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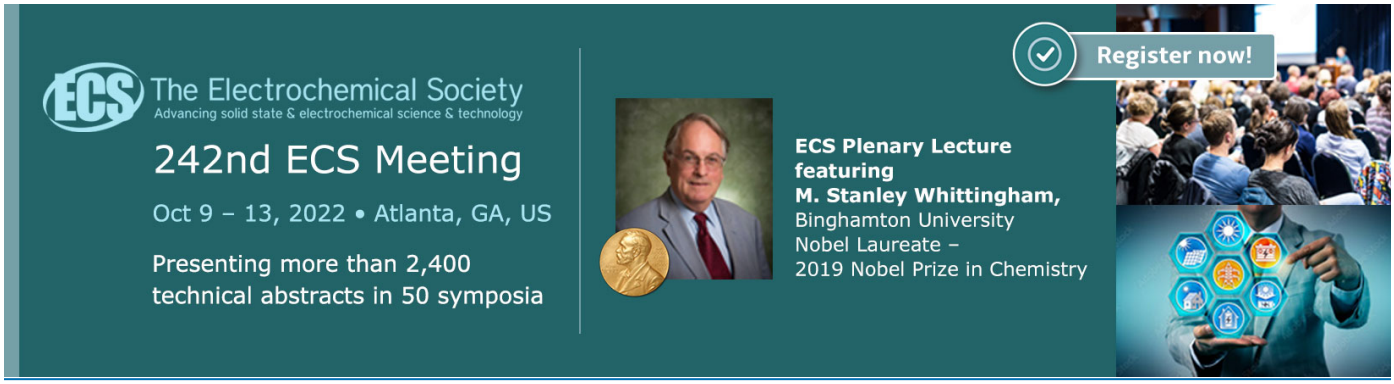

Dose Response Curve Using Chemically Induced PCC Assay in Peripheral Cells Irradiated In-vitro to 5 MeV Alpha Particles Emitted From Radon Source

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Abstract. Bio-dosimetry is defined as estimation of dose received by individuals based on biological end points induced by ionizing radiation. By using cytogenetic assay, it is possible to quantify the absorbed dose. High linear energy transfer (LET) radiation produces dense ionizations along their path and causes more clustered DNA damage compared to low LET. Radon is a natural radioactive element present in the atmosphere mainly produced during decay of uranium. The main objective of the present study is to construct dose response curve by using Premature Chromosome Condensation (PCC) techniques in blood cells exposed in-vitro to radon exposure. Blood samples were drawn from healthy non-smokers (n=4), aged between 28 to 42 years and exposed to twenty doses of radon ranged between 0 to 5.5 mGy. A total of about 24000 metaphase spreads were counted to find the DNA damages include ring chromosomes (RC), dicentric chromosomes (DC) and acentric fragments (AF). As the dose increases gradual increase was observed in RC, DC and AF with the slope of 0.001, 0.005 and 0.064 respectively. So far, no calibration reference curve was generated for low dose radon using chemically induced PCC assay and hence this study is considered as first of its kind.

1. Introduction

Bio-dosimetry is defined as estimation of dose received by individuals using biological end points induced by ionizing radiation. High linear energy transfer (LET) radiation produces dense ionizations along its path and causes more clustered DNA damage. The energy produced by high LET radiation is more effective per unit dose when compared with the low LET in causing cellular damage. Radon (High LET radiation) is a natural radioactive element present in the atmosphere mainly produced during decay of uranium. When radon is inhaled for many years, it causes lung cancer.

In order to identify and to estimate the radiation effects on DNA damage, cytogenetic biomarkers showed to be accurate methods [1]. The chromosome damage caused by radon was examined in-vitro by using cytogenetic assays and also it is possible to quantify the absorbed dose.

There are various cytogenetic techniques includes dicentric assay, micronuclei, translocation using Fluorescence In-Situ Hybridization analysis (FISH) and Premature Chromosome Condensation (PCC) analysis [2]. Among these techniques, dicentric chromosomes are considered as one of the most important indicators of radiation exposure and strongly correlate with absorbed dose. But the disadvantage of the assay is it does not suit for higher dose as the metaphase cells available for scoring is very less at higher dose.

Techniques that cause chromatin/chromosome to condense prior to mitosis by adding chemical solution is termed as Premature Chromosome Condensation (PCC) analysis. This analysis is regarded as a substitute to DC analysis. It is possible to estimate low and high doses of ionizing radiation using the above mentioned technique.

Though the PCC technique was introduced during 1975, it was not popular because the efficiency of the PCC assay was highly dependent on virus activity and there was a risk of infection while handling the virus during experiments [3]. Subsequently chemical induced fusion - PCC protocol with polyethylene glycol as cell fusion substance was presented by Pantelias and Maillie [4]. However, this method was observed to be more challenging, since this method necessitates substantial amount of mitotic arrested inducer cells.

To overcome these limitations, drugs such as Cantharidin or Catharidic acid, sodium Metavanadate, 2-amino purine, Staurosporine and serine/threonine are used as protein inhibitors and Calyculin A induced PCC technique was introduced [5]. Moreover, IAEA has suggested chemically induced PCC technique as one of the standard protocols for cytogenetic dosimetry [2]. IAEA has given recommendation to generate calibration reference curve for each technique by every cytogenetic bio-dosimetry laboratory.

The objective of this study is to generate calibration reference curve with PCC techniques in blood cells exposed in-vitro to radon exposure. This study is considered as first of its kind to generate calibration reference curve for low dose radon exposure using chemically induced PCC assay.

2. Material and Methods

2.1. Sample collection

The study has been approved by the Institutional Ethics Committee (IEC-NI/18/JAN/63/01). After obtaining informed consent, peripheral cells were obtained from healthy persons (n=4), aged between 28 to 42 years and irradiated to twenty doses of radon ranged between 0 to 5.5 mGy by means of easy, transferable irradiation set up available at SQ&RMG, IGCAR.

2.2. Irradiation set up

Blood samples were irradiated by using the irradiation setup established at SQ&RMG of IGCAR. Three-way cock was used to evacuate air from the bottle which contain blood sample and to inject radon. Blood bottle was connected to one end of the three-way cock using a needle, second end was connected to a 60-ml syringe and third end was connected to a radium source or a vacuum pump. Blood sample was irradiated with 60 ml of radon gas. Lucas cell was used to measure the radon activity. Lucas cell was irradiated with 60 ml of radon gas and after 3 h, radon activity was measured using alpha counter.

3. Result

Concentration of radon gas present in Lucas cell was counted using alpha counter and calculated the radon dose for each experiment. A total of about 24000 metaphase spreads were counted to find the DNA damages include ring chromosomes (RC), dicentric chromosomes (DC) and acentric fragments (AF). Rings include both acentric and centric rings. As the radon dose increases, gradual increase in RC, DC and AF were found in calyculin induced PCC analysis.

Table 1 shows the percentage of cells in different phases of cell cycle such as G1, S, M, M/A and PCC index for radon exposed sample after 48 hours of incubation. Radon is known to induce considerable DNA damage in human cells. Initially most of the cells are in G1 and S phase. Because of DNA damage, cell cycle check points are activated which prevents damaged cells enter into another phase. After DNA repair mechanisms (24 to 48 hours), cells gradually move to M and M/A phase which indicates cell cycle progression.

Figure 1, 2 and 3 provides the dose response curve for PCC rings, dicentrics and acentric fragments in radon exposed lymphocytes. Figure 4 shows dose response curve for total aberrations.

Table 1. Percentages of PCC cells at different phases of cell cycle and PCC index.

Dose	Cells scored	G1%	S%	M%	M/A%	PCC Index
Control	4000	2.4	4.2	42	15	63
0.2	1000	3	2.9	33	20	58.9
0.3	1000	2.1	1.5	30	23	56.6
0.5	1000	2.8	2.2	28	23	56
0.8	1000	2	1.7	28	22	53.7
1.3	1000	2.2	1.6	24	28	55.8
1.8	1000	2.1	1.8	22	27	52.9
2	1000	2	1.7	23	28	54.7
2.4	1000	2.3	2	23	28	55.3
2.8	1000	2.8	2.1	24	29	58
3	1000	2.9	2	24	25	53.9
3.1	1000	3.2	2.8	23	26	55
3.3	1000	3.1	2.7	22	28	55.8
3.7	1000	3	1.7	21	31	56.7
3.8	1000	3	1.9	22	30	57
4	1000	2.7	1.8	23	30	57.5
4.3	1000	2.3	1.4	19	29	51.7
4.6	1000	2.4	1.5	18	29	50.9
4.8	1000	2.5	1.8	20	32	56.3
5	1000	2.4	2	18	31	53.4
5.5	1000	1.8	1.6	17	33	53.4

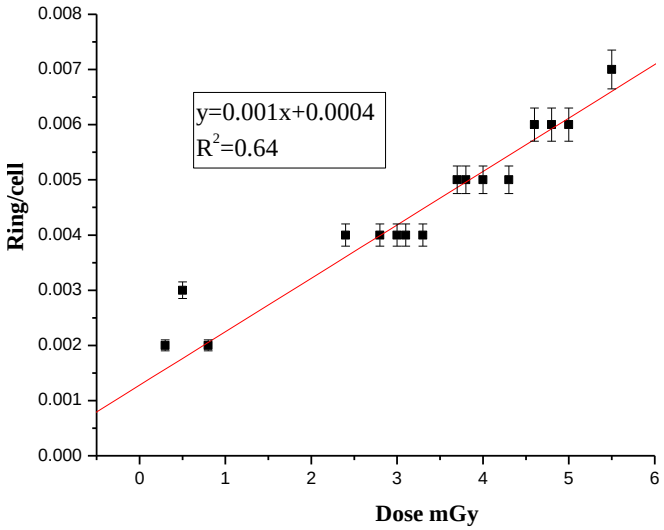

Figure 1. Calibration curve for PCC ring in peripheral cells irradiated in-vitro to radon.

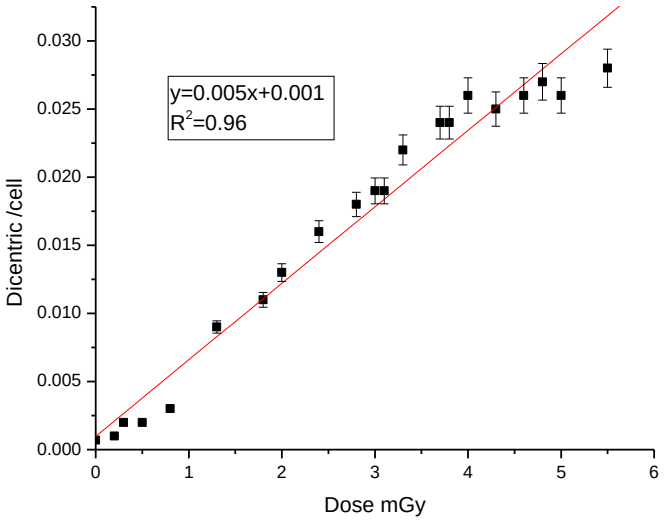

Figure 2. Calibration curve for PCC dicentric in peripheral cells irradiated in-vitro to radon.

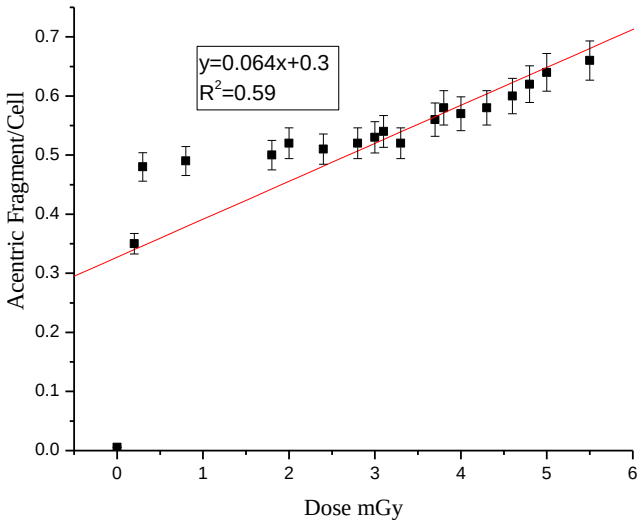

Figure 3. Calibration curve for PCC acentric fragment in peripheral cells irradiated in-vitro to radon.

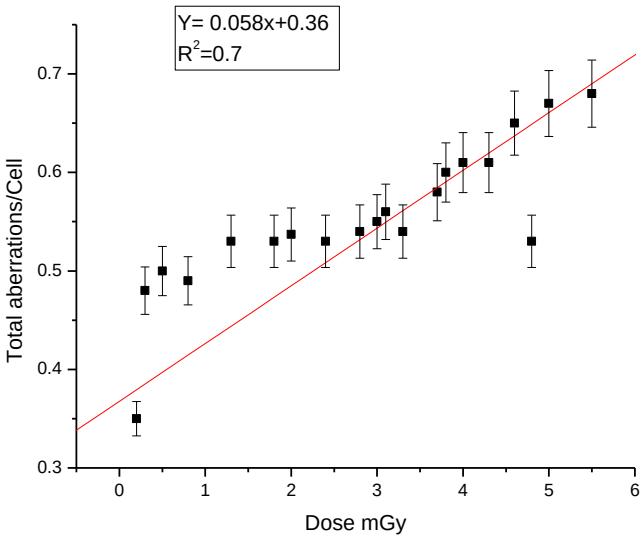

Figure 4. Calibration curve for PCC total aberrations in peripheral cells irradiated in-vitro to radon

4. Discussion

Calyculin induced PCC assay is found to be very simple and reliable assay to study the chromosome aberrations and also to estimate the radiation dose received by individuals [6]. When compared with the dicentric assay, PCC assay can be used to estimate the higher doses owing to the advantage of chromosome condensation before the cells reaching to metaphase which avoids interphase cell death. Hence in PCC assay, more numbers of lymphocytes are present for scoring even at higher doses; led to the reliable dose estimation during accidental scenarios. PCC analysis was used for dose calculation in three sternly exposed workers in Tokaimura nuclear accident [7]. This study became popular as this was the first to use the PCC analysis in real accidental conditions. This assay also used to find the high dose received by victims during medical treatment.

There are many reports on dose response curve for low LET radiation [8-10] and for high-LET radiation using PCC assay [8, 11] and none of them for alpha particles. However, there is one recent report about the dose response for α-particles with the maximum dose of 2.5 Gy [12]. But till date no report exists for dose response curve of radon using PCC technique. Hence the present study was carried out to assess the DNA damage induced by low dose radon exposures and also to construct the dose response curve by using PCC technique.

Present study confirms increase in aberrations such as DC, RC and AF even with very low doses of radon (5.5 mGy). Induction of rings in blood cells were identified to be at much lower frequency compared to that of dicentrics, which makes scoring of rings as a realistic end point for high dose exposure [2]. There are published reports to indicate the frequency of rings is less compared to other aberrations [13]. Similar observation was found in the present study. Lower frequency of rings with a slope of 0.001 was observed in the present study when compared with other aberrations. Linear dose dependent increase in dicentrics was found in this study with a slope of 0.005; indicates that the induction of rings is 20 % that of dicentrics. Alternatively, inductions of dicentrics are 5-fold higher than that of rings.

When the ‘u’ value is within ±1.96, signify that the aberrations follow a Poisson distribution [14]. It is known that the high LET radiations show over dispersion of dicentrics [15]. In the present study, over dispersion of dicentrics was observed beyond 4.6 mGy. Puig et al [12] showed an initial increase in dicentric distribution with over dispersion for 0.05 Gy, 0.1 Gy and there was decline till 2.5 Gy. Many other authors had similar observation in the over dispersion [13, 16]. Puig et al [12] observed over dispersion in dicentric frequency for the entire dose range since the minimum dose was 50 mGy.

Linear dose dependent increase in acentric fragments was detected in this study with the slope of 0.064. Frequency of acentric fragments confirms over dispersion. Similar observation for acentric fragments was obtained by Puig et al [12].

Individual chromosomes at different phases of cell cycle can be distinguished by PCC assay. The present study shows reduction in percentage of M-phase PCC cells and increase in M/A cells as the dose increases. From table-1 it can be inferred that 51-63% of lymphocytes showed clearly condensed chromosomes. Hosseini et al [17] observed 30-60% and 39-60% of clearly condensed chromosomes respectively.

Though there are published reports for the applicability of PCC assay for high dose exposures, the present study confirms the usefulness of PCC technique for low dose exposures. Since the induction of dicentrics is 5-fold higher than that of rings, dicentric assay is widely used for low dose exposure and PCC assay is used for high dose exposure.

5. Conclusion

The present study reveals that significant chromosomal aberrations (DC, AF and RC) can be induced by very low doses of radon. Linear dose dependent increase in rings and dicentrics found in this study clearly indicates that the PCC analysis can be effectively used to estimate the dose even for very low doses of radon exposure.

References

[1] Bilban M and Jackopin CB 2005 *Mutagenesis.* **20(3)** 187-191
[2] IAEA 2011 Technical Reports International Atomic Energy Agency Vienna, Austria.
[3] Rao PN and Johnson RT 1972 *Mutat. Res.* **448** 35-45
[4] Pantelias GE and Maillie HD 1983 *Somatic. Cell. Genet.* **9**5 33-547
[5] Gotoh E, Asakawa Y and Kosaka H 1995 *Biomedical. Res.* **16** 63-68
[6] KandaR, Hayata I and Lloyd DC 1999 *Int. J. Radiat. Biol.* **75(4)** 441-446
[7] Hayata I, Kanda R, Minamihisamatsu M, Furukawa M and Sasaki S 2001 *Radiat. Res.* **42** S149-155
[8] Lamadrid AI, Garcıa O, Delbos M, Voisin P and Roy L 2007 *J. Radiat. Res.* **48** 1-6
[9] Balakrishnan S, Shirsath K, Bhat N and Anjaria K 2010 *Mutat. Res.* **699** 1-2
[10] Puig R, Barrios L, Pujol M, Caballın M R and Barquinero JF 2013 *Mutat. Res.* **75** 71-77
[11] Lee R, Nasonova E, Hartel C, Durante M and Ritter S 2011 *Radiat. Environ. Biophys* **50** 371-381
[12] Puig R, Pujol M, Barrios L, Caballín MR and Barquinero JF 2016 *Int. J. Radiat. Biol.* **92(9)** 493-501
[13] Barquinero JF, Stephan G and Schmid E 2004 *Int. J. Radiat. Biol*. **80(2)** 155-164
[14] Papworth DG 1970 *Curr. Top. Radiat. Res.* **6** 129-194
[15] IAEA Technical Reports Series No. 405. 47. 2001 International Atomic Energy Agency, Vienna.
[16] Schmid and Roos 2009 *Radiat. Environ. Biophys.* **48 (2)** 181-187
[17] Hosseini S, Mozdarani H 2004 *Iranian. J. Radiat. Res.* **2(1)** 15-20