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# ANALYSIS OF CHROMOSOMAL ABNORMALITIES: A STUDY OF PARTIAL EXPOSURE TO X-RAYS

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## ABSTRACT

Biological dosimetry is used in case of supposed accidental overexposure. The most commonly used biomarkers for assessing the absorbed dose are unstable chromosomal abnormalities. In a case of a partial body exposure, the frequencies of those abnormalities varies according to the area of the exposed body and may be substantially different from a total exposure of the body with an identical dose. The present study aimed to evaluate the frequency of chromosomal changes simulating, with blood samples, partial (25%, 50%) and full body irradiation (100%) in X-ray beam. The irradiation was performed at Metrology Service (CRCN-NE / CNEN) with a bundle of 250kVp X-rays, resulting in the absorbed dose of 1.0 Gy. Prior to obtain the metaphases, irradiated blood was mixed with non-irradiated blood, and then the mitotic metaphases for the chromosomal analyzes were obtained by culturing lymphocytes and the slides were stained with 5% Giemsa. It was observed that there was an increase in dicentric frequency when the dose percentage increases in both subjects (0.024 and 0.049 in subject 1 and 0.016 and 0.038 in subject 2) after irradiation. The cellular distribution was “contaminated” only at dose 25% of the first individual who had a prolongation of the distribution. The Qdr and Dolphin methods were used to estimate partial absorbed dose, but the Qdr method was not efficient and whereas the Dolphin method was efficient when the individual had a prolonged cell distribution. It is necessary to increase the number of observations to be sure of the observed behaviors.

## 1. INTRODUCTION

Ionizing radiation (IR) has high enough energy to ionize atoms and molecules. Ionization is due to the fact that the radiation has enough energy to break the chemical bonds or expel electrons from the atoms after collisions. To characterize the interaction of ionizing radiation with matter, Linear Energy Transfer (LET), which is defined as "the amount of energy dissipated per unit of path length" is used. The radiations considered low LET are: X-rays and γ-rays. In this case, the ionization of any absorbed dose will be randomly distributed between the cells as well as the DNA lesions, which leads one to assume that there is the same probability of generating chromosomal alteration for each cell. It is concluded that the chromosomal abnormalities will be randomly distributed between the cells [1].

Biological dosimetry is a technique used to determine exposure to radiation and calculate the absorbed dose through biomarkers. After the individual undergoes the irradiation process, different cytogenetic assays are usually used to estimate the absorbed dose. This type of dosimetry is used in situations where the whole body absorbed dose is unknown or uncertain, as in the case of accidental or occupational exposures. It is also used in cases where the dose estimation by means of physical dosimetry was not possible or even in the cases where the dosimeter (dosimetric films, thermoluminescent dosimeters) was used. It is used to aid in the diagnosis of physicians in relation to the dose absorbed by the potentially exposed individual, and thus contributes to a more reliable diagnosis and prognosis on the part of physicians [2].

The most common chromosome aberrations are: (1) Dicentric, it is an exchange between the centromeric pieces of two broken chromosomes which in its complete form. It is accompanied by an acentric fragment composed of the acentric pieces of these chromosomes; (2) Ring chromosome, it is an exchange between two breaks on separate arms of the same chromosome and is also accompanied by an acentric fragment; and (3) Acentric fragment, this aberration can be formed independently of the exchanges described above and as such are usually referred to as excess acentrics. It can be terminal or interstitial deletions of varying sizes but it is not always possible to determine their origin and so they are combined with other aberrations [2].

In homogenous, full-body exposures, it is assumed that all lymphocytes in the body received approximately the same dose. In nonhomogeneous exposures, where in the lymphocyte population there is a mixture of exposed and non-exposed doses, the distribution of dicentrics between cells tends to be overspread and this is used as a signature of non-homogeneity. While clinical signs and symptoms can be developed to determine the severity of radiation damage from a specific organ to an organ system, the analysis of dicentrics in circulating lymphocytes can give a good estimate of the percentage of full body exposure [3,4,5].

However, partial exposure biodosimetry based on peripheral blood lymphocytes believes that (I) the exposure was uniform, or (II) the exposure was relatively short, and therefore the mixing of the exposed blood pools with unexposed blood is limited. In these situations, to estimate the dose received and the fraction of the irradiated body, the International Atomic Energy Agency manual recommends two methods: the contaminated Poisson method (Dolphin method) and the Qdr method. Both are based considering that the changes observed are in damaged cells. In the case of radiation-related accidents, it is important to know whether the exposure was partial or full-body as well as to estimate the absorbed dose because this information can help doctors with the patient's prognosis [3,4,5].

The aim of this research is to evaluate the frequencies of chromosomal changes of two voluntaries, simulating total (100%) and partial (25%, 50%) irradiations of the body in an X-ray beam with an absorbed dose of 1Gy.

## 2. MATERIALS AND METHODS

### 2.1 Blood Samples

Six heparinized blood samples (10 mL) were obtained from two healthy donor. Each sample was divided equally (5mL + 5mL) between two culture tubes, the first was considered control sample that was not exposured to irradiation and the second was considered irradiation sample.

## 2.2 Irradiation

Each blood sample was irradiated at Metrology Service (CRCN-NE/CNEN) with 250 kVp X-ray beams. The dose rate was 0.275 Gy/min and with an absorbed dose of 1Gy.

The study was done simulating partial irradiations and also simulating whole body irradiation. Then a mixture of irradiated and non-irradiated blood was performed at the proportions of 25% and 50% prior to cell culture, maintaining the total volume of 5ml in each tube for the samples of partial irradiations, while for the whole body there was no mixing, keeping only the irradiated blood.

## 2.3 Cell Culture

Lymphocytes were cultured for 48 hours in RPMI 1640 media (Gibco), supplemented with 20% (v/v) fetal calf serum, 1% (v/v) phytohemaglutinin (Gibco) and 0,05 µg/mL Colcemid (Gibco) was added 46 hours after culture started. The cells were harvested by centrifugation of the samples and the hypotonic shock (0.075 M KCl) was realized for 15 min at 37°C. The cells were fixed in methanol:acetic acid (3:1). Finally, cells were dropped on clean slides and stained with a 5% Giemsa solution (Sigma).

## 2.4 Scoring Criteria and statistical analysis

About 500 complete metaphases were examined for each irradiation samples. It is recommended that only complete metaphases be recorded, i.e. those with 46 or more pieces. If the cell contains unstable aberrations, then it should balance. For example, a spread containing a dicentric should also have an acentric fragment, yet still count to 46 pieces [2]. After the microscopic analysis and counting of the chromosomal changes, calculations were made to verify the number and frequency with which the alterations appeared, being counted separately dicentric, dicentric + rings and acentric fragments. The cell distribution was made, where it was seen how many alterations by metaphase were found and thus the cells were separated according to this quantity. By establishing the mean and the variance of each sample it was possible to calculate their respective dispersion indexes and all percentages were tested to evaluate their conformity to the Poisson model, using the Papworth u test. With the help of the Dose Estimate program the partial dose estimation was done using the contaminated Poisson and Qdr methods.

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# 3. RESULTS AND DISCUSSION

In this work, 3,135 metaphases were analyzed, the cellular distribution corresponding to the chromosomes associated with ring chromosomes is shown in table 1. It was observed that in the percentage of 25% referring to the first individual there was overdispersion with u value above 1.96, demonstrating the called "Poisson contamination", while the other percentages followed the Poisson distribution (u values between -1.96 and 1.96).

**Table 1. Cellular distribution of dicentric chromosomes associated with ring chromosomes.**

| Cellular Distribution |
|---|

| Donor | % | 0Dic+R | 1Dic+R | 2Dic+R | 3Dic+R | 4Dic+R | Y[1] | Var[2] | Var/Y[3] | U[4] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 25 | 535 | 11 | 1 | | | 0.024 ± 0.007 | 0.027 | 1.132 ± 0.058 | 2.273 |
| | 50 | 487 | 24 | 1 | | | 0.051 ± 0.010 | 0.052 | 1.028 ± 0.061 | 0.459 |
| | 100 | 531 | 60 | 2 | | | 0.108 ± 0.013 | 0.103 | 0.956 ± 0.058 | -0.76 |
| 2 | 25 | 492 | 8 | | | | 0.016 ± 0.006 | 0.016 | 0.986 ± 0.059 | -0.237 |
| | 50 | 480 | 20 | | | | 0.040 ± 0.009 | 0.038 | 0.962 ± 0.062 | -0.617 |
| | 100 | 444 | 39 | | | | 0.081 ± 0.013 | 0.074 | 0.921 ± 0.064 | -1.24 |

1. Mean; 2. Variance; 3. Dispersion index; 4. u values; Dic. Dicentrics; R. Rings

For X-ray irradiation, it produces a damage distribution that is very well represented by the Poisson distribution [6]. The u test is a standard unit of the dispersion index, which for the Poisson distribution must be the unity. Greater than 1.96 indicates overdispersion and less than -1.96 indicates subdispersion, which is a biological organism, it is very difficult to happen, possibly a problem with the sample [2]. Intercellular distributions of dicentric + rings obtained are in accordance with the Poisson distribution model, expected model for blood samples exposed to low LET radiation in whole body irradiations.

However, for partial irradiations it was expected that the samples would suffer "Poisson contamination", fact that only occurred in the sample of 25% of individual 1. This contamination occurred because the relation between the cells with one type of alteration and the one with more than one type is very close so, there is no way to "compensate" for the error and avoid "Poisson contamination". Therefore, the sample of 25% of the first individual was the only one that behaved as expected, in the case of partial exposures.

The other five doses didn't following the Poisson distribution, therefore there was no "stretching" of the cell distribution. This was probably due to the number of cells tested, the dose being relatively low (1Gy) and a low dose rate (0.275 Gy/min), which increases the time for DNA repair performance. Possibly these three factors are not sufficient stimulus to cause the dispersion required for the Qdr and Dolphin calculations.

In works such as Fernandes et al. (2008), Senthamizhchelvan et al. (2009) and Vaurijoux et al. (2012), a dose greater than 2 Gy was used, a dose rate greater than 0,3 Gy/min was used and a larger number of cells was counted, therefore more cells with two or more alterations appeared. Then, probably, if a larger dose had been used or a larger dose rate or the number of cells analyzed had been increased, a larger number of cells with two or more chromosomal alterations could arise and therefore present Poisson contamination.

It is observed in Table 2 that in the Qdr method in the first individual in the two samples the dose was lower than the actual dose and the standard deviation was greater than the doses estimated in the two samples. In the second individual, it was not possible to obtain a result in the percentage of 25%, since the frequency of dicentrics was very low, making it impossible to calculate. In the 50% percentage of the second individual, what happened in the first individual was repeated: the estimated dose was lower than the actual dose and the standard deviation was higher.

In the contaminated Poisson method the first donor had the estimated dose close to the actual one and maintained the standard deviation lower than the dose. The fraction of irradiated cells in the percentage of 25% was of 14.6%, whereas in that of 50% it was of 64%. The fraction of the irradiated body, in the percentage of 25%, came closer to reality with a value of 22%, while that of 50% estimated a fraction of 71% of the irradiated body. It was not possible to calculate the estimated dose of the second individual because there was no prolonged cellular distribution, occurring only metaphases counted with a dicentric.

**Table 2. Absorbed doses estimated by partial dose estimation methods.**

| Donor | % | Qdr | | | | | Contaminated Poisson | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Dose (Gy) | SD | Ratio | Fraction of irradiated cells | Fraction of the irradiated body | Dose (Gy) | SV | Fraction of irradiated cells | Fraction of the irradiated body |
| 1 | 25 | 0.013 | 0.398 | 0.232 | 0.102 | 0.103 | 1.4 | 0.353 | 0.146 | 0.223 |
| | 50 | 0.422 | 0.471 | 0.361 | 0.135 | 0.155 | 0.924 | 0.281 | 0.643 | 0.717 |
| 2 | 25 | - | - | - | - | - | - | - | - | - |
| | 50 | 0.151 | 0.437 | 0.282 | 0.135 | 0.142 | - | - | - | - |

(-) can't get result.

The Qdr method underestimated all doses and the standard deviation was above the estimated dose, which makes the result not applicable for this scenario. The expected method would be to estimate the dose close to the real one, which was 1Gy, which did not happen in any sample that could be calculated. The 25% sample of the second individual could not be calculated because the dicentric frequency was very low. This method uses, in addition to the number of dicentrics and the number of metaphases counted, the number of ring chromosomes, the number of damaged cells and the number of acentric fragments. The Qdr method was probably unable to reliably calculate the dose because the dose and dose rate used were low compared to other studies such as Fernandes et al. (2008), Vaurijoux et al. (2012) and Senthamizhchelvan (2009) reducing the number of chromosomal changes [7,8,9].

The contaminated Poisson method was only able to calculate the dose of the first individual. In the second, the cellular distribution was not prolonged, having only cells with one type of alteration. In the first individual, the doses were estimated to be close to the actual as expected and the fraction of the irradiated body approached more at the 25% dose, while the fraction of irradiated cells was closer to 50% as compared to the method Qdr. In the contaminated Poisson method, only the number of dicentrics and the number of metaphases analyzed are counted. Thus, the longer the cell distribution, the easier it is to calculate the estimated dose, and this prolongation occurs with higher doses or even higher dose rates.

## 4. CONCLUSIONS

In view of the results obtained it was possible to observe that the Qdr mathematical method used to estimate the dose for partial irradiation does not seem to be efficient when dealing with low doses. The contaminated Poisson method proved to be efficient when the subject presented a prolonged cellular distribution. However, it is necessary to increase the number of observations (more individuals, more metaphases) to be sure of the behaviors observed, and it may be necessary to develop a mathematical method for this type of scenario.

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