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An assessment of immediate DNA damage to occupationally exposed workers to low dose ionizing radiation by using the comet assay

Angélica Martínez,*,** Matthew Coleman,*** Carlos Alejandro Romero-Talamás,**** Sara Frías*

* Departamento de Investigación en Genética Humana, Instituto Nacional de Pediatría.
** Laboratorio de Enfermedades Complejas, Instituto Nacional de Medicina Genómica.
*** Physics and Life Sciences, Lawrence Livermore National Laboratory, Livermore California, USA.
**** University of Maryland, College Park, MD, USA.

ABSTRACT

Background. Several cytogenetic studies have shown an increased frequency of chromosomal aberrations for workers exposed to low dose ionizing radiation, however the dose, type of radiation and management vary among the areas of work; it is possible that this variation may generate different quantity of DNA damage, detectable within the first hours after exposure of the personnel. In this study we assessed early DNA lesions caused by exposure to low doses of ionizing radiation in 41 workers from the departments of Radiology, Nuclear Medicine and Radiotherapy and a group of 20 healthy unexposed individuals, all from the same Institution. **Material and methods.** Blood samples were obtained from exposed and unexposed subjects for analysis of DNA damage using the comet assay. The migration of the comet's tail was compared before and after the workday, as well as among the groups; the relationship between DNA migration and the exposure dose of the month was also obtained. **Results.** A significant increase in damage to DNA was seen after workday for the occupationally exposed group ($p < 0.01$) as compared with the samples before workday as well as with those from the unexposed group. A positive correlation was found between the monthly dose of radiation and the migration length of DNA before and after the workday ($p < 0.01$). There were significant differences in the length of the comet tails among workers from different departments: workers from Radiology (28.6 µm) have less DNA damage than those from Nuclear Medicine and Radiotherapy (92.5 µm, 63.4 µm respectively) departments. **Conclusions.** All the workers occupationally exposed showed an increase in DNA fragmentation after the workday. The amount of radiation in all three services is different, in Nuclear Medicine and Radiotherapy the workers showed a greater monthly dose of exposure and greater DNA damage than the Radiology workers. The longer tails were observed in Nuclear Medicine where radionuclides are

Evaluación del daño inmediato al DNA en trabajadores ocupacionalmente expuestos a bajas dosis de radiación ionizante mediante ensayo cometa

RESUMEN

Introducción. *Diversos estudios citogenéticos han demostrado un incremento en la frecuencia de aberraciones cromosómicas en trabajadores expuestos a bajas dosis de radiación ionizante en comparación con los individuos no expuestos; sin embargo, el manejo, el tipo de radiación y las dosis utilizadas varían entre las diferentes áreas de trabajo y es posible que esta variación genere diferentes cantidades de daño al DNA, detectable dentro de las primeras horas de exposición. En este estudio se evaluaron las lesiones tempranas del DNA inducidas por exposición ocupacional, mediante la técnica de electroforesis unicelular alcalina (ensayo del cometa) en 41 individuos ocupacionalmente expuestos, que laboran en tres diferentes departamentos: Radioterapia, Medicina Nuclear y Radiología, así como en 20 individuos sanos no expuestos, todos trabajadores de la misma institución.* ***Material y métodos.*** *Se tomaron muestras de sangre periférica antes y después de la jornada laboral de todos los individuos quienes aceptaron participar voluntariamente en el estudio. Se obtuvo el promedio de la migración de la cola del cometa y se compararon los datos obtenidos antes y después de la jornada laboral, entre los individuos expuestos y no expuestos y entre los diferentes departamentos; se hizo la correlación entre la migración del DNA y la dosis de exposición mensual.* ***Resultados.*** *Se observó un incremento significativo en el daño al DNA después de la jornada laboral en el grupo expuesto ($p < 0.01$). La correlación entre la dosis mensual y la migración del DNA fue positiva y significativa al inicio ($p < 0.01$) y al final ($p < 0.05$) de la jornada laboral en los individuos expuestos. El análisis por áreas de exposición mostró que los*

used; these radioactive substances are handled and administered to patients orally or intravenously by the workers, which implies a different type of exposure and radiation, this may explain the differences found in this study. Most of the DNA damage detected by the comet assay is repaired, however a part of it may result in stable chromosomal rearrangements that may represent a long-term health risk. It is important to sensitize exposed workers on their responsibility of working with radiation and the improvement of the hospital safety practices.

Key words. Comet assay. Occupational exposure. Ionizing radiation. DNA damage.

trabajadores de Radiología presentaron una menor migración del DNA (28.6 µm) en comparación con los individuos de Medicina Nuclear y Radioterapia (92.5 µm, 63.4 µm respectivamente). ***Conclusiones.*** *En todos los trabajadores ocupacionalmente expuestos a radiación se encontró un incremento en la fragmentación del DNA al terminar su jornada laboral. La cantidad de radiación en los tres departamentos es diferente, en Medicina Nuclear y Radioterapia, los trabajadores mostraron una dosis mensual mayor, así como un mayor daño en su DNA en comparación con los trabajadores de Radiología. Las colas de cometa más largas se encontraron en los trabajadores de Medicina Nuclear en donde se utilizan radionúclidos; estas substancias son manejadas y administradas oral o intravenosamente por los trabajadores, lo cual implica un tipo de exposición diferente a la radiación, esto podría explicar las mayores diferencias encontradas en este estudio. El daño detectado por el ensayo cometa se repara en su mayoría, pero puede generar aberraciones estables y representar un riesgo para salud a largo plazo, por lo que es importante sensibilizar a los trabajadores expuestos sobre la responsabilidad que implica trabajar con radiaciones e implementar medidas de seguridad mas eficientes para el personal.*

Palabras clave. *Ensayo del cometa. Exposición ocupacional. Radiación ionizante. Daño al DNA.*

INTRODUCTION

Humans are naturally exposed to ionizing radiation (IR) from cosmic rays, and artificially through diagnostic procedures, medical treatments or occupationally during work shifts. It is well known that IR produces DNA damage through different mechanisms: by loss of bases, by denaturing of certain segments of crossed bridges between protein-DNA and DNA-DNA, by single-strand breaks (SSB), double strand breaks (DSB), and damage to purine and pyrimidine bases.[1-3] This early damage may lead to chromosomal aberrations and thus to increased risk of mutagenesis and carcinogenesis. Cytogenetic studies in workers occupationally-exposed to ionizing radiation have demonstrated an increase in the frequency of chromosomal aberrations in comparison to non-exposed individuals, showing a linear relationship between the frequency of chromosomal aberrations in lymphocytes and the total accumulated dose of radiation.[4-13] These chromosomal aberrations (translocations, dicentric chromosomes, rings, centric fragments) are the result of an erroneous repair of the DNA lesions produced by radiation.[10-15]

In this study, we assessed early DNA lesions in lymphocytes from hospital workers exposed to low doses of radiation using the alkaline comet assay; The comet assay, is a very sensitive, rapid, and simple technique that detects DNA damage within individual cells.[16] Better defining the early occupational exposure effects may lead to the improvement of hospital safety practices.

MATERIAL AND METHODS

Subjects

Three Departments where people are occupationally exposed to ionizing radiation were studied; all of the workers assigned to these areas by the time of this study, accepted to participate: six subjects from the Nuclear Medicine Department (NM), four from Radiotherapy (RT) and 31 from the Radiology Department (RX) of the National Institute of Pediatrics (INP) in Mexico City; during their work, all of them wore individual dosimeters (optical stimulated luminiscence, OSL) and the readings were done monthly. Unexposed group was selected from administrative areas of the INP, who had never been occupationally exposed to IR and was matched to the exposed subjects with respect to age and sex.

All individuals who agreed to participate in the study signed a consent letter. All of them were in good health and had completed a detailed questionnaire concerning their habits, lifestyles and medical records (age, medications, smoking, drinking habits, X-ray exposure). Persons that had previously received chemotherapy, radiotherapy, or had been diag-

nosed with cancer were excluded from the study. Individuals with an acute illness (i.e. diarrhea or respiratory infection) were excluded from the study for that day and were included for later sampling when they presented in healthy condition.

Blood sampling

Venous blood was obtained from each person by venipuncture while fasting before starting work, and again six hours later at the end of their work day, with at least two hours after having eaten to avoid changes in DNA migration due to ingestion of C vitamin, which has been observed to reduce the DNA damage.[17]

The alkaline comet assay

The alkaline comet assay was performed basically as described by Tice, *et al.*[16] Electrophoresis, which allowed for fragmented DNA migration was carried out for 20 min at 25 V and 300 mA. After the electrophoresis, the slides were neutralized with 0.4 M Tris, pH 7.5, stained with 50 µL of ethidium bromide (20 µg/mL) and analyzed with a fluorescence microscope (Olympus. 40x objective lens). The extent of DNA damage was assessed from the DNA migration distance, which was derived by subtracting the diameter of the nucleus from the total length of the comet. Fifty randomly selected cells were examined for each replicate, for each sample or subject, and the slides were scored blind to remove any possibility of bias in the analysis.

Statistical Analysis

Statistical evaluations were performed by SPSS package version 15.00. To detect differences between the samples obtained before and after workday for each group, the Wilcoxon test was used. Multiple comparisons among the different groups were done using the Kruskal Wallis and the comparisons between two groups were performed by the Mann Whitney U test. In our second round of analysis, we used the Pearson's correlation coefficient to look for a correlation between variables of DNA migration *versus* a) the monthly dose of exposure to IR, and b) people's ages. Multiple lineal regression analysis and analysis of collinearity for independent variables were performed to detect confounders associated with DNA damage. The level of statistical significance was set at $p < 0.05$.

RESULTS

The comet's tail migration measurements for all groups before and after the workday are shown in figure 1 and tables 1 and 2. Before the workday, the unexposed group had a range of DNA migration between 1.3 to 39.17 µm and the average was 15.5 ± 2.4 µm. Afterwards, the range of DNA migration was 6.3 to 34.06 µm (average = 15.2 ± 1.92 µm). The unexposed group differences between the start and end of the workday were not statistically significant (Wilcoxon test, $p = 0.85$, figure 1 and table 1). However, the occupationally exposed groups showed significant differences between the start and end of

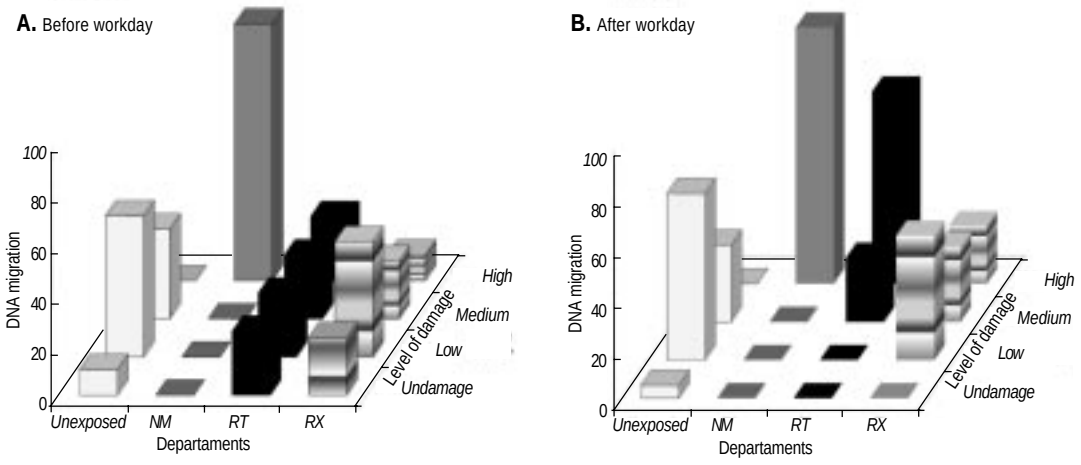

Figure 1. *Distribution of individuals from the four different departments, according with the level of damage: arbitrary stratification in four categories: undamaged (< 5 µm), low (6-20 µm), medium (21-40 µm) and high (Ã 41 µm) damage. In spite of all exposed groups showed a significant increase after workday, we observed differences regards the level of damage: DNA migration for unexposed and NM workers before workday is similar to After workday, while RT and RX had marked changes in the levels of damage: undamaged level disappear in both departments in After workday.* ***A.*** *Before workday.* ***B.*** *After workday.*

Table 1. Age, Gender and average DNA migration in non-exposed Individuals.

Code	Age/Gender	DNA Migration (microns) Before Work Shift	After Work Shift
1	19/F	39.17	34.06
2	22/F	29.0	18.6
3	23/F	30.99	24.89
4	23/F	13.5	13 2
5	24/M	7.7	9.5
6	25/F	29.0	24.52
7	26/M	20.63	11.55
8[d]	31/M	17.1	23.0
9[d]	35/F	5.7	9.6
10	35/F	14.2	18.6
11[d]	36/F	4.4	6.8
12[d]	41/M	1.3	7.6
13	43/F	6.38	3.78
14	45/F	15.6	7.4
15[s]	45/F	6.21	8.4
16	48/M	24.8	24.22
17	50/F	6.8	7.5
18	50/F	7.6	6.3
19	50/M	23.0	25.22
20	55/F	7.6	18 9
n = 20	x = 36.3 ± 2.6	x = 15.5 ± 2.4*	x = 15.2 ± 1.9*

x: values are the average ± standard error. [d]: individuals drank alcoholic beverages on occasion. [S]: individuals smoked 10 or less cigarettes a day. *: Wilcoxon test p = 0.85.

the workday (Wilcoxon test, NM RX and RT: $p < 0.01$, table 2), with marked differences among the departmental groups (Figure 1). The wide standard errors estimated in this study (after the workday: NM = 92.5 ± 19.2 ± µm, RT = 63.4 ± 15.4 µm, and RX = 28.6 ± 3.6 µm) indicate there is great inter-individual variability in DNA migration. However, the relative levels of migrations were preserved before and after work in each individual.

In a comparison of the samples taken before the workday, we observed significant difference among the groups of individuals (Kruskal Wallis, $p < 0.002$, tables 1, 2 and figure 1); NM is different to all other groups ($p < 0.001$), while no significant differences were found between RT *vs.* RX , RX *vs.* unexposed group, RT *vs.* unexposed group (Mann Whitney U $p > 0.05$).

We also found significant differences after the workday when we compared departmental groups to the unexposed group ($p < 0.01$).

The results also demonstrated that there is a positive correlation between DNA migration *versus* the monthly exposure dose of IR and DNA migration before the workday ($r = 0.37$, $p = 0.001$), as well as after ($r = 0.28$, $p < 0.003$) the workday in individuals exposed to IR. With respect to age, the results demonstrated that unexposed group had a negative correlation before their workday ($r = -0.53$, $p = 0.01$), while after workday this negative correlation was not significant ($r = -0.30$, $p = 0.12$). In the exposed group, this negative correlation was observed before $r = -0.30$, and after $r = -0.39$ the workday ($p < 0.01$). The regression analysis showed that alcohol intake or smoking, have not a significant contribution to DNA damage observed in all studied groups.

DISCUSSION

Ionizing radiation is a genotoxic agent for all living creatures. It is capable of causing genetic damage even at very low doses, which is why it may be necessary to assess any possible DNA damage levels in individuals chronically exposed at their place of employment.[11,18-25] In the present study, the comet assay was used to assess DNA damage in peripheral blood samples from technical workers occupationally exposed to low doses of radiation at INP México City for comparison with a group of unexposed workers within the same institution. The results were analyzed by comparing the values before and after a normal workday. Data was assessed in terms of differences in the DNA migration distances according to work site. We found a higher DNA migration after the workday in all exposed groups, as compared with the DNA damage before the workday and with the unexposed group. The amount of radiation in all three departments is different; in Nuclear Medicine and Radiotherapy departments, the workers showed a greater monthly dose of exposure and greater DNA damage than the Radiology workers.

All studied groups showed large inter-individual variability that was considered as an inherent individual characteristic, however the values for the comet's tail length before and after work day remained within the same range for each individual, sustaining in both groups their coherence with baseline levels, it has been proposed that the inter-individual variability can be due to factors such as individual's life-style.[26,27]

According to the questionnaire completed by the individuals, the population was healthy, with a few individuals positive for relatively low alcohol and tobacco consumption. Alcohol consumption was positi-

Table 2 . Age, Gender, Exposure dose and average DNA migration in Individuals exposed to ionizing radiation.

Group	Age/Gender	Monthly Exposure Dose (mSv) /years of work	DNA Migration (microns) Before the work shift	After
Nuclear Medicine Department				
1	25/F	0.15/1	58.52	102.88
2	30/F	0.19 /10.5	54.89	94.01
3[d]	30/F	0.29/4.6	47.94	61.17
4	33/M	0.26/7	66.99	180.3
5	52/M	0.16/17	42.09	60.7
6	52/F	0.18/28	48.87	56.03
n = 6	x = 37 ± 4.8	x = 0.21 ± 0.02	x = 54.05 ± 3.7*	x = 92.5 ± 19.02*
Radiotherapy Department				
7	23/F	--/5	82.62	103.64
8[d]	29/M	0.8/4	38.8	68.2
9	37/F	0.16/4	3.5	51.0
10	50/F	0.19/14	19.0	30.8
n = 4	x = 34.8 ± 5.6	x = 0.4 ± 0.2	x = 35.9 ± 17.1*	x = 63.4 ± 15.4*
Radiology Department				
11	20/F	0.34/5[+]	27.0	68.9
12	22/F	0.28/2	38.4	74.0
13[d]	25/F	0.14/1	21.22	32.66
14[d]	26/M	0.05/4.6	3.51	6.48
15	27/F	0.20/5	31.4	58.5
16[s]	28/M	0.05/5	4.5	15.4
17	30/F	0.13/2	27.43	36.81
18	31/F	-/1.7	9.0	22.0
19	31/F	0.10/2	6.7	18.34
20	31/M	0.06/-	6.8	20.9
21	32/F	0.19/2	5.52	17.51
22[s]	34/F	0.25/10	54.11	56.54
23	35/F	0.8/17	48.01	44 27
24	35/F	0.12/6	45.96	70 98
25[d]	37/M	0.11/7[+]	7.6	18 28
26[d]	38/M	0.04/3	8.8	18.1
27	38/F	0.22/3	24.26	62 24
28	38/F	0.27/3	6.0	23.68
29[d]	39/M	0.30/-	2.7	9.0
30[d]	39/M	0.08/3	4.7	14.3
31	40/M	0.19/3	13.5	20.5
32[s]	40/F	0.16/10	1.3	16.2
33	41/F	0.15/-	6.8	17.5
34[d]	42/F	0.13/-	2.6	12.2
35	42/F	-/1.6	7.3	25.7
36[d]	43/F	0.12/17	3.8	10.4
37[d]	45/M	0.14/5	17.3	18.8
38[sd]	45/F	0.10/22	10.9	25.3
39[s]	46/F	0.12/24	6.15	8.2
40	49/F	0.11/23	14.3	17.5
41	49/M	0.07/-	23.6	25.5
n = 31	x = 36.1 ± 1.4	x = 0.17 ± 0.02	x = 15.8 ± 2.6*	x = 28.6 ± 3.5*

x: values are the average ± standard error; [+]:months; [d]: individuals drank alcoholic beverages on occasion; [S]: individuals smoked 10 or less cigarettes a day; *: Wilcoxon test $p < 0.01$

ve in 3/20 unexposed individuals and in 12/41 individuals exposed to low doses radiation, although all stated they were occasional drinkers. Kalaiselvi, *et al*., have observed that alcohol induces a significant increase in the percentage of cells with DNA damage in normal individuals.[28] In the present study no differences were found between the non-drinkers and occasional drinkers. Similarly, with respect to smoking, 1/20 unexposed and 5/41 exposed individuals were smokers and consumed an average of 1-10 cigarettes daily. Studies related to the induction of DNA damage due to smoking have demonstrated a significant association between the increase of number of cells with DNA damage and tobacco consumption in lymphocytes of cardiologist exposed to radiation.[29] In the present study, there was no association between alcohol and tobacco consumption and DNA damage. It is probable that individuals in this study did not reach toxic consumption levels would have produced a detectable difference with our methodology.

When comparing the three exposed groups and the unexposed group, significant differences were found between the DNA migration values from the unexposed group *versus* exposed groups at the start of the workday. That is, NM and RT groups had a DNA damage baseline that was well above the unexposed group, whereas the RX group had a baseline comparable to the unexposed group. This may suggest that during the workday, the cells from the RX group receive IR in their work environment but have lower exposure levels of DNA damage that are efficiently repaired and returned to baseline levels overnight.

Unexposed individuals had similar before *versus* after DNA migrations indicating little to no occupational increase in DNA damage levels, while those occupationally exposed showed a significant increase in DNA migration at the end of the workday. This indicates that, the protection equipment and practices presently used by these individuals needs to be revised to avoid permanent DNA damage.

To the best of our knowledge, there are no other published reports comparing the DNA damage effects of low dose IR exposures before and after a normal workday. However, our results are comparable to studies carried out in individuals chronically exposed to low doses radiation where a significant increase in DNA damage was seen in comparison to unexposed individuals.[3,7-9,11-15,18-31]

As shown in table 2, the amount of radiation in all three services is different. In MN and RT there is a greater monthly dose of exposure and therefore, greater DNA damage in comparison to the RX individuals. The differences found can also be related to the types of exposure and quality of radiation. In MN radionuclides are used; these radioactive substances are handled by the workers and administered to patients orally or intravenously. These materials are continuously emitting alpha, beta, and/or gamma radiation from the containers and from patient's body.[32] Within the RT department, radiation is of the gamma type and the radiation dose is therapeutic, exposing workers to high doses of IR. At RX, low diagnostic X radiation doses are used with short exposure times. It is likely that these factors may have contributed to the differences we observed in DNA migration distances in the three exposed departmental groups.

A positive and significant correlation was observed between the monthly exposure total dose of radiation and DNA migration rates of samples taken before and after the workday in individuals, showing that at a greater dose caused greater amounts DNA damage. These results show that there is cumulative damage tied to high levels of exposure with a difference between the start and the end of the workday. Several published reports have shown a correlation between increase in sister chromatid exchanges, micronuclei or chromosome aberrations, and IR dose;[7,8,11,21,24,25,33] because DNA breaks is the previous lesion to chromosomal damage, it is important to detect sensitive individuals with different levels of risk for producing an increased level of stable chromosomal aberration.

In this study, a negative correlation was found between DNA migration before workday and age in unexposed group. Studies about age and DNA migration show controversial results, since some authors have not found a relationship,[23,34] while others have shown that as age increases, so does the levels and types of DNA damage.[1,8]

In conclusion, we found that occupationally exposed workers showed an immediate increase in DNA damage over a period of one workday, especially in NM and RT workers. It is important to note that a part of this damage may not be repaired and could lead to the development of cancer and/or reproductive problems. In the future it will be important to look at other experimental endpoints of DNA damage, across a much larger population as well as over a greater time period to better understand the health risk of these exposure. This information may be used to contribute to the improvement of the hospital safety practices.

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Correpondence and reprint report:

Sara Frias
Laboratorio de Citogenética,
Instituto Nacional de Pediatría.
Av. del Imán No. 1,
Torre de Investigación 6º. Piso
Col. Insurgentes-Cuicuilco,
04530, México, D.F.
Phone and Fax: + (5255) 1084-5533
E-mail: sarafrias@yahoo.com

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