dosimetry, Chromosomal aberration analysis for dose assessment. 1986**, 260,** 1-69.
6. Fenech, M. & Morley, A.A. Measurement of micronuclei in lymphocytes. *Mutation Research,* 1985, **147**, 29-36.
7. Prosser, J.S.; Moquet, J.E.; Lloyd, D.C. & Edwards, A.A. Radiation induction of micronuclei in human lymphocytes. *Mutation Research,* 1988, **199**, 37-45.
8. Stephan, G. & Pressl, S. Chromosome aberrations in human lymphocytes analysed by fluorescence *in situ* hybridisation after *in vitro* irradiation and in radiation workers, 11 years after an accidental radiation exposure**.** *Int. J. Radiat. Biol.,* 1997, **71**, 293-99.
9. Lucas, J.N.; Poggensee, M. & Straume, T. The persistence of chromosome translocations in a radiation worker accidentally exposed to tritium. *Cytogenet. Cell Genet.,* 1992, **60**, 255-56.

10. Lucas, J.N.; Hill, F.S.; Burk, C.E.; Cox, A.B. & Straume, T. Stability of translocation frequency following whole body irradiation in rhesus monkeys. *Int. J. Radiat. Biol.,* 1996, **70**, 309-18.
11. Littlefield, L.G.; Travis, L.B.; Sayer, A.M.; Voelz, G.L.; Jensen, R.H. & Boice, J.D. Cumulative genetic damage in haematopoietic stem cells in a patient with a 40-year exposure to alpha particles emitted by thorium dioxide. *Radiation Research,* 1997, **148**, 135-44.
12. Natarajan, A.T.; Vyas, R.C.; Wiegant, J. & Curado, M.P. Cytogenetic follow-up study of the victims of radiation accident in Goiania (Brazil). *Mutation Research,* 1991, **247**, 103-11.
13. Lindholm, C.; Salomaa, S.; Tekkel, M.; Paile, W.; Koivistoinen, A.; Ilus, V. & Veidebaum, T. Biodosimetry after accidental radiation exposure by conventional chromosome analysis and FISH. *Int. J. Radiat. Biol.,* 1996, **70**, 647-56.
14. Venkatachalam, P.; Paul, S.F.D..; Mohan Kumar, M.N.; Prabhu, B.K.; Gajendiran, N. & Jeevanram, RK. Estimation of dose in cancer patients undergoing fractionated radiotherapy using TL, DC and MN frequency. *Mutation Research,* 1999, **429**: 1-12.
15. Ohtaki, K.; Shimba, H.; Awa, A.A. & Sofuni, T. Comparison of type and frequency of chromosome aberrations by conventional and G-staining methods in Hiroshima atomic bomb survivors. *J. Radiat. Res.,* 1982, **23**, 441-49.
16. Schmid, E.; Zitzelsberger, H.; Braselmann, H.; Gray, J.W. & Bauchinger, M. Radiation induced chromosome aberrations analysed by FISH with a triple combination of composite chromosome specific DNA probes. *Int. J. Radiat. Biol.,* 1992, **62**, 673-78.
17. Finnon, P.; Llyod, D.C. & Edwards, A.A. Fluorescence *in situ* hybridisation detection of chromosomal aberrations in human lymphocytes: applicability to biological dosimetry. *Int. J. Radiat. Biol.,* 1995, **68**, 429-35.
18. Garty, G.; Chen, Y.; Salerno, A.; Turner, H.; Zhang, J.; Lyulko, O.; Bertucci, A.; Xu, Y.; Wang, H.; Simaan, N.; Randers-Pehrson, G.; Yao, Y.L.; Amundson, S. & Brenner, D.J. The RABIT: a rapid biodosimetry tool for radiological triage. *Health Phys*., 2010, **98**, 207-17.
19. Rogakou, E.P.; Nieves, N.W.; Boon, C.; Pommier, Y. & Bonner, W.M. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *Bio Chem.,* 2000, **275**, 9390-395.
20. Campisi, J. & Fagagna, F. Cellular senescence: when bad things happen to good cells. *Nat. Rev. Mol. Cell Bio.,* 2007, **8**, 729-40.
21. Yunis, J.J. High resolution of human chromosomes. *Science.,* 1976, **191**, 1268-270.
22. Savage J.R.K. Curve fitting by maximum likelihood method*, Radiat. Bot.,* 1975, **15**, 127-31.
23. Edwards, A.A. & Dennis, J.A. Polyfit-A computer program for fitting a specified degree of polynomial to data points. *NRPB-M11,* 1990, 1-18.
24. Lloyd, D.C.; Edwards, A.A. & Prosser, J.S. Chromosome aberrations induced in human lymphocytes by *in vitro* acute x and α-radiation. *Radiat. Prot. Dosim.,* 1986, **15,** 83-88.
25. Paul, S.F.D.; Venkatachalam, P. & Jeevanram R.K. Biological indicators for radiation absorbed dose: A Review**.** *IGC.,* 1996**,** 172.
26. Venkatachalam, P.; Paul, S.F.D.; Mohan Kumar, M.N.; Prabhu, B.K.; Gajendiran, N.; & Jeevanram, R.K. Comparison of chronic exposures received by radiation workers using different biological end-points with the doses recorded by TLD. *Australasian Radiol.,* **2002**, 45, 464-71.
27. Wilkins, R,C.; Romm, H.; Kao, T.; Awa, A.A.; Yoshida, M.A.; Livingston,, G.K.; Jenkins, M.S.; Oestreicher, u.; Pellmar, T.C & and Prasann, G.S.; Interlaboratory comparison of the DC assay for radiation biodosimetry in mass casualty events. *Radiation Research,* 2008, **169**, 551-60.
28. Fenech, M. & Morley, A.A. Cytokinesis block MN method in human lymphocytes: effect of *in vivo* ageing and low dose x-irradiation. *Mutation Research,* 1986, **161**, 193-201.
29. IAEA technical report: Cytogenetic analysis for radiation dose assessment–technical reports series, 2001: **405** IAEA, Vienna.
30. Solomon F.D. Paul, Venkatachalam, P. & Jeevanram, R.K. A comparative study of synchronised and conventional culture method on micronuclei dose-response curve. *Mutation Research,* **1997,** 391, 91-98.
31. Tucker, J.D.; Morgan, W.F.; Awa, A.A.; Bachinger, M.; Blakey, D.; Cornforth, M.N.; Littlefield, L.G.; Natarjan, A.T. & Shasserre, C. A proposed system for scoring structural aberrations detected by chromosome painting. Cytogenet. *Cell Genet.,* 1995, **68**, 211-21.
32. ISCN, An internationals system for human cytogenetic nomenclature, edited by L.G. Shaffer and N. Tommerup; S. Karger, Basel, 2005, 1-127.
33. Tucker, J.D. & Sneft, R. Analysis of naturally occurring and radiation induced breakpoint locations in human chromosomes 1, 2 and 4**.** *Radiation Research,* 1994, **140**, 31-36.
34. Tucker, J.D.; Ramsey, M.J.; Briner, J.F.; Olsen, L. & Moore, D.H. On the frequency of chromosome exchanges in a control population as measured by chromosome painting. *Mutation Research,* 1994, **313**, 193-202.
35. Van Diemen, P.C.M.; Massdam, D.; Vermeulen, S.; Darroudi, F. & Natarajan, A.T. Influence of smoking habits on the frequencies of structural and numerical chromosomal aberrations in human peripheral blood lymphocytes using fluorescence *in situ* hybridisation (FISH) technique. *Mutagenesis***,** 1996, **10**, 487-95.
36. Garcia-Sagredo, J.M.; Vallcorba, I.; Lopez-Yarto, A.; Sanchez-Hombre, M.D.C.; Resino, M. & Ferro, M.T. Chromosome painting in biological dosimetry: Assessment

of the ability to score stable chromosome aberrations using different pairs of paint probes. *Environ. Health Pres.,* 1996, **104** (3), 475-77.

37. Knehr, H.; Zitzelsberger, H.; Braselman, H. & Bauchinger, M. Analysis for DNA-proportional distribution of radiation-induced chromosome aberrations in various triple combination of human chromosomes using fluorescence *in situ* hybridisation. *Int. J. Radiat. Biol.,* 1994, **65**, 683-90.
38. Van-Bull, P.P.W.; Zandman, I.M.; Natarajan, A.T. & Boei, J.J.W.A. Non-random chromosomal involvement in radiation induced translocations in mouse spermatogonial stem cells. *Mutagenesis,* 1996, **11**, 391-93.
39. Oscar, F.C.; Alicia, L.; Michel, N. & Andre, N. H2AX: the histone guardian of the genome. *DNA Repair,* 2004, **3**, 959-67.
40. de Lemos Pinto M.M,; Santos, N.F & Amaral, A. Current status of biodosimetry based on standard cytogenetic methods. *Radiat. Environ. Biophys*, 2010, **49**, 567-81.
41. Vinnikov, V.A,; Ainsbury, E,A,; Maznyk, N.A,; Lloyd DC & Rothkamm K, Limitations associated with analysis of cytogenetic data for biological dosimetry. *Radiation Research.* 2010, **174**, 403-14.

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