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The cytokinesis-blocked micronucleus assay: Dose estimation and inter-individual differences in the response to γ-radiation

A.C. Antunes, V. Martins, J. Cardoso, L. Santos, O. Monteiro Gil*

IST/ITN – Instituto Superior Técnico, Universidade Técnica de Lisboa, Estrada Nacional 10, ao km 139,7, 2695-066 Bobadela LRS, Portugal

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ABSTRACT

Biological dosimetry plays an important role in case of a radiation accident or incident, either when it is the only way to estimate the dose or when it is used to complement physical dosimetry. A cytogenetic study was conducted in a group of 16 Portuguese individuals by use of the cytokinesis-blocked micronucleus (CBMN) assay. A dose–response curve for micronuclei yield was established with a linear-quadratic model: $Y = (0.0122 \pm 0.0010) + (0.0241 \pm 0.0023)D + (0.0193 \pm 0.0007)D^2$. Also, baseline values for the micronucleus formation in the 16 donors were analyzed, with results in close agreement with those from other laboratories. A validation experiment was carried out with three individuals. The real and the estimated doses obtained with the dose–response curve were in very good agreement, allowing the use of the micronucleus dose–response calibration curve in biological dosimetry for estimation of radiation dose in case of overexposure.

The results obtained for the cytogenetic endpoints, studied in the same group of 16 individuals, were also analyzed as a function of age and gender. A higher inter-variability was observed for the higher dose points and differences in response were identified between genders, above 2 Gy, for all endpoints.

1. Introduction

The widespread use of ionizing radiation for medical, industrial, research and other purposes increases the risk of accidental human exposure. Around 400 episodes of overexposure involving high acute radiation doses have been formally reported by the International Atomic Energy Agency (IAEA) [1,2]. In fact, according to UNSCEAR [3], the use of ionizing radiation in medical applications, such as diagnostics and radiotherapy, has seen a remarkable increase in the last decades. Also, the occurrence of large-scale radiological accidents, or terrorist attacks with ionizing radiation have become a realistic concern [1,4].

Biological dosimetry plays an important role in case of radiation accidents or incidents either when it is the only way to estimate the dose or when it is used to complement physical dosimetry [5,6]. Among the radiation-induced chromosomal aberrations (CA), dicentric chromosomes and micronuclei are the most useful endpoints for quantitative analysis in cases of radiological accidents [7]. Analysis of dicentric chromosomes in peripheral blood lymphocytes is considered the 'gold standard' technique for biological dosimetry [8] because of the very low background level of dicentrics in the healthy general population, and the specificity of the response to ionizing radiation [9–11]. However, this analysis is time-consuming and requires highly skilled scorers to evaluate the samples [2,5]. The cytokinesis-blocked micronucleus (CBMN) assay, developed by Fenech and Morley in 1985 [12], is an alternative method to evaluate the biological damage induced by ionizing radiation [13–17]; it allows quantification of both chromosome breakage and loss [18,19]. The CBMN assay has the advantage, over the CA assay, of a more rapid evaluation of samples, not requiring highly trained scorers [5]. The uncertainty on the dose assessment is not significantly different between the dicentrics and micronuclei for radiation doses exceeding 1 Gy [15]. The main disadvantage of micronucleus formation is the lack of specificity for ionizing radiation, since it is known that its frequency is affected by other factors such as age, gender, diet and exposure to chemical agents [20]. Despite this, in the last years the CBMN assay has become a thoroughly validated and standardized technique to evaluate exposure to ionizing radiation [19,21,22] at doses above 0.2–0.3 Gy of X- or γ-rays [6,17,23]. Moreover, several studies have been conducted to validate the use of the CBMN assay for triage in cases of mass casualty events, where a large number of individuals are involved. For this purpose, the approaches identified are based in the automation of the assay and in the reduction of the number of cells scored [2,24,25]. According to McNamee et al. [5], scoring of only 200 binucleated cells per subject is sufficient to detect radiation doses above 1 Gy.

The use of CBMN assay in biological dosimetry needs an appropriate dose–response curve. As there are inter-laboratory differences in the dose–response of micronucleus formation due to

* Corresponding author. Tel.: +351 219946344.
E-mail address: octavia.gil@ctn.ist.utl.pt (O. Monteiro Gil).

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the use of different protocols and scoring criteria, any laboratory that intends to carry out biological dosimetry should determine its own *in vitro* calibration curve [6]. In this context, an *in vitro* dose–response curve has been established at our Institute (IST/ITN) for the CBMN assay.

Additionally, a particularly interesting feature of bio-dosimetry has been not only to obtain estimates of the absorbed dose with adequate calibration curves, but also to find a way to demonstrate inter-individual radiosensitivity [26,27] and a possible correlation with age and gender. The influence of age and gender in baseline micronucleus frequencies has been reported in several studies [28–30]. Women are known to have higher baseline micronucleus frequencies than men. In an international study involving nearly 7000 subjects, the baseline micronucleus frequency in women was 19% higher than in men [30,31]. Aging has also been positively correlated with an increase in the frequency of micronuclei [29,30].

2. Materials and methods

2.1. Subjects

To establish the dose–response curve for the CBMN assay, 16 healthy non-smoking individuals without previous exposure to ionizing radiation for at least the last six months, were studied. All donors explicitly agreed to participate in this study and answered a questionnaire about demographic data, smoking habits, exposure to ionizing radiation, intake of drugs and diet. Peripheral blood samples were taken from each donor by venipuncture. The subjects, ranging in age from 20 to 59 years, were divided in two age groups, from 20 to 39 and 40 to 59 years, each one consisting of 4 men and 4 women. To validate the dose–response curve, three further individuals were selected, one woman and two men aged 31 (donor A), 42 (donor B) and 48 (donor C) years, respectively. Donor B is an exposed radiation worker (cumulative dose of 3.02 mSv between 1992 and 2012), while the other two had no previous history of radiation exposure.

2.2. Irradiation conditions

The blood samples were irradiated *in vitro* at the Metrology Laboratory of Ionizing Radiation at IST/ITN. Samples were placed at a distance of 80 cm from the ^{60}Co radiation source and were irradiated at room temperature with an AECL ELDORADO 6 irradiator in a square radiation field of 8 cm × 8 cm. Dose rates ranged from 157 mGy/min to 126 mGy/min, due to the decay of the ^{60}Co source. For each donor, a non-irradiated control was included and six different doses were studied: 0.25, 0.50, 0.75, 1.00, 2.00 and 3.00 Gy. Also, a dose of 5 Gy was given to blood samples of 12 individuals. The blood samples of donors A, B and C were used to validate the calibration curve and were irradiated with 1.75 Gy.

2.3. Cytogenetic analysis

The CBMN assay was performed according to the method described in Monteiro Gil et al. [32]. Briefly, aliquots of heparinized peripheral blood were cultured in RPMI-1640 supplemented with fetal bovine serum, antibiotics (Sigma, St. Louis, MO) and heparin (B Braun, Queluz de Baixo, Portugal). Lymphocytes were stimulated with phytohaemagglutinin (Gibco, Grand Island, NY) and incubated for 72 h at 37 ± 1 °C. Cytochalasin B (Sigma, St. Louis, MO) was added after 44 h of culture. Cells were harvested by centrifugation, washed twice and submitted to a mild hypotonic treatment. Centrifuged cells were placed on dry slides and smears were prepared. Slides were fixed with cold methanol/acetic acid (3:1) and stained with Giemsa 4%.

For each individual, a total of 1000 bi-nucleated cells per dose were analyzed in terms of micronucleus frequency, according to the criteria of the IAEA [6]. For the validation samples 2000 binucleated cells were scored per individual. The nuclear division index (NDI) was calculated according to the formula: $NDI = (M_1 + 2M_2 + 3M_3 + 4M_4)/N$, where M_1–M_4 represents the number of lymphocytes with 1–4 nuclei and N is the total number of viable cells scored [6,33].

2.4. Dose estimation and statistical analysis

The NDI standard error (SE) was calculated as described by the IAEA [6]. To analyze if micronucleus formation followed a Poisson distribution, the dispersion index (σ^2/y) and the normalized unit of this index (u) were used [34]. Dose–response calibration curves were constructed with the Chromosome Aberration Calculation Software (CABAS, v.2) [35] and the Dose Estimate software, v.5.1 [36]. The corresponding 95% confidence intervals were calculated according to Merkle's simplified approach as described in IAEA [6] and in Szłuińska et al. [37]. The goodness-of-fit and the chi-squared test for homogeneity were performed with the Dose Estimate software [36]. Doses given to the three validation samples were estimated with the Dose Estimate software [36] and the 95% confidence limits include the combined Poisson and calibration-curve errors. Statistical analysis was also performed with the paired Student's t-test, the one-way analysis of variance (ANOVA) and the Spearman's test.

3. Results

The data for micronucleus formation obtained from 16 healthy donors were pooled to construct a calibration curve for the CBMN assay. Prior to the construction of the calibration curve, the chi-squared test for homogeneity was applied to all doses. For the pooled micronucleus data, homogeneity between donors was verified only for the zero dose ($p > 0.05$). Also, dose–response curves were constructed for men and women and for the 20–39 and 40–59 age groups, separately. The same test was conducted to analyze homogeneity between donors when the data were split by age and gender. For both males and females, homogeneity was determined only for 0 Gy. For both age groups homogeneity was found in the 0, 0.25 and 0.50 Gy dose groups and for the older group (40–59 years) homogeneity between donors occurred also at 1 Gy. Despite the inhomogeneity found at most of the radiation doses, results obtained for the 16 healthy donors were pooled to construct the calibration curves.

In a total of 124,000 bi-nucleated cells analyzed, 16,231 micronuclei were found and a statistically significant dose-dependent increase in micronucleus formation was observed ($p < 0.01$). To determine the accuracy of the dose estimation from the established calibration curves for micronucleus formation we conducted an *in vitro* irradiation of peripheral blood simulating whole-body exposure at 1.75 Gy. After blind scoring 2000 binucleated cells for donors A, B and C, doses were estimated by referring the micronucleus frequency to the calibration curves. Table 1 presents the number of cells scored, the micronucleus frequencies and distributions after irradiation with γ-rays at different doses, both for the pooled data and for the validation samples. The NDI (±SE), the u-value and the dispersion index are also indicated. The distribution of micronuclei was tested for Poisson distribution using the u-test [34] and revealed over-dispersion in all doses, with u-values between 6.23 and 11.16. Only two of the validation samples (donors A and B) were consistent with Poisson, with the u-value between ±1.96. The dispersion-index values are close to 1, ranging from 1.069 and 1.125 for the pooled data, showing

Table 1
Number of micronuclei and their distribution in binucleated human lymphocytes of blood samples exposed *in vitro* to different doses of ^{60}Co.

	Cells scored	Mn	Distribution of Mn in binucleated cells						NDI ± SE	*u*	σ^2/y
			0	1	2	3	4	5			
Data from 16 donors											
Dose (Gy)											
0.00	16,000	196	15,812	180	8	0	0	0	1.78 ± 0.20	6.23	1.069
0.25	16,000	327	15,693	289	16	2	0	0	1.79 ± 0.18	10.23	1.114
0.50	16,000	435	15,595	378	24	3	0	0	1.79 ± 0.18	11.16	1.125
0.75	16,000	656	15,392	562	45	0	1	0	1.77 ± 0.17	10.25	1.115
1.00	16,000	879	15,178	765	57	0	0	0	1.75 ± 0.16	6.70	1.075
2.00	16,000	2302	13,968	1791	213	27	1	0	1.73 ± 0.16	10.45	1.117
3.00	16,000	4041	12,556	2902	491	48	2	1	1.64 ± 0.16	6.50	1.073
5.00	12,000	7395	6789	3454	1398	300	50	9	1.50 ± 0.13	8.58	1.111
Validation samples											
Donor A	2000	230	1788	195	16	1	0	0	1.84 ± 0.14	1.61	1.051
Donor B	2000	235	1785	195	20	0	0	0	1.53 ± 0.14	1.69	1.053
Donor C	2000	221	1805	171	22	2	0	0	1.85 ± 0.16	4.55	1.143

Mn – micronuclei.
NDI – nuclear division index.
SE – standard error.
σ^2/y – dispersion index.

Table 2
Coefficient values and goodness of fit parameters calculated with Dose Estimate software, for the pooled data and related to age and gender.

	$c \pm$ SE	$\alpha \pm$ SE (Gy^{-1})	$\beta \pm$ SE (Gy^{-2})	χ^2	*df*	*p*
Total	0.0122 ± 0.0010	0.0241 ± 0.0023	0.0193 ± 0.0007	8.6	5	0.127
Men	0.0108 ± 0.0020	0.0266 ± 0.0049	0.0203 ± 0.0014	19.4	5	0.002
Women	0.0136 ± 0.0013	0.0224 ± 0.0028	0.0177 ± 0.0008	6.1	5	0.295
20–39 years	0.0103 ± 0.0019	0.0174 ± 0.0044	0.0209 ± 0.0012	17.5	5	0.004
40–59 years	0.0139 ± 0.0011	0.0313 ± 0.0027	0.0174 ± 0.0008	4.5	5	0.485

SE – standard error.
df – degrees of freedom.

no variation with dose. An analysis of variance study (ANOVA) was performed and a significant decrease of the pooled NDI values with increasing radiation doses was observed ($p < 0.01$). Also, a significant difference of NDI between genders was identified ($p < 0.01$) with men having higher NDI values (data not shown). Moreover, a correlation between NDI and micronucleus frequency was observed in the Spearman's rank-order test on the data of the 16 donors ($p < 0.01$) and also when the values were grouped by age ($p < 0.05$) and gender ($p < 0.01$). In addition, the data obtained at the individual donor level were analyzed, and a correlation between NDI and micronucleus formation ($p < 0.05$) was found for 5 of the 16 donors.

The fitted coefficients of the constructed dose–response curves are shown in Table 2, taking into account the pooled data and the data split by age and gender. The coefficients were calculated with CABAS and Dose Estimate softwares, which yielded the same results. Differences appeared only in the SE values, since the Dose Estimate software corrects these for under- and over-dispersion. The resultant fitted curve for micronucleus formation, obtained with Dose Estimate considering the data from the 16 donors is $Y_{Mn} = (0.0122 \pm 0.0010) + (0.0241 \pm 0.0023)D + (0.0193 \pm 0.0007)D^2$, ($\chi^2 = 8.6$, degrees of freedom = 5, $p = 0.127$) and shown in Fig. 1, with the corresponding 95% confidence intervals adjusted according to IAEA recommendations [6]. Dose estimates for the validation samples were obtained with the dose–response curve of the pooled data and the gender- and age-specific calibration curves (Table 3). The values for the dose estimates ranged from 1.623 ± 0.107 Gy to 1.860 ± 0.108 Gy. The real dose fell within the 95% confidence interval of the estimated dose for all donors and for all calibration curves.

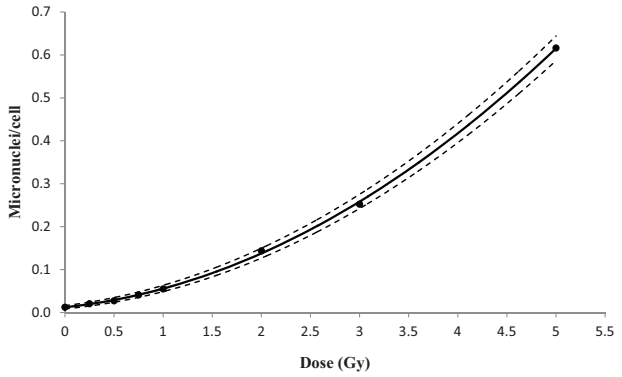

Fig. 1. Dose–response curve for γ-rays using the CBMN assay. Coefficient fitted values of the linear quadratic function $Y = c + \alpha D + \beta D^2$ are $c = 0.0122 \pm 0.0010$; $\alpha = 0.0241 \pm 0.0023$ and $\beta = 0.0193 \pm 0.0007$. Observed frequencies of micronuclei are represented by ●. Solid line represents the fitted dose–response curve. 95% confidence intervals are represented by dashed lines.

Table 3
Dose estimates for the validation study.

Donor	Curve	Estimated dose (Gy) ± SE	95% dose confidence interval (Gy)
A	Total	1.767 ± 0.093	1.584–1.949
	Women	1.843 ± 0.106	1.636–2.050
	20–39 years	1.860 ± 0.108	1.649–2.071
B	Total	1.793 ± 0.093	1.611–1.976
	Men	1.729 ± 0.122	1.489–1.969
	40–59 years	1.701 ± 0.107	1.491–1.911
C	Total	1.717 ± 0.093	1.535–1.899
	Men	1.656 ± 0.122	1.417–1.894
	40–59 years	1.623 ± 0.107	1.413–1.832

SE – standard error.

Table 4
Variation with dose of different cytogenetic endpoints according to age and gender.

Dose (Gy)	Total (Mean ± SD)	Women (Mean ± SD)	Men (Mean ± SD)	20–39 years (Mean ± SD)	40–59 years (Mean ± SD)
Micronuclei/binucleated cell					
0.00	0.012 ± 0.004	0.013 ± 0.003	0.012 ± 0.004	0.011 ± 0.003	0.013 ± 0.004
0.25	0.020 ± 0.007	0.021 ± 0.008	0.020 ± 0.006	0.015 ± 0.003	0.026 ± 0.006
0.50	0.027 ± 0.009	0.028 ± 0.009	0.026 ± 0.010	0.020 ± 0.005	0.034 ± 0.006
0.75	0.041 ± 0.013	0.045 ± 0.014	0.037 ± 0.012	0.037 ± 0.012	0.045 ± 0.013
1.00	0.055 ± 0.012	0.053 ± 0.012	0.057 ± 0.012	0.050 ± 0.014	0.060 ± 0.007
2.00	0.144 ± 0.034	0.128 ± 0.030	0.160 ± 0.031	0.139 ± 0.030	0.148 ± 0.039
3.00	0.253 ± 0.073	0.234 ± 0.052	0.272 ± 0.089	0.239 ± 0.044	0.266 ± 0.095
5.00	0.616 ± 0.099[a]	0.577 ± 0.124[b]	0.645 ± 0.075[c]	0.624 ± 0.101[d]	0.602 ± 0.111[e]
Chromosomal aberrations/cell					
0.00	0.038 ± 0.021	0.034 ± 0.013	0.043 ± 0.027	0.036 ± 0.022	0.041 ± 0.022
0.25	0.055 ± 0.024	0.061 ± 0.028	0.048 ± 0.020	0.047 ± 0.012	0.063 ± 0.031
0.50	0.098 ± 0.047	0.085 ± 0.048	0.110 ± 0.046	0.100 ± 0.062	0.095 ± 0.032
0.75	0.164 ± 0.051	0.179 ± 0.051	0.149 ± 0.051	0.180 ± 0.053	0.149 ± 0.048
1.00	0.239 ± 0.045	0.232 ± 0.026	0.246 ± 0.059	0.246 ± 0.043	0.231 ± 0.048
2.00	0.732 ± 0.070	0.749 ± 0.089	0.715 ± 0.042	0.714 ± 0.057	0.750 ± 0.081
3.00	1.534 ± 0.277	1.724 ± 0.171	1.344 ± 0.229	1.558 ± 0.291	1.511 ± 0.280
Dicentric chromosomes/cell					
0.00	0.001 ± 0.002	0.001 ± 0.002	0.001 ± 0.002	0.001 ± 0.002	0.001 ± 0.002
0.25	0.008 ± 0.007	0.009 ± 0.007	0.008 ± 0.006	0.006 ± 0.004	0.010 ± 0.008
0.50	0.017 ± 0.014	0.015 ± 0.013	0.019 ± 0.017	0.019 ± 0.018	0.015 ± 0.011
0.75	0.036 ± 0.017	0.041 ± 0.022	0.030 ± 0.005	0.038 ± 0.020	0.033 ± 0.013
1.00	0.058 ± 0.019	0.055 ± 0.015	0.061 ± 0.024	0.058 ± 0.020	0.058 ± 0.020
2.00	0.210 ± 0.032	0.221 ± 0.030	0.198 ± 0.032	0.201 ± 0.030	0.219 ± 0.033
3.00	0.455 ± 0.098	0.514 ± 0.085	0.395 ± 0.071	0.472 ± 0.112	0.438 ± 0.085

SD – standard deviation.
[a] 12 individuals.
[b] 5 individuals.
[c] 7 individuals.
[d] 8 individuals.
[e] 4 individuals.

Table 4 presents the results for micronuclei, CA and dicentrics of the 16 individuals studied grouped by age and gender. The results of CA assay were from a previous study conducted in the same group of individuals [38]. For all groups, the results for the three endpoints analyzed, from 0 Gy to 3 Gy, are the average results for eight individuals. For the results of 'number of micronuclei/bi-nucleated cell', the number of individuals in the 5-Gy dose group varies depending on the different groups analyzed. Analysis of variance was performed. Women showed higher values of dicentrics/cell and chromosomal aberrations/cell than men, for 2 and 3 Gy. For the 3-Gy dose the difference between men and women is statistically significant ($p < 0.01$) for both endpoints. No variation with age was seen. Concerning the 'number of micronuclei/cell', the average baseline for the 16 donors is 12 micronuclei/1000 binucleated cells. Baseline micronucleus frequencies are higher for women and for the older age group. For the doses of 2, 3 and 5 Gy, men show more micronuclei/cell than women. Also, for all doses except 5 Gy the older age group shows higher micronucleus frequencies. No significant differences were found between the 16 donors or between genders were found. Significant differences in micronucleus frequencies were found between age groups for 0.25 and 0.5 Gy ($p < 0.01$).

4. Discussion

To construct a γ-radiation dose–response calibration curve by use of the CBMN assay, we pooled the data from the 16 donors to take into account the variability between individuals, since it is known that the micronucleus frequency is affected by factors such as age, gender, diet and exposure to chemical agents [20]. The results obtained from the 16 donors were also divided by age and gender and the corresponding calibration curves were constructed. The spontaneous level of micronuclei assessed from the 16 healthy donors ranged from 7 to 18 (data not shown), with a mean value of 12 micronuclei per 1000 binucleated cells, in good agreement with baseline values obtained in other laboratories [39,40] and with what was expected since baseline values ranging from 0 to 40 micronuclei per 1000 binucleated cells have been reported [6]. In the present work a decrease of NDI was detected with increasing radiation doses; similar results were reported by Bolognesi et al. [2]. We also found a correlation between NDI and micronucleus formation in the data of the 16 donors ($p < 0.01$) and also in the age ($p < 0.05$) and gender ($p < 0.01$) sub-groups. Although the NDI is not sufficiently robust for direct application as a biological dosimeter, it can be employed to indicate alterations to the cell cycle that can be induced by exposure to ionizing radiation [6]. Furthermore, it has been reported that the NDI can be an useful tool in dose-estimation after accidental high-dose exposures (exceeding 5–7 Gy), in combination with the CBMN assay [41].

All doses from the pooled data present u-values above 1.96, which indicates over-dispersion and hence deviation from a Poisson distribution. Several other studies also showed the same tendency for over-dispersion in micronucleus distribution [39,42,43]. Unlike the dicentrics, the distribution of micronuclei in irradiated cells commonly exhibits over-dispersion, since it is difficult to determine if the exposure is a total- or a partial-body irradiation. However, in a study with cancer patients after hemi-pelvic irradiation, Senthamizhchelvan et al. [7] were able to identify partial exposures, due to the high σ^2/y values, which varied between 2.52 and 2.93.

The values of the α and β coefficients obtained here are comparable with other studies, despite a certain degree of variation between different laboratories [39,42,44–46]. It is thus strongly recommended that every laboratory has its own dose–response curve [6]. When we distributed the data by age and gender, no significant variations in the calibration curve parameters were found, although a clear difference can be observed in the α coefficients between the calibration curves for the two age groups. Also, between males and females the calibration-curve coefficients differ, although less clearly.

A validation experiment was performed with three individuals to test the accuracy of the established dose–response curve to estimate radiation doses. For all cases the 95% confidence interval encompassed the actual dose. For donor A, the age and gender calibration curves gave over-estimates when compared with the values obtained with the pooled dose–response curve, while for donor B the use of the age and gender curves resulted in under-estimates of the real dose. For donor C, the dose-estimates were lower than the real dose for all calibration curves, and the dose estimated with the pooled dose–response curve was the closest to the real dose. Based on these results the micronucleus dose–response calibration curve for the 16 individuals is the more suitable to be used in biological dosimetry for estimation of dose in case of an accidental overexposure to radiation.

We analyzed the results obtained from the 16 individuals as a total and also divided by age and gender (Table 4), with the CBMN and the CA assays [38]. Women and the older age group presented the higher baseline micronucleus levels, in accordance with the known differences in age and gender in baseline micronucleus frequencies [20,30,47], but without statistical significance, probably mainly due to the small size of the study population. A correlation with age can also be found in micronucleus formation for all doses except 5 Gy, most likely as a result of the different number of individuals in each age group for this dose. The older age group presents higher micronucleus rates, with statistical significance for 0.25 and 0.50 Gy ($p < 0.01$). When the data for micronucleus frequency are split-up by gender, men show higher numbers of micronuclei/cell than women for 2, 3 and 5 Gy, but this is without statistical significance. Analyzing the data of dicentrics/cell and chromosomal aberrations/cell by gender, women present higher yields than men for 2 and 3 Gy, and for the 3-Gy dose this difference is statistically significant ($p < 0.01$). A study performed by Marcon et al. [49] with 31 individuals found no influence of age and gender in the number of CA after 2 Gy (γ-rays).

A larger inter-individual variability was seen at 3 Gy, for dicentrics and total CA, and at 3 and 5 Gy for micronuclei. In a preliminary study concerning CA, with six of the donors included in the present work, we already found a similar inter-individual variability for the 3-Gy dose [48]. The inter-variability observed in both cytogenetic assays, for the higher dose points (above 2 Gy), shows a notable difference in response to high doses of ionizing radiation between individuals, which may be associated with differences in genotype [49–51]. In this work inter-individual variability was observed in the level of different cytogenetic biomarkers induced by high doses of ionizing radiation. Moreover, the analysis of the results of the 16 individuals in this study suggests a difference between genders in the biological response to higher doses of ionizing radiation, and further work on this subject can contribute to clarify this idea.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

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