

Linear Dose Response of Acrocentric Chromosome Associations (Aca) To Gamma Irradiation In Human Lymphocytes

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Abstract

Purpose: The frequency of acrocentric chromosome associations (ACA) was studied to determine the possible dose-response relation with low doses of gamma irradiation in lymphocytes.

Methods: Peripheral blood collected from three healthy donors were irradiated with 0, 0.1, 0.25, 0.5, 0.75, and 1 Gy gamma radiation. Chromosomal preparations were made after 48 hrs culture as per the standard guidelines.

Results: The average number of ACA and ACA % were increased significantly with an increase in a dose. The D-G and D-D type of association was most prominent and showed a dose-dependent increase. The ACA frequency in irradiated lymphocytes showed an increase concerning the dose. The fitted regression equation was $y=0.4759x+0.1663$ ($R^2=0.9635$; $p=0.0005$). An assessment of dicentric chromosomes (DC) was carried for the same slides. The correlation curve was prepared for ACA frequencies versus DC frequencies, resulting in a regression equation as $y=8.659x+0.237$ ($R^2=0.8275$; $p=0.0119$).

Conclusion: Our results showed an increase in frequencies of ACA in irradiated lymphocytes with an increase in radiation dose and followed a similar linear trend with DC frequency, thus, ACA may serve as a candidate cytogenetic biomarker for radiation biodosimetry especially for low radiation doses.

Introduction

Acrocentric chromosome associations (ACA) have been widely studied for their incidence in diverse health conditions like Down's syndrome [1-2], mental retardation [3], reproductive disorders [4-5], aging [6], smallpox vaccine [7], asthma [8], Alzheimer's disease [9] and oral squamous cell carcinoma [10]. The epidemiological findings suggest that low radiation exposure can be a possible cause of non-disjunction in humans; however, there are minimal scientific studies to provide strong experimental evidence for this observation. It was reported that exposure to a low dose of radiation produces mitotic non-disjunction in human lymphocytes. The authors also observed that the abnormal segregation was not only induced in irradiated cells but also in cells that were incubated with irradiated cell-free plasma or serum, and chromosomes 21 and X were found to be most susceptible to non-disjunction [11]. Later, it has been suggested that radiation could cause an increase of satellite associations through the aging phenomenon [12].

Ionizing radiations are responsible for the induction of DNA double-strand breaks (dsb) and consequently, chromosomal aberrations are produced. It has been reported that inter-satellite fibers or connectives of short arms in acrocentric chromosomes consist of nucleoprotein B, and it was identified as DNA complementary to rRNA i.e. ribosomal DNA [13]. Recently, it was revealed linkages between rDNA on heterologous chromosomes through super-resolution microscopy and suggested that the dynamic DNA loci contribute to corporal inter chromosomal networks that are an essential and a persistent character of genome organization [14]. The chromosomal associations involving 2-5 acrocentric chromosomes were noticed in Uranium miners [15]. Significantly higher values of ACA and maximum occurrence of D-G type associations were reported in occupationally exposed workers and spaceflight astronauts [16-18]. Chromosome aberrations (CA) and micronuclei (MN) frequencies are the most popular cytogenetic parameters utilized for estimation of absorbed dose in case of radiation accidents [19-20]. The dicentric chromosome assay (DCA) was initially designed in 1962 and is now considered as a "gold standard" of radiation biodosimetry due to its high specificity and being neutral of gender, age, or region [21-22]. However, it is necessary to take note that the DCA technique needs well-trained manpower and is time-consuming [22-24].

The ACA represents early chromosomal instability/damage, merits attention, and was advocated to be considered as an indicator of chronic ionizing irradiation especially to low doses [17-18]. Therefore, looking at its significance as a

candidate cytogenetic biomarker for radiation biodosimetry, the present study was designed for assessing the frequency of ACA in human metaphases induced by gamma irradiation and construction of dose-response curve.

Methods And Materials

Sample Collection:

The whole blood obtained from healthy donors was used to study variations in frequencies of ACA and DC concerning radiation doses through chromosome preparation. The study was approved by the Institutional ethical committee (IEC/38/Research/17). The scope and significance of the study were explained before sample collection to each donor and informed consent was obtained. The donors were 32-55 years old with no history of chronic disease, viral infection within 6 months, or exposure to toxic chemicals or radiation. The heparinized vacutainer tubes were used to collect approximately 10 ml peripheral blood by venipuncture from each donor.

Irradiation:

Blood samples were aliquoted into six cryo vials with 1 ml each in a biosafety cabinet. The samples were then exposed to a ^{60}Co γ - rays Teletherapy unit (Model Bhabhatron II). They were irradiated with 0 (sham irradiation), 0.1, 0.25, 0.50, 0.75 and 1.0 Gy, with dose rate of 0.38 Gy /min, SSD of 120 cm, and field size 20 x 20 cm². Immediately after irradiation, all samples were brought back to the culture laboratory and kept at 37°C for 2 hours. This duration allowed DNA repair as per the standard practice in all biodosimetry laboratories for dicentric chromosomal and cytokinesis blocked micronuclei assays [22].

Culture set up:

After 2 hrs, lymphocyte cultures were initiated using the established standard method [22-23]. Briefly, the cultures were set up by pouring 8 ml culture medium RPMI 1640 (GIBCO) in a T25 flask, 100 μ l antibiotic Penstrep (GIBCO) was added to this medium. It was then supplemented with 2 ml fetal bovine serum (GIBCO, final concentration 20% v/v) and mixed gently. 1 ml of whole blood was inoculated into the medium and mixed, immediately followed by the addition of 400 μ l of phytohaemagglutinin (PHA-M, GIBCO), and then incubated at 37°C for 48 hrs. After 24 hrs, 200 μ l of colchicine (Sigma) with a final concentration of 0.02 μ g/ml to arrest metaphases was added and re-incubated at 37°C for another 24 hrs. Following centrifugation, the pellet was suspended in 0.075 M KCl and kept for 25 min. at 37°C (hypotonic treatment), again centrifuged and fixed in chilled Carnoy's fixative (1 part acetic acid: 3 part methanol). Aliquots of each cell suspension were made and stored at 4°C. The cell suspension was dropped onto pre-cleaned, chilled slides, and air-dried. The staining of slides was performed with 4 % Giemsa stain solution (pH 6.8 phosphate buffer) for microscopic observations.

Scoring of slides:

ACA Scoring- The coded slides were scored for the occurrence of ACA following the criterion of Zang and Black [25]. Briefly, the chromosome associations were considered:

1. If the distance between centromeres of the chromosomes was less than the length of the long arm of the largest G chromosome.
2. Longer distance was accepted when short arms were attached through visible threads.
3. If the associating chromosomes were heading towards each other with longitudinal congregation then a larger distance was accepted (up to the length of the long arm of the largest D chromosome).

DC Scoring- The standard dicentric chromosomal assay (DCA) guidelines of the International Atomic Energy Agency (IAEA) were followed [22].

1. A complete metaphase with 46 chromosomes, all with a single centromere.
 2. If metaphase contained unstable aberrations (dicentric) and was accompanied by an acentric fragment, yet the total count remains 46.
 3. Every acentric is not linked with a dicentric or centric ring that increased the chromosome count past 46.
 4. Each tricentric corresponds to two dicentrics, accompanied by two acentric/fragments.
- Minimum 100 metaphases for ACA and 500 metaphases for DC, from each dose level, were analyzed under X1000 using oil immersion. The scored data was entered in score sheets. After completion of scoring, all the slides were decoded, and a dose-response curve was constructed.

Statistical analysis:

For statistical analysis of results, the Student's "t" test was applied; the dose-response curve was fitted by linear regression analysis using GraphPad software (version 8). Dose versus yield with standard error for ACA and DC were calculated by using Dose Estimate v5.3.

Results

The occurrence, combination/types, and association/cell (%) in human lymphocytes irradiated with gamma radiation were presented in Table 1 and Table 2. The average number of ACA and ACA % was observed to be significantly higher in all exposed groups in a dose-dependent manner. The D-G type of association was most prominent in all the doses studied and showed a dose-dependent increase, followed by the D-D type. However, other types of ACA like G-G, 2D-G, 2G-D, 2G-2D, 3D, and 3G were not consistent and the difference observed was non-significant (Table 1, Fig. 1 and Fig. 3). The total number of associations and ACA frequency in metaphases of human lymphocytes irradiated with 0, 0.1, 0.25, 0.5, 0.75, and 1.0 Gy of gamma radiation also showed an increase with increasing dose (Table 2). A linear fit model was followed for the dose-response effect between ACA frequency and radiation dose. The fitted regression equation was $y=0.4759x+0.1663$ ($R^2=0.9635$; $p=0.0005$), where y is the ACA frequency induced by radiation dose x in Gy (Fig. 4A).

Table 1
Dose versus yield (frequencies) for ACA and DC in metaphases of gamma irradiated lymphocytes.

Dose (Gy)	ACA				DC			
	Total Metaphases Scored	Total Number of ACA	Yield (ACA Frequency)	S.E.	Total scored metaphases	Total Number of DC	Yield (DC Frequency)	S.E.
0	540	62	0.115	0.015	2000	1	0.001	0.001
0.1	580	127	0.219	0.019	2020	12	0.006	0.002
0.25	580	200	0.345	0.024	2020	20	0.01	0.002
0.5	580	237	0.409	0.027	1540	31	0.02	0.004
0.75	580	302	0.521	0.030	2320	56	0.024	0.003
1.0	620	388	0.626	0.032	2040	114	0.056	0.005

Dose versus yield with standard error for ACA and DC calculated by using Dose Estimate v5.3.

Table 2
Variations in ACA and DC pattern observed in metaphases of gamma irradiated lymphocytes.

Sr. No.	Group	ACA			DC		
		Average scored metaphases	Average number of ACA	Associations per cell (%)	Average scored metaphases	Average number of DC	DC per cell (%)
1	0 Gy	180.00±11.55	20.66±0.88	11.60±1.07	666.67±166.67	0.33±0.33	0.0003±0.0003
2	0.1 Gy	193.33±6.67	42.33±2.19 (p=0.0008)	21.87±0.40 (p=0.0008)	673.33±163.44	4.00±1.00 p=0.02	0.60±0.20 p=0.04
3	0.25 Gy	193.33±6.67	66.67±3.28 (p=0.0002)	34.48±1.15 (p=0.0001)	673.33±163.44	6.67±1.45 p=0.01	1.00±0.20 p=0.007
4	0.5 Gy	193.33±6.67	79.00±5.57 (p=0.0005)	41.03±3.63 (p=0.001)	513.33±6.67	10.33±0.67 p=0.0002	2.00±0.20 p=0.0006
5	0.75 Gy	193.33±6.67	100.67±4.91 (p<0.0001)	52.03±1.24 (p<0.0001)	773.00±139.20	18.67±2.60 p=0.002	2.50±0.30 p=0.001
6	1.0 Gy	206.67±17.64	129.33±19.55 (p=0.005)	61.94±5.14 (p=0.0007)	680.00±160	38.00±11.50 p=0.03	5.43±0.33 p<0.0001
<p>Each value represents Mean ± SEM (n=3). The paired Student's "t" test was used to make statistical comparison. Radiation exposed Groups were compared with the unexposed group (0 Gy).</p>							

A parallel but independent assessment of dicentric chromosomes was also carried for the same slides. An average number of DC and DC per cell (%) were observed to be significantly higher in all exposed groups in a dose-dependent manner (Table 2, Fig. 2). The total number of DC and DC frequency in metaphases of human lymphocytes irradiated with 0, 0.1, 0.25, 0.5, 0.75, and 1.0 Gy gamma radiation showed an increase with increasing dose. The dose-response effect between DC frequency and radiation dose, followed a linear fit model up to radiation dose 0.75 Gy, afterward, it follows the linear-quadratic model. The fitted regression equation was $y=0.0480x-0.0013$ ($R^2=0.8880$; $p=0.0049$), where y is the DC frequency induced by radiation dose x in Gy (Fig. 4B). The correlation curve was prepared for ACA frequencies (Y-axis) versus DC frequencies (X-axis) in human lymphocytes (Fig. 4C), resulting in a regression equation as $y=8.659x+0.237$ ($R^2=0.8275$; $p=0.0119$).

Discussion

Various structural chromosomal alterations are induced by exposure to ionizing radiation, mainly due to telomere shortening and DNA double-strand breaks (dsb) that ultimately produce dicentric chromosomes, micronuclei, nucleoplasmic bridges as well as chromosome end fusion. Although the incidence of associations between short arms of acrocentric chromosomes has been reported as satellite associations long ago [26-28], recent cytogenetic literature has a minuscule number of publications in this aspect concerning biodosimetry.

In the present study, average ACA, and ACA % showed a significant increase with the increasing dose of radiation. The D-G and D-D type of associations were majorly recorded in all doses studied and showed a dose-dependent increase, but other types of ACA were poorly consistent. In a cytogenetic study of uranium miners from the Western Carpathians, it was observed a wide array of chromosomal aberrations, the most frequent being chromosomal associations involving 2-5 acrocentric chromosomes [15]. The associations between chromosomes 13, 14, 15, and 22 with triradial formations

were prominent. Yadav and Seth [16] showed significantly higher values of ACA, further maximum occurrence of D-G type associations was noted while the 3-D type was minimum in workers who were occupationally exposed to X-rays. Around 2.5 fold increases in the frequency of ACA in occupationally exposed hospital workers was recorded [17]. Recently, a significantly higher satellite association was observed in astronauts who had participated in a spaceflight [18].

In the present study, the total number and frequency of ACA in metaphases of irradiated human lymphocytes with 0, 0.1, 0.25, 0.5, 0.75, and 1.0 Gy of gamma radiation showed an increase with increasing dose. The dose-response effect between ACA frequency and radiation dose followed a linear fit model. Only a few studies are available on the dose-effect relationship between ACA and radiation exposure. For the first time, the effect of low dose X-irradiation on acrocentric chromosome satellite associations was studied and observed that irradiation may influence the composition of the satellite association complexes, however, results were inconsistent [29]. In another study, an increase in acrocentric association with increase of radiation dose was reported for 0-1 Gy radiation exposure, however, exposure to 1-4 Gy showed an inverse relation with dose [30].

A “gold standard” of radiation biodosimetry i.e. dicentric chromosome assay (DCA) [22] was used successfully for dose assessment in incidents like Chernobyl, Goiania, and Fukushima [31-33]. However, the DCA technique needs well-trained manpower and is time-consuming which has proved to be the main hindrance in its application [22-24]. In the case of MN, the analysis is influenced by various factors and induced by several agents, thus not radiation specific [34-37]. It is also important to record that dicentric aberrations are hardly induced by low radiation doses thus putting an additional constraint on the popular DCA method [38-41]. The difficulty in estimating dose-response curves for low dose range was expressed and was concluded that DCA as well as translocation analysis after gamma irradiation particularly less than a 0.1 or 0.05 Gy was devoid of accuracy and showed poor dose-response [42]. However, accuracy could be improved by analyzing more than 5000 cells for the low dose range [43], which might be impractical in case of a radiation disaster situation.

In the recent years, developments are in progress for complete automation of DCA for radiation emergencies through proper validation and optimization, by the combined use of image processing and machine learning techniques [44-47]. The latest study by Alsbeigh et al., [48], highlighted the observation that DC is underestimated in accidental radiation exposures with doses of less than 1 Gy, wherein lies an impending risk of developing late stochastic effects [49]. Since it is a fact that physical inter-chromosomal connections in ACA are the first to break during genomic instability [14], the parameter captured our attention and the results of the present analysis are indicative that ACA can serve as a precise and sensitive tool for generating biodosimetry curves, especially for low dose ionizing radiation exposure. Further investigations are warranted for multicentric studies and inter-laboratory comparisons for its validation as a sensitive, prospective cytogenetic biodosimetric marker. The combined use of improved image processing techniques and machine learning may also be considered for ACA scoring for dosimetry purposes.

Conclusion

Our results showed an increase in frequencies of ACA in irradiated lymphocytes with an increase in radiation dose and followed a similar linear trend with DC frequency, thus, ACA may serve as a candidate cytogenetic biomarker for radiation biodosimetry especially for low radiation doses. Also, ACA has advantages of scoring over DC in terms of the total number of cells thus saving time and can be further developed as a tool for triage biodosimetry.

Declarations

Conflict of Interest: None.

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Author Contributions: RMS and NKC have designed the study. RMS and PG generated, analyzed the data, and wrote the manuscript. All authors have reviewed and approved the final version of the manuscript.

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Figures

Figure 1

Metaphase spreads showing different types of ACA in gamma irradiated lymphocytes. (a) Normal metaphase (b) D-D type ACA (c) D-G type ACA (d) G-G type ACA (e) 2D-G type ACA (f) 2G-D type ACA (g) 2D-2G type ACA (h) 3D type ACA (i) 3G type ACA

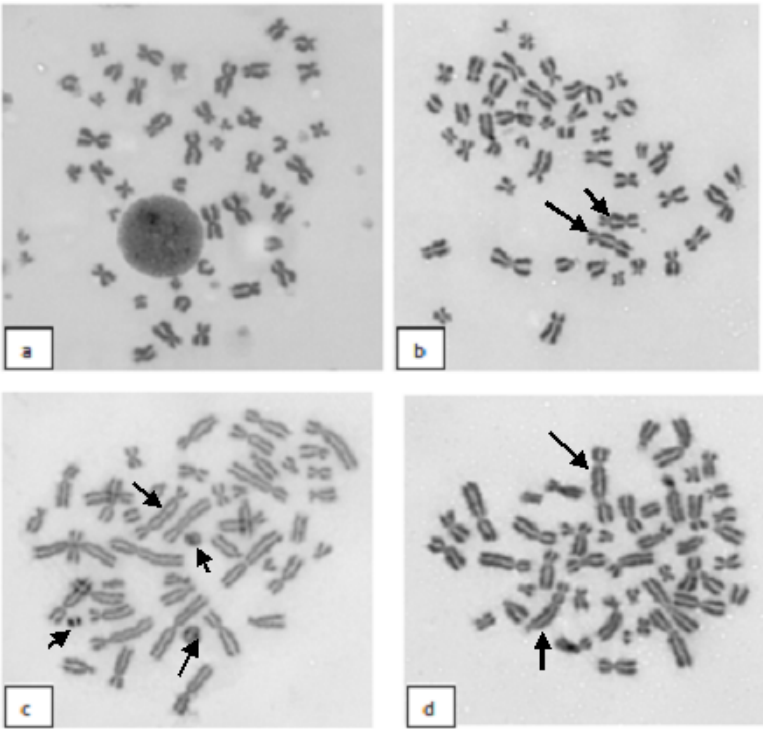


Figure 2

Metaphase spreads showing normal, dicentrics, ring, and tricentric chromosomes in gamma irradiated lymphocytes. (a) Normal metaphase, (b) dicentric chromosome, (c) dicentric, acentric, and ring chromosome (d) tricentric chromosomes

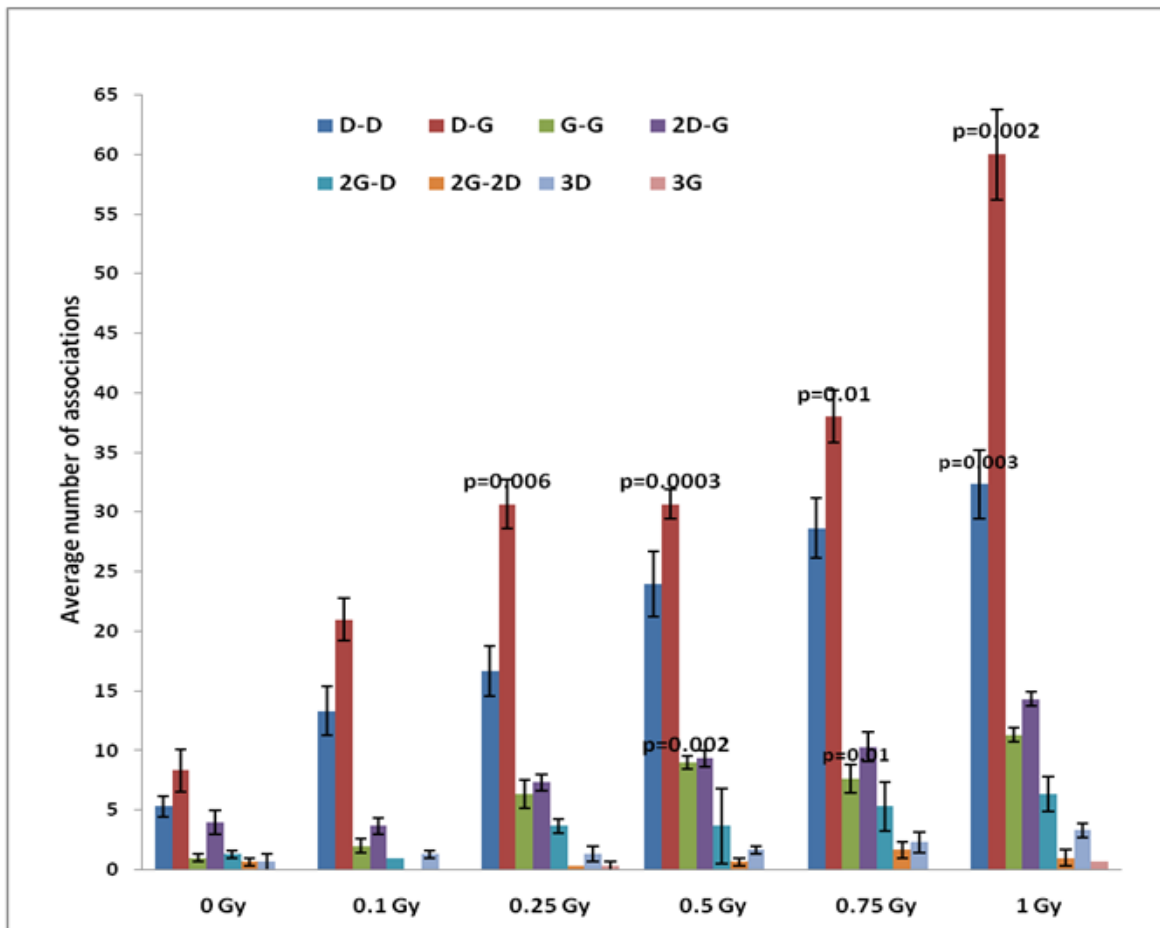


Figure 3

Average number of various types of ACA observed in metaphases of human lymphocytes exposed to different doses of gamma radiation. Each value represents Mean \pm SEM (n=3). The paired Student's "t" test was used to make statistical comparison. Radiation exposed Groups were compared with the unexposed group.

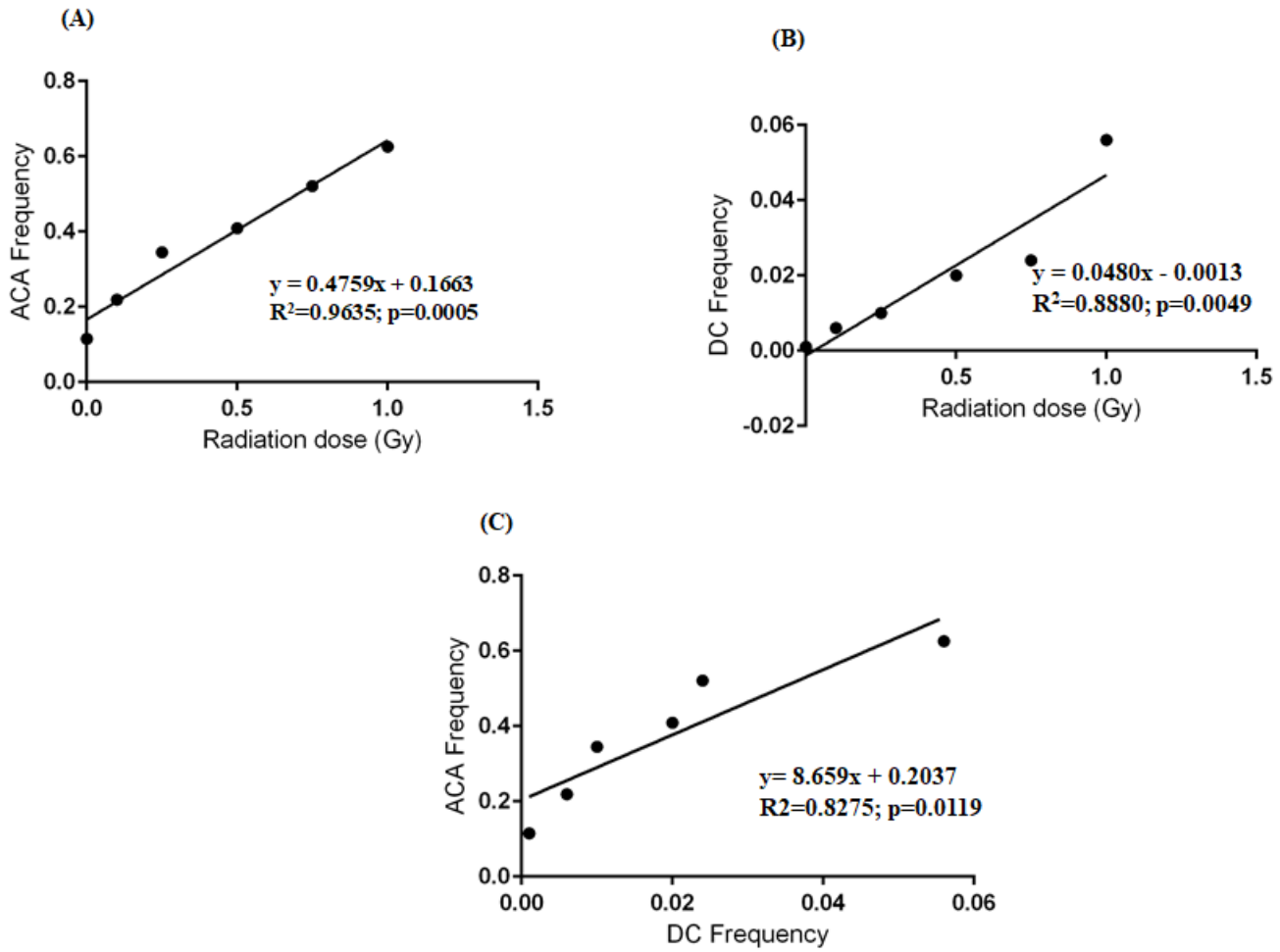


Figure 4

(A) Dose-response curve of ACA frequencies, (B) Dose-response curve of DC frequencies and (C) correlation curve of ACA frequencies versus DC frequencies in human lymphocytes.