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Unexplained Overexposures on Physical Dosimetry Reported by Biological Dosimetry

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***Abstract*— The Medical Service of the Radiation Protection Service from the University Hospital La Fe (Valencia, Spain), carries out medical examinations of the workers occupationally exposed to ionising radiation. The Biological Dosimetry Laboratory is developing its activity since 2001. Up to now, the activities have been focused in performing biological dosimetry studies of Interventionists workers from *La Fe Hospital*. Recently, the Laboratory has been authorized by the Health Authority in the Valencian Community.**

Unexplained overexposures of workers and patients are also studied. Workers suspected of being overexposed to ionising radiation were referred for investigation by cytogenetic analysis. Two of these were from Hospitals of the Valencian Community and one belonged to an uranium mine from Portugal. Hospital workers had a physical dose by thermoluminiscence dosimeters (TLD) that exceeded the established limit. The worker of the uranium mine received a dose from a lost source of Cesium 137 with an activity of 170 mCi. All three cases showed normal values after the hematological analysis.

Finally, the aim of this study consist to determine whether the dose showed by the dosimeter is reliable or not. In the case of workers that wore dosimeter, it is concluded that the doses measured by dosimeter are not corresponding to real doses. Hospital worker with a physical dose of 2.6 Sv and 0.269 Sv had an estimated absorbed dose by biological dosimetry of 0.076 Gy (0 - 0.165 Gy) and 0 Gy (0 - 0.089 Gy), respectively. In case of the mine worker an estimated absorbed dose of 0.073 Gy (0 – 0.159 Gy) was obtained by biological dosimetry. In all cases we used the *odds ratio* to present the results due to a very low frequency of observed aberrations [1].

I. INTRODUCTION

Biological dosimetry, based on the study of chromosomal aberrations, mainly the dicentric assay, has become a routine component of accidental dose assessment [2]. The aim of biological dosimetry is to estimate the dose and the associated uncertainty when a person is occupationally exposed, or in a radiological accident or for suspected or verified overexposures both workers and public that no dosemeter was worn. Always if it is possible, together with other information such as clinical symptoms and the physical absorbed dose.

This process requires the use of the maximum-likelihood method for fitting a calibration curve. For the estimation of doses, each laboratory must have its own dose-effect curve [3]. The main objective of a dose-effect curve is its use in radioprotection. The analysis of unstable chromosomal aberrations (dicentrics, fragments and rings) in metaphases of cultured peripheral blood lymphocytes is a long established method of biological dosimetry for ionizing radiation.

Cytogenetical analysis of peripheral blood lymphocytes can provide a biological estimation of the dose received in exposures to ionizing radiation [4]. Information of the absorbed dose and its distribution in the body has great importance for an early assessment of irradiations consequences in exposed individuals. The absorbed dose is related with appropriate in vitro dose response calibration curves and is expressed in Gray (Gy). The procedure adopted by our lab is to score 500 metaphases and if no dicentrics are observed, the dose is close to zero. If one or more dicentrics are seen in 500 cells, the score is often extended to 1000 cells or occasionally more.

The present study was performed for unexplained overexposures to ionizing radiations. The difference in the circumstances of three workers were the presence or absence of the dosimeter and the place were the incident took place. Physical doses are expressed in Sieverts (Sv) and are obtained from personal dosimeters (TLD).

II. MATERIAL AND METHODS

A. CULTURE CONDITIONS

Peripheral blood samples were obtained by venipuncture from individuals occupationally exposed to ionizing radiation, according to the general principles of health and safety at work in Spain. A complete hematological analysis was done.

Physical annual doses were recorded by thermoluminiscence dosimeters (TLD) in those cases corresponding to Hospital workers.

Lymphocytes from a peripheral blood sample are stimulated using phytohemaglutinin (PHA) to enter their division cycle. To select first division metaphases was added to the cultures 12 µg /ml of bromodeoxyuridine. Colcemid was added 2 h before harvesting.

The stain technique used was *Fluorescence plus Giemsa stain technique (FPG)* and details of the protocol and scoring criteria are given by *IAEA* 1989 [5]. If a secondary constriction is suspected when the analysis is performed, a C-Band stain is used to discriminate a secondary centromer or dicentric chromosome [6].

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B. SCORING CRITERIA

After Hoechst plus Giemsa staining, chromosomal analysis was carried out exclusively on first-division metaphases containing 46 centromeres or more. Chromosome abnormalities were classified as follows: dicentric chromosomes were only considered when the acentric fragment was presented. A translocation was recorded only when the morphology of the derivative chromosome was clearly indicative of this kind of rearrangement. The frequency of this type of aberration could be underestimated because unbanded preparations were used. Chromosome breaks (csb) and acentrics were recorded together. Other abnormalities like chromatid breaks (ctb) and gaps were also taken into account. Numerical abnormalities were only considered when hyperploidy was observed.

C. STATISTICAL ANALYSIS

To check if the distribution of dicentrics followed a Poisson distribution, the dispersion index DI (σ^2/ y) and the normalized unit of this index (*U*) were used [7]. A value of *U* greater than 1.96 indicates overdispersion at the 5% level of significance. Statistical analyses were carried out using the chi-square test and linear regression.

When the frequency of dicentric chromosomes is determined, a statistical analysis was realized using a software called CABAS. The program called as CABAS consists of the main curve-fitting and dose estimating modules for calculating the dose in cases of partial body exposure, for estimating the minimum number of cells necessary to detect a given dose of radiation and for calculating the dose in the case of a protracted exposure [8].

The dose is estimated fitting a linear-quadratic dose–response relationship by the method of maximum-likelihood with the number of aberrations observed.

In this study, the fitted coefficients using a dose-effect curve for X and γ-ray are showed in Table I. Figure 1 represents dose-effect calibration curve for γ-rays.

In all cases we used the *odds ratio* to present the results because is probably more appropriate as on the balance of probabilities for a very low frequency of observed aberrations [1].

TABLE I
COEFFICIENTS USED TO ESTIMATING THE DOSE FOR DICENTRICS BY X [9] AND γ-RAY (data not published).

Radiation Quality	Case	$Cx10^{-2}$±SEx 10^{-2}	$\alpha x10^{-2}$±SEx10^{-2}	$\beta x10^{-2}$±SEx10^{-2}
X-Ray	1, 2	0.09±0.04	3.43±0.68	5.70±0.42
γ-Ray	3	0.07±0.06	4.13±0.58	4.44±0.33

C: background frequency of dicentrics, α: linear coefficient, β: quadratic coefficient

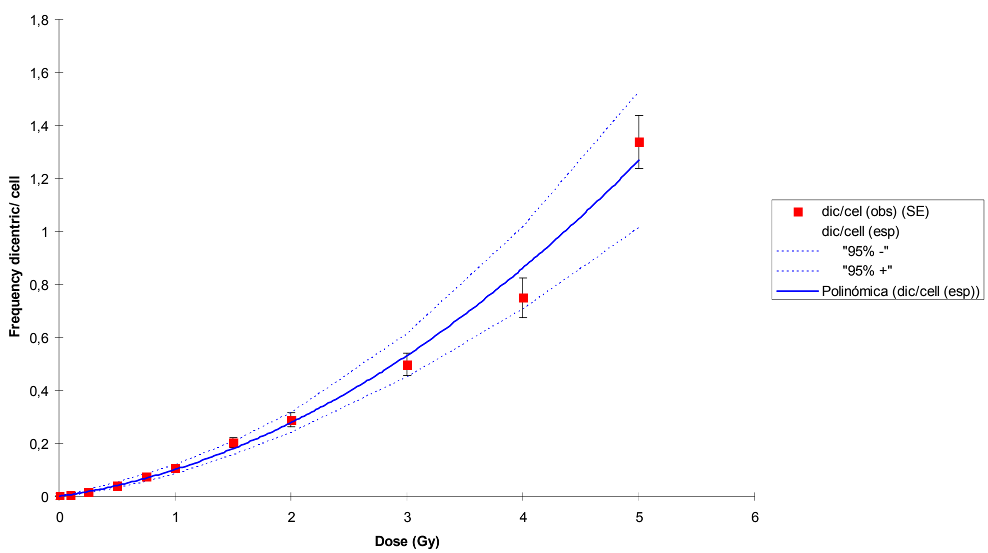

Fig. 1. Dose-effect calibration curve by γ rays to estimate the dose of case 3 (obs: observed, exp: expected and SE: standard error).

III.RESULTS

Table II shows the cytogenetic results obtained in exposed workers, with the analysis of dicentrics.

In relation to hematological results, workers had normal values and their analysis did not indicate a possible overexposure.

In the three individuals studied, a total of 3330 metaphases were analysed in solid stain preparations and 500 metaphases in C-bands stain for case 2.

CASE 1

It is described as a possible overexposure of a professional worker due to ionising radiations. Dated on June 2007, it was communicated that the lecture of the dosimeter of a worker exposed to ionizing radiation during the period March to May exceeded the established dose according to the Spanish legislation. The TLD lecture was 2.6 Sv.

TABLE II
CYTOGENETIC RESULTS OBTAINED IN EXPOSED WORKERS, WITH *FPG TECHNIQUE*

Exposed Worker	1	2	3
Cells scored	1000	1330	1000
Dicentrics (dic)	4	1	4
Translocations (t)	2	-	1
Acentric fragments (ace)	14	8	18
Chromosome breaks (csb)	-	-	-
Ace + csb	-	-	-
Total structural Chromosome-type aberrations	20	9	23
Chromatid breaks (ctb)	1	1	
Gaps (g)	3	2	5
Ctb + g	-	-	-
Ring (r)	2	-	-
Hyperploidy	-	-	-

A biological dosimetry study was performed in October 29th 2007 analizing dicentrics chromosomes uniformly stained. The results showed 4 dicentrics per 1100 cells (the frequency of dicentrics shows a Poisson distribution, Table III). The estimated absorbed dose was 0.076 Gy (95% confidence level, 0 – 0.165 Gy).

TABLE III
POISSON DISTRIBUTION OF CASE 1

O dic	1 dic	DI	Test *U* [7]
1096	4	0.997	-0.074

dic: dicentric, DI: dispersion index

CASE 2

It is described as a possible overexposure of a professional worker due to ionising radiations. Dated on June 2008, the personal TLD dosimeter of a worker exposed to ionizing radiation showed a lecture of 0.269 Sv corresponding to February 2007. The value exceeded the annual dose limit stablished by the Spanish Legislation.

Using biological dosimetry, the frequency of dicentrics was 1 out of 1330 cells. Frequency of dicentics follows a Poisson distribution but data are not showed. Chromosomes, particularly those of B group, can sometimes contain a secondary constriction or a band between sister chromatids giving the appearance of a second centromere. There are some methods to discriminate this fact. One of these stained methods is C bands [6]. After analizing 500 stained metaphases with C bands, no chromosome dicentric was observed.

So we can confirm the results obtained in the metaphases with uniformly stain. The estimated dose was 0 Gy (95% confidence level, 0 – 0.089 Gy) (Figure 2 shows dose and confidence limits by CABAS program).

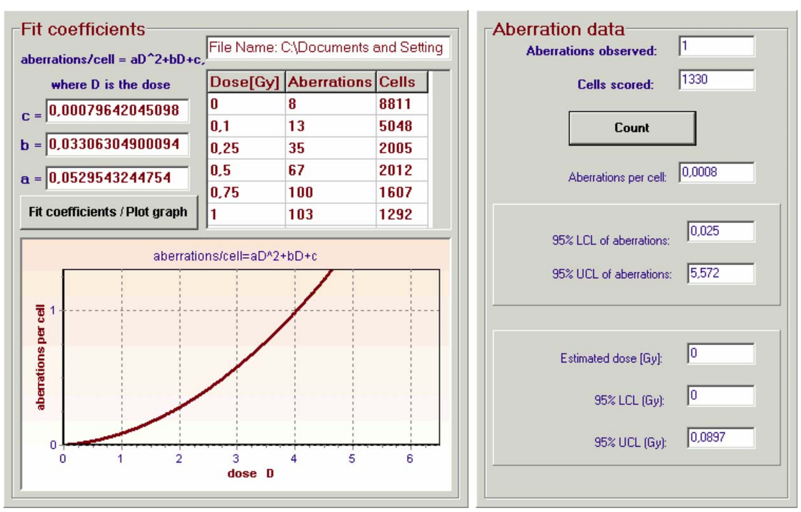

Fig 2. CABAS Program. Graph shows dose-effect calibration curve by X rays [9] and dose and 95% Confidence limits for case 2.

CASE 3

It is described as a possible overexposure of a professional worker due to ionising radiations. Dated on October 2006, a Portuguese Mine Company related an overexposure of a professional worker due a lost source of Cs-137. Once the source was located and the worker was informed, the Nuclear and Technological Institute of Portugal decided to perform a biological dosimetry study.

A study of biological dosimetry using the method of uniformly stained chromosomal dicentrics was done. The results showed 4 dicentrics out of 1000 cells. The estimated dose was 0.073 Gy (95% confidence level, 0 – 0.159 Gy) (the frequency of dicentrics shows a Poisson distribution, Table IV).

Circumstances of the incident:

Years of employment: 8, Source: Cs 137, 170 mCi, Not wearing dosimeter, Average time (with the lost source): 1

month, source was 50 cm below a bridge where the man was working. No clear idea of exposure duration, level or dose. Triage based on medical signs not precise enough.

TABLE IV
POISSON DISTRIBUTION OF CASE 3

O dic	1 dic	DI	Test *U* [7]
996	4	0.997	-0.077

dic: dicentric, DI: dispersion index

IV. DISCUSSION

The activity of the Biological Dosimetry Laboratory is to estimate absorbed doses of professional workers exposed to ionising radiation, mainly to those cases where physical dosimetry is too high or unexplained and to radiological accidents.

The basal frequency in control individuals is one or two dicentrics per 1000 cells, and increased yields are related to the exposure to ionizing radiation. This basal frequency was obtained for case 2. A significant increase of different types of chromosome aberrations compared to background levels has been described in several populations exposed occupationally to low and protracted doses of ionizing radiation [9]. These workers with lower levels of exposure, showed a increases for acentric fragments.

Radiation accidents are fortunately few, in three years the Health Protection Agency (HPA) investigated 23 persons where 15 of them showed no evidence of radiation exposure, for to the rest their averaged whole body doses were low [10].

The present work has been used to validate that the method is effective enough in order to study possible overexposures of professional workers. Biological dosimetry allows us to distinguish whether the physical dosimetry value received by the worker is true or it was just a bad use of the personal dosimeter.

In *case 3*, the biological dosimetry study was used to identify the real risk of an accidental exposure in a quantitative way and it allows an adequate perception of the risk for the worker.

Finally, we suggest that people involved in such occupational exposure work should receive periodic training in radioprotection to avoid these incidents.

REFERENCES

[1] M. Szłuińska, et al. "Presenting statistical uncertainty on cytogenetic dose estimates". *Radiation Protection Dosimetry*. 123, 443-449, 2006

[2] P. Voisin, et al. "Towards a standardization of biological dosimetry by cytogenetics. Cell Mol Biol (Noisy-le-grand). Jul;48(5):501-4. 2002

[3] Lloyd, D.C., Edwards, A.A., *Chromosome aberrations in human lymphocytes: Effect of Radiation quality, dose and dose rate*, Radiation-Induced Chromosome Damage in Man. Edited by Ishihara, T., Sasaki, M.S., (Alan R. Liss, New York 1983) p. 23-49.

[4] J. F. Barquinero, et al. "Biological dosimetry in simulated in vitro partial irradiations" *Int. J.Radiat. Biol*., 71:435-440, 1997

[5] *IAEA*. Biological Dosimetry: Chromosomal aberration analysis for dose assessment", *Technical Reports Series* "Nº. 260, Viena, International Atomic Agency, 1986

[6] **T**.S. Fernandes, et al. "A comparison of different cytological stains for biological dosimetry". *Int J Radiat Biol*.;84(8):703-11. 2008

[7] D.G. Papworth. "Curve fitting by maximum likelihood". *Radial. Bot*. 15: 127-140, 1975

[8] J. Deperas, et al. "CABAS: a freely available PC program for fitting calibration curves in chromosome aberration dosimetry". *Radiation Protection Dosimetry*. 124, No. 2, pp. 115–123, 2007

[9] A. Montoro, et al. "Biological dosimetry in a group of radiologists by the analysis of dicentrics and translocations".*Radiation research* 164, 612–617, 2005

[10] D C Lloyd, et al. "Doses in Radiation Accidents Investigated by Chromosomal Aberration Analysis XXIV: Review of Cases Investigated, 2003-2005". HPA-RPD-012, 2006