Unstable chromosomal aberrations and micronuclei analyses in the biomonitoring of workers occupationally exposed to ionising radiation

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Abstract: The quantification of unstable chromosomal aberrations and micronuclei in peripheral blood lymphocytes is a method commonly used in biodosimetry by cytogenetic analysis, especially when physical dosimetry cannot be performed. In this context, the aim of this research was to compare these methods in the biomonitoring of health professionals occupationally exposed to ionising radiation. In parallel, the C-banding technique was applied to confirm the presence of unstable chromosomal aberrations (dicentrics and rings). For this, samples of peripheral blood from health professionals of three hospitals (Recife – Brazil) were collected and lymphocyte cultures were carried out based on classical cytogenetic techniques. The number of cells scored per subject was the same (1000) for each assay. Among the individuals, those who do not usually wear a lead apron had higher frequencies of unstable chromosomal aberrations and micronuclei than the ones who carefully observe the radioprotection rules.

Keywords: biomonitoring; chromosome aberrations; cytogenetic analysis; micronuclei.

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1 Introduction

The fundamental physical quantity for the monitoring of individuals exposed to ionising radiation (IR) is the absorbed dose, which is defined as the average energy deposited by the radiation per unit of mass of the irradiated volume (ICRP, 1990). Knowledge about levels of doses is important in radiological protection in order to evaluate the consequences to the health of exposed people (Amaral, 2002). In general, the absorbed dose can be directly determined by physical dosimeters (such as film or TLD badges, semiconductors and ionisation chambers) or indirectly, by numerical models (Germain, 1995).

In most cases of real or suspected accidental exposures to IR, however, physical dosimetry cannot be performed for retrospective estimates, principally due to the lack of information about the irradiation conditions. In such situations, biological dosimetry (biodosimetry) has been proposed as an alternative method, which is based on the investigation of cellular and molecular changes (biomarkers) induced by IR, in order to

correlate them with the radiation dose (Bonassi and Au, 2002; IAEA, 2001). In particular, scoring of chromosomal aberrations from peripheral blood lymphocytes has been developed into a valuable biodosimetric tool in radiological protection (Lloyd, 1998).

This has proved its value on many occasions. For example, in real cases of overexposure to IR, information on biological effects assists the medical team in the planning of therapy of the exposed people and alerts them to the deterministic health consequences that could arise in the following weeks and months (IAEA, 2001; Lloyd, 1998; Voisin et al., 2001). For exposures below the level where treatment is needed, as in the case of workers occupationally exposed to IR, chromosome damage information is important in counselling irradiated persons on risks of developing late biological effects, such as cancer (IAEA, 2001; Lloyd, 1998). Finally, the assay can relieve a lot of anxiety when persons wrongly suspect that they have been irradiated (Lloyd, 1998).

Stable and unstable chromosomal aberrations are the most extensively investigated effects in cytogenetic dosimetry. Stable chromosomal aberrations (translocations, deletions and insertions) are gross structural changes on the chromosomes that tend to remain in the cells for a long period of time. Generally they can pass successfully through cell divisions and therefore they are recommended for retrospective evaluations (Lloyd, 1998; Natarajan, 2002; Ramalho et al., 1995; Voisin et al., 2002). On the other hand, the assay for unstable chromosomal aberrations (dicentrics, rings and fragments) is well established and accurate, especially when the blood samples are obtained within a few weeks after radiation exposure (IAEA, 2001; Lloyd, 1998; Voisin, 2001).

The union of two different chromosomes forms a dicentric after irradiation. The union of the extremities of the same chromosome forms a ring and the fragment is the part of the chromosome without the centromeric region (IAEA, 2001). Besides the use of this biomarker, there is another easier and potentially faster cytogenetic technique, namely the micronucleus test (Voisin et al., 2000). Micronuclei are formed from chromosomal fragments or lagging chromosomes at an anaphase, and are not included in the principal nuclei of daughter cells. They are therefore seen as distinctly separate objects within the cytoplasm of the binuclear cell (IAEA, 2001).

However, the analysis of alterations involving visualising the centromeric region is sometimes difficult. For this reason, techniques such as fluorescent *in situ* hybridisation (FISH) and C-banding were developed to aid the identification of the heterochromatic region, which accompanies the centromeres of all human chromosomes, except Y (Guerra and Souza, 2002).

In this context, the aim of this research was to compare the analysis of unstable chromosomal aberrations and micronuclei in the biomonitoring of workers occupationally exposed to IR. Another objective was to evaluate the use of the C-banding technique as a quality control tool to confirm the presence of dicentrics and rings.

2 Materials and methods

2.1 Subjects

Eight health professionals (four males and four females) from three different hospitals in Recife (Brazil), were investigated. They were chosen based on their work functions:

- 1 auxiliary nurse
- 1 administrator
- 1 technician
- 3 medical physicists
- 2 orthopedic traumatologists.

The first two were selected as control subjects who do not work with radiation and the other six subjects have received radiation doses.

All subjects gave written informed consent before the beginning of this study for approval from the hospital ethical practices committees and completed a questionnaire about work and lifestyle. Table 1 summarises some information obtained from the questionnaires. None of the subjects had received medical irradiation as patients.

Table 1 Data of the professionals studied

Professionals	*Hospitals*	*Job*	*Time of work (years)*	*Age (years)*	*Sex*
A	I	Auxiliary nurse	9	48	F
B	I	Administrator	10	39	F
C	I	Technician	5	34	M
D	I	Medical physicist	8	33	F
E	I	Medical physicist	2	43	M
F	II	Medical physicist	18	41	M
G	III	Traumatologist	13	36	M
H	III	Traumatologist	15	43	F

2.2 Lymphocyte culture

2.2.1 Unstable chromosomal aberrations

Five ml blood samples were collected into heparinised tubes and peripheral lymphocytes were cultured for 48 h in a humidified atmosphere containing 5% CO_2 in air at 37°C. Whole blood (0.3 ml) of each subject was added to a culture medium that consisted of 4 ml of RPMI 1640 supplemented with 20% fetal calf serum and 2% phytohaemagglutinin (Cultilab, Campinas-SP, Brazil). To block the cells in the metaphase stage, Colcemid (Sigma-Aldrich, Irvine, UK) was added for the last two hours of culture at a final concentration of 0.1 μg/ml. The lymphocytes were harvested by centrifugation and hypotonic shock with 75 mM KCl. Following this, the cells were washed and fixed with 3:1 methanol: acetic acid. Slides were made for each sample and stained with 10% Giemsa and mounted. The slides were examined with an optical microscope (Olympus BX 60) and cells were classified as aberrant if they had one or more unstable chromosomal aberrations (dicentrics, rings and fragments).

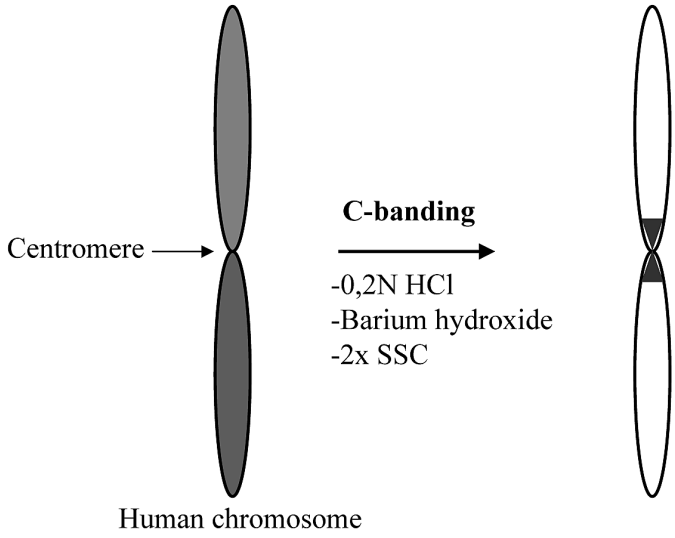
2.2.2 Micronuclei

The lymphocytes were cultured as described above but for 72 h. The culture medium consisted of 4 ml of RPMI 1640, supplemented with 27% of fetal calf serum and 1.8% of phytohaemagglutinin (Cultilab, Campinas-SP, Brazil). Cytochalasin B (Sigma-Aldrich, Irvine, UK) was added to the medium at a final concentration of 5 μg/ml for the last 28 h of culture to inhibit cytokinesis. The hypotonic shock was applied with NaCl 0.9% and the cells were fixed with 3:1 methanol: acetic acid. Slides were made for each sample and stained with 10% Giemsa, mounted and examined with an optical microscope (Leitz Wetzlar MPV2) and the frequency of micronuclei was scored in binucleate cells.

2.3 C-banding

The metaphase preparation slides previously scored for unstable aberrations were restained by the C-banding technique, to make evident the centromeric region of human chromosomes (Figure 1) and confirm the presence of dicentrics and rings. The slides were placed in 0.2 N HCl at room temperature for 30 min, followed by 5% barium hydroxide at 60°C for 30 sec–1 min and then 2×SSC at 60°C for a further 45 min (Prosser, 1975).

Figure 1 Diagram of C-banding technique



3 Results and discussion

3.1 Aberration frequencies

All the frequencies of unstable chromosomal aberrations and micronuclei are summarised in Table 2.

Table 2 Frequencies of unstable chromosomal aberrations and micronuclei each in 1000 cells of the study subjects

Workers	*Dc*	*R*	*Fr*	*FUCA*	*MN*	*FMN*
A	0	0	0	0.000	5	0.005
B	0	0	0	0.000	9	0.009
C	1	0	2	0.003	14	0.014
D	3	0	2	0.005	18	0.018
E	2	1	2	0.005	14	0.014
F	6	1	0	0.007	40	0.040
G	1	0	0	0.001	2	0.002
H	9	1	1	0.011	33	0.033

Notes: Dc – dicentrics; R – centric rings; Fr – fragments; FUCA – frequency of unstable chromosomal aberrations; MN – micronuclei; FMN – frequency of micronuclei.

Analyses of unstable chromosomal aberrations and micronuclei in lymphocytes of the auxiliary nurse (A) and administrator (B), who are not occupationally exposed to radiation, served to indicate the contribution of the background radiation in hospital activities. No unstable aberrations were observed in 1000 metaphases from these two people and their micronucleus frequencies, also in 1000 binucleate cells, can be considered as baseline and were similar to results obtained elsewhere with an unexposed control individual (Magnata, 2002). Considering different populations, the spontaneous frequency of dicentrics does not vary significantly, being of the order of 1 per 2000 lymphocytes (Bonassi and Au, 2002; Voisin, 1997).

Table 2 shows that, compared with persons A and B, the results for the technician C and three physicists (D, E and F) were all elevated. D and E primarily work with diagnostic X-ray equipment in hospital I while F works in the nuclear medicine department of hospital II. During questioning, F declared that he did not always use a lead apron, which might account for his cytogenetic aberration frequencies being higher than those of the other two physicists.

With regard to the two traumatologists, G had a lower frequency of unstable chromosomal aberrations and micronuclei than H. Both work with X-rays including fluoroscopy procedures in hospital III. At interview, G confirmed that he followed the radioprotection rules, while H admitted that he rarely uses a lead apron during the surgical procedures. These results are in agreement with Zakeri and Assaei (2004), who observed a higher frequency of unstable chromosomal aberrations and micronuclei in cardiologists, nurses and technicians who do not always use a lead apron in angiocardiography clinics in Iran.

Also in both cases the traumatologists' exposures were to their hands frequently in the primary beam and, for person G, these repeated exposures were sufficient to have caused chronic radio-dermatitis over both hands. For person H, who worked less, there was one small patch of dermatitis noted, but for this person, working routinely without an apron, the whole body was regularly exposed to scattered radiation. It is this exposure in H that is reflected in the frequency of chromosomal damage shown in Table 1, representing whole body irradiation. In situations such as person G, with exposure confined to a very small percentage of the body volume, cytogenetics is inefficient in detecting this, so that an aberration frequency consistent with normal background is unsurprising.

The hospitals' safety regulations state that radiation exposed staff should wear lead aprons and also personal dosimeter badges. However, it had already been noted that most of the radiation workers in these hospitals do not always strictly adhere to these requirements during their routine procedures (Zakeri and Assaei, 2004). As a result, an investigation of radioprotection conditions of work on the basis of personal physical dosimetry may lead to a misinterpretation of safety conditions. It was for this reason that cytogenetics was proposed as a complementary method for the monitoring of such workers.

The *Comissão Nacional de Energia Nuclear* (CNEN) is the Brazilian authority on radiation safety, which has endorsed the ICRP recommended dose limits of 20 mSv per year for occupational exposures, averaged over five years, with the restriction that the dose should not exceed 50 mSv in a single year (CNEN, 2005).

CNEN requires that all professionals occupationally exposed to ionising radiation must wear dosimeters, such as thermoluminescent or film badges or other devices used for recording cumulative dose (CNEN, 2005). These data must not be released to workers or the public and therefore have not been included in this paper. However, it may be stated that all personal physical dosimetric reports showed no instance of a person having exceeded the dose limit. However, as discussed above, adherance to the regulations concerning wearing of dosemeters is haphazard and when local officials were questioned about how often non-wearing would happen, the answer was 'very frequently'. It was added by some officials that this is particularly so when staff were undertaking non-routine emergency procedures.

In Brazil, as in all countries, monitoring by physical dosimeters is the legally required method of personal dosimetry for radiation workers and it is the results of this monitoring that becomes the official record of their cumulative radiation exposure (CNEN, 2005; ICRP, 1990). The issuing and processing of film or TLD badges is simple and relatively cheap so that they can be applied to many thousands of workers. Badges are very sensitive to low levels of radiation and accurately record the dose. However this monitoring suffers from one major disadvantage, namely that the dose on the badge may not truly reflect the dose to the individual. Persons may choose not to wear their badge, badges may be accidentally left near radiation sources or persons may work with beams of radiation so that the exposure to a primary beam or to scattered radiation incident on the body is heterogeneous. The badge is monitoring just a few square centimetres of the body at the wearing position and so in heterogeneous fields it is difficult to relate this to the average whole body dose.

In contrast, biological cytogenetic monitoring uses circulating blood lymphocytes which are ubiquitous throughout the body. The effect is therefore to indicate an averaging of the body exposure. One drawback of biological monitoring is cost, which prevents it being used routinely on all workers in a way analogous to the dosimeter badge. It is therefore better deployed, as in the present study, where there are grounds for concern.

Another important difference is that the badge record is cumulative throughout the working life of the individual. On the other hand, chromosome monitoring by unstable aberrations or micronuclei is using a signal that declines with time as lymphocyte renewal occurs. Fortunately the cell types used have a relatively long lifespan. In persons with normal haematology the replacement half time is around three years, although individual variability will exist. This means that, in effect, aberrations or micronuclei are indicating radiation exposures received during the most recent few years (IAEA, 2001) and, therefore, in the context of the present study, would not indicate exposure to persons C–H during the early stages of their careers.

3.2 Comparison between methods

The time required to obtain cells in metaphase to evaluate the frequency of unstable chromosomal aberrations is shorter than that to evaluate micronuclei in binucleate cells. However the analysis of chromosomal aberrations is more laborious and time consuming than for micronuclei. In 2 h it was possible to count about 50 metaphases and 500 binucleate cells, similar to values reported by Voisin et al. (2000). Therefore, the time required for the analyses of unstable chromosomal aberrations was ten times higher than that required for the micronucleus assay (Table 3).

Whilst the micronucleus assay is clearly more time and therefore cost efficient Table 4 summarises the relative advantages and limitations of the two assays.

Table 3 Comparison between the methods of unstable chromosomal aberrations (UCA) and micronuclei (MN)

Method parameters	*UCA*	*MN*
Blood volume/culture	0.3 mL	0.3 mL
Culture time	48 h	72 h
Colcemid add	46 h	X
Cytochalasin B add	X	44 h
Number of observed cells	1000	1000
Staining	Giemsa	Giemsa
Cells scoring time	40 h	4 h

Table 4 Advantages and limitations of the analysis of unstable chromosomal aberrations and micronuclei

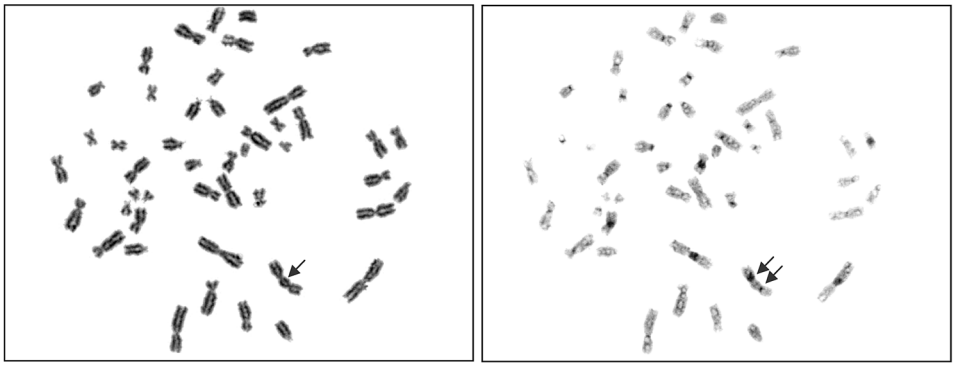
	Advantages	*Limitations*
MN	Faster and easier microscope analysis	Higher background – less specific to radiation. Can be induced by chemicals too
UCA	Less background – more specific to radiation because few chemicals and drugs induce dicentrics and rings	Slow and laborious microscope analysis, requiring more skill

These comparisons suggest the use of micronucleus assay for a preliminary investigation of a cohort of people with suspected overexposure to ionising radiation, followed-up by the analysis of chromosomal aberrations to confirm such exposures and assess the radiation dose more accurately.

3.3 C-banding

The C-banding technique was effective in confirming the presence of dicentric chromosomes, as shown in Figure 2. Figure 2a shows a metaphase and the presence of a possible dicentric chromosome (arrow) with Giemsa block staining. Figure 2b shows the same metaphase and confirmation of the dicentric by the C-banding technique, where denser staining highlights the regions of constitutive heterochromatin (centromeres).

Figure 2 Metaphases presenting one probable dicentric chromosome (A) and its confirmation by C-banding (B)

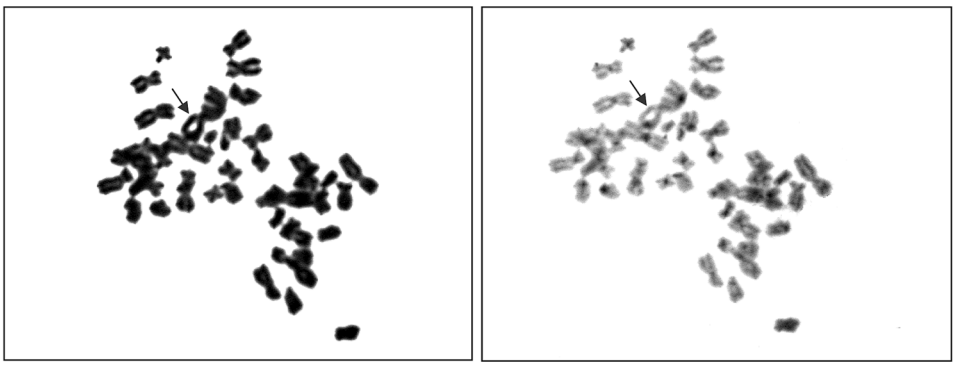


(a) (b)

The same enhancement of staining by C-banding could confirm ring chromosomes.

Figure 3a shows a ring (arrowed) in a poorly spread metaphase somewhat over-stained with Giemsa and in Figure 3b it can be seen that the centromeric region becomes much more easily distinguished after C-banding.

Figure 3 Metaphase containing one possible ring chromosome (A) and its better appearance after C-banding (B)



(a) (b)

The results obtained with C-banding are similar to those that can be obtained with fluorescence *in situ* hybridisation (FISH) using a pan-centromeric probe but C-banding is much cheaper and requires less complicated laboratory processing.

4 Conclusions

The results obtained in this study of a relatively small number of individuals clearly showed that the frequencies of unstable chromosomal aberrations and micronuclei in

lymphocytes of the peripheral blood from health professionals occupationally exposed to ionising radiation are correlated with their individual conditions of radioprotection. In contrast, routine personal physical monitoring with dosimeter badges had given no cause for concern. The study has shown that there is room for improvement in enforcing observance of good radiation protection practices. The demonstration of actual biological effects in exposed staff is a salutatory lesson that may be used to improve the perception of risk and the radiation safety ethos of these professionals. Comparison between the biological assays showed that the analysis of micronuclei is faster than that of unstable chromosomal aberrations, leading to a suggestion for the use of the micronucleus assay as a preliminary screening test in the investigation of suspected accidental exposures to ionising radiation. The C-banding technique was useful in removing ambiguity in the microscope identification of some dicentric and ring chromosomes.

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Notes

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