SEGURIDAD y Medio Ambiente
Year 32 N127 Third Quarter 2012
FUNDACIÓNMAPFRE

Exposure to radiation and genetic damage

Safety

Genotoxic assessment in dentistry students exposed to X rays while taking dental radiographs

This research project aims to ascertain whether chronic exposure to X rays during clinical diagnoses carried out by dentistry students as part of their academic training might produce any genetic damage. To this end two biotests were carried out: firstly, the micronucleus assay on cells of the buccal mucous to evaluate both clastogenic and aneugenic effects and other abnormalities of the nucleus that might be used as markers of cell damage; and secondly the single cell gel electrophoresis test or comet assay in peripheral blood samples to determine any damage to the single or double chain of the DNA. Two cohorts of dentistry students were analysed, one exposed group and another control group. The exposed cohort involved final-year students who were taking patient X rays during a chronic 3-year exposure period. The control cohort involved first-year students who were not X ray exposed. Results show a significant increase in micronuclei ($p < 0.05$) and of cells in apoptosis and other abnormalities of the nucleus in chronically radiation-exposed students but there were no differences in comet-assay DNA damage readings as compared with the control group. The conclusion that can be drawn from these results is that the dose received and the X ray exposure time have a cytotoxic effect on cells of the buccal mucous and produce minimum DNA damage in peripheral blood cells.

By **D. FRANCO DE DIANA.** Biology graduate. Director of the Basic Sciences Department of the Universidad Católica «Nuestra Señora de la Asunción» (deidamia_franco@uca.edu.py).
J. SEGOVIA. graduate. Senior research fellow of the Universidad Católica «Nuestra Señora de la Asunción»
D. CASTIGLIONI. Biology graduate. Senior research fellow of the Universidad Católica «Nuestra Señora de la Asunción».
C. VEGA. Doctor in microbiology. Biology graduate. Senior research fellow of the Universidad Católica «Nuestra Señora de la Asunción» Director of the *Centro para el Desarrollo de las Ciencias* (CEDIC).
N. LÓPEZ ACOSTA. Statistical mathematics graduate. Research fellow of the *Facultad de Ciencias Exactas y Naturales* of the Universidad Nacional de Asunción.
A. GÓMEZ. Master in Biological Sciences from the Centro para el Desarrollo de las Ciencias (CEDIC).

The main effect of ionising radiation occurs in the DNA molecule and is tied up with the dose and exposure time. Any dose however slight, implies some risk, so the exposure should be reduced to the minimum possible. Important factors to bear in mind here, however, are the chronic exposure time to which workers and persons in occupational training are exposed such as healthcare degree students, and also the variation in radiosensitivity from one person to another, depending on age gender and exposure time[1].

X ray exposure during panoramic radiographs in adult and child patients produces genotoxic effects in gingival cells increasing chromosome damage and cell death. These panoramic dental X rays should therefore be sought only in very necessary cases, since this procedure cannot be considered as low risk or risk-free[2].

Tolbert *et al* (1992) put forward the use of the micronucleus assay to evaluate the genotoxic effect, whether caused by chromosomal breakage or abnormal mitotic cell divisions, and also to detect other atypical nuclear abnormalities such as pyknosis, karyorrhexis or karyolysis, which are indicators of cell damage[4][5].

Another useful test for detecting damage to the DNA chain is single cell gel electrophoresis or the comet assay, which assesses the damage to single and double DNA chains in cell populations without the need of cell proliferation studies. It is a potentially sensitive tool for showing up genotoxic damage induced by various toxins within their radius of action[6].

In Paraguay both teachers and students in the dentistry degree course are frequently exposed to radiation during image based diagnoses. Furthermore the exposure is often without any type of protection because the radioprotection biosecurity habit is not strictly practised at this level, despite the existence of standards and legislation regulating this type of activity. Bearing this factor in mind we have carried out micronucleus assays on buccal smears and peripheral blood samples to gauge DNA damage by means of the comet assay. The smears and blood samples came from dentistry students exposed chronically to low X ray doses.

Material and Methods

The research was designed as a cross sectional analytical observational study on exposed and non-exposed cohorts, who were first asked to fill out a survey to find out their state of health, their smoking, drinking and drug-taking habits exposure time and radioprotection methods while taking X rays.

Exposed Population

Students of both sexes aged 24 to 29 carrying out clinical practices in their dentistry degree and who were exposed to ionising radiation while taking dental patient X rays for a period of at least 3 years.

Non exposed population

Dentistry students still in the basic part of the course who have not yet been taking dental radiographs and have therefore suffered no X-ray exposure.

Data collection instruments and techniques

Genotoxic analysis

The genotoxic effect was evaluated by means of two biotests: the micronucleus assay on buccal smears and the comet assay.

The buccal smears and peripheral bloods samples of each individual, both of the control and exposed population, were taken simultaneously from June to October.

Exfoliated buccal cell assay

An evaluation was made of whether chronic X ray exposure during the clinical practice of dentistry students causes genetic damage

A sample of buccal cells was obtained by scraping the inside of the cheek with a spatula. The smear was then spread on the microscope slide. The samples were fixed with a 3:1 mixture of methanol/acetic acid and coloured with the Feulgen technique. Microscope observation was carried out by using special filters to bring out the cytoplasm. Two thousand cells were counted per individual and a determination was made of the frequency of micronuclei in both populations, the control and exposed, according to Tolbert (1992)[7] and Fenech (1999)[8].

Comet assay to measure DNA damage

The comet assay was carried out according to Singh et al. (1988) with small modifications[9] and optimising tweaks[10]. Two µl of peripheral blood was taken from the ring finger of each participant. The samples were suspended in 0.5% of low melting point (LMP) agarose and pipetted onto a slide previously covered with a layer of normal melting point (NMP) agarose at 1% and kept at 4°C for 10 minutes; it was then submerged in a lysis buffer (2.5M Nacl, 100 µm Na2EDTA, 10 µm Tris- Hcl Ph 10,1, Triton - X - 100 and 10% DMSO) for one hour at 4°C in the dark to provoke lysis and DNA breakdown. The slides were then exposed to alkaline buffer (1 mM Na2EDTA, 300mM NAOH buffer) Ph >13 for 20 minutes to degrade the DNA.

For the purpose in hand two biotests were conducted:

Each slide was submitted to electrophoresis for 20 minutes at 25 V and 300 m A in the same buffer and then rinsed in 0.4 Try-Hcl buffer (Ph 7.5) to eliminate excess alkali and remove detergents. The samples were fixed with alcohol p.a and the

micronucleus assay of buccal smears and single cell gel electrophoresis assay or comet assay of peripheral blood samples

slides were finally tinged with ethidium bromide (10 µg/ml) before being examined under a 400 x fluorescence microscope.

100 cells per agarose plate were evaluated by visual check and by means of the software Casp Lab Comet Assay free (http// casp lab comet assay software).

Evaluation by visual check

This was carried out according to the criteria of Speit (1995) [12] and Collins (2004) [13] , wherein cells are classified on the assumption that gentoxic damage varies directly with the number of DNA breaks outside the nucleus. To quantify DNA damage a cell damage category was established on the basis of the comet length:

- Category 0: undamaged cells (5 µm).
- Category I: cells with low damage level (5-20 µm).
- Category II: cells with medium damage level (between 20 and 40 µm).
- Category III: cells with high damage level (between 40 and 95 µm).
- Category IV: maximally damaged cells (higher than 95 µm).

Once the percentage of cells in each category has been established, a calculation is then made of the damage index (N) fo each sample of the individual:

N= number of cells in category I + 2 X number of cells in category II + 3 X number of cells in category III + 4 X number of cells in category IV

Evaluation by the software Casp Lab Comet Assay IV

Tail moment values were obtained for measuring the DNA percentage in the comet tail and the tail moment olive describing the heterogeneity of the response within a cell population, since it calculates the DNA distribution variations within the tail measured in µm.

Statistical Analysis

The SPSS statistical package was used for the exploratory analysis of micronucleus data in buccal smears for the contro group (n=31) and the exposed group (n=30). The results were analysed statistically by means of the student's T test, applying thereafter the non parametric Mann-Whitney U test to cross check the hypothesis.

The analysis of DNA damage with the comet assay was carried out by comparing Mann-Whitney U readings for two samples regardless of DNA damage data and tail moment and tail moment olive values.

Results

Demographic variables of the studied populations

Control or non-exposed population

Dentistry students aged 18 to 20 taking the first year of their degree course. 84% of the population is female (25/31) and 16% (5/31) is male.

43.3% (13/30) of the population have some family cancer precedent, be it lung, prostate, uterus or, predominantly, breast cancer. 40% of the analysed population drinks on a casual basis.

Exposed population

The exposed population consists of 30 dentistry students currently taking the 6th year of their degree course. Their ages range from 23 to 29; 83% of this population is female (25/30) and 17% (5/30) is male. 56.7% of the studied population drinks moderately.

267% (8/30) of the population has some type of family cancer precedent, be it bone, prostate, breast or, predominantly colon. All surveyed students report that they have been working for at least three years in the X ray room; none of the respondents uses any type of protection against the X rays.

Table 1 shows the most important demographic factors of the studied population.

Table 1. Variables of the studied population.

Demographic and clinical variables	Exposed population	Non-exposed population
Gender	80% (24/30) female 20% (6 /30) male	84% (25/31) female 16% (5/31) male
Age	23 - 29 años	17 - 20 años
Casual drinking	56,7%	40%
A family cancer precedent	26,7%	43,3%
Time of X ray exposure for dental radiographs	3 years	0 exposure

Evaluation of the genotoxic effect by means of the buccal smear micronucleus assay

Table 2 shows the micronuclei frequency found in the non-exposed population and some nuclear abnormalities. Table 3 shows the readings for micronuclei frequency and nuclear abnormalities in the exposed population. Table 4 compares the mean micronuclei frequencies in both populations: 1.68 ± 1.99 for the control population and 6.67 ± 5.2 for the exposed population and nuclear abnormalities. Application of the student's T test and non parametric Mann-Whitney U test for cross checking of the hypothesis throws up the value of p= 0.006, where significant values are considered to be those below 0.05. The difference in the micronuclei frequency between both populations is therefore significant, ruling out the null hypothesis and confirming the alternative. Nuclear abnormalities like pyknosis, karyorrhexis and condensed chromatin are taken as parameters of genotoxic and cytotoxic evaluation.

Table 2. Frequency of micronuclei (MN) and nuclear abnormalities in cells of the non-exposed (control) population

Estudiante número	Edad	Sexo	MN/2000 cel	Anormalidades nucleares					
				BN	Cariolisis	Cariorrexis	*Broken eggs*	Cromatina condensada	Picnosis
1	18	F	2					1	1
2	19	F	1					0	2
3	19	F	4					1	0
4	18	F	1	2	2	4		1	0
5	19	F	4					2	1
7	18	F	0					0	0
8	17	F	0	2				0	0
9	18	M	0					0	1
10	18	F	2					0	0
11	18	F	1				2	1	0
12	18	F	0				0	0	
13	18	F	2	2			6	0	0
14	19	F	3	0	1	2	3	0	0
15	20	M	1					0	1
16	19	F	9			35	15	1	1
17	18	M	2	5			6	0	0
18	18	F	1					0	0
19	19	F	4			3		0	0
20	19	F	0	1	1		6	0	0
21	19	F	2				5	1	1

22	18	F	1				2	0	1
23	19	F	2					1	0
24	19	F	0		12		9	0	1
25	19	M	5				2	0	0
26	18	M	0					2	1
27	19	F	3					0	1
28	20	F	0				1	0	2
29	19	F	2					1	0
30	20	F	0		3		4	0	1
31	18	F	0				3	0	0
		Medias	1,68	0,39	0,61	1,42	2,06	0.37	0,48
		desv. típ.	1,99	1,05	2,216	6,302	3,434	0,6	0,63

BN = bi nucleate.

Table 3. Frequency of micronuclei and nuclear abnormalities in cells of the exposed population

Estudiante número	Edad	Sexo	MN/2000 cel	Anormalidades nucleares					
				BN	Cariolisis	Cariorrexis	*Broken eggs*	Cromatina condensada	Picnosis
1	24	F	6	6	2	1	10	1	1
2	25	F	0	0	8	16	10	1	2
3	27	F	0	6	2	28	5	4	4
4	27	F	6	14	12	20	7	10	8
5	25	F	4	14	10	24	12	1	2
6	24	F	6	10	6	32	7	2	2
7	24	F	6	2	1	16	4	2	1
8	23	F	6	12	6	20	12	1	8
9	24	F	4	10	12	36	24	4	2
10	37	M	12	6	6	35	12	1	2
11	24	F	10	12	12	2	2	1	2
12	24	F	6	4	10	14	28	8	12
13	24	F	0	6	1	1	1	1	2
14	24	F	8	4	8	8	6	5	8
15	24	M	26	4	4	4	8	4	12
16	24	F	6	1	1	6	2	1	0
17	24	F	8	1	1	1	10	1	1
18	27	F	6	0	1	6	4	0	0
19	24	F	4	0	1	2	7	1	1
20	24	F	16	0	1	1	12	0	1
21	24	F	0	0	1	1	1	1	1

22	24	F	10	0	1	2	2	0	0
23	26	F	2	1	1	4	5	0	0
24	29	F	6	4	1	1	4	0	1
25	25	F	4	0	0	1	4	1	2
26	24	F	2	0	2	2	6	0	0
27	24	F	12	10	10	36	12	4	8
28	25	M	8	6	15	20	26	2	10
29	25	F	10	6	10	30	18	1	1
30	26	M	6	10	12	28	22	5	0
		Medias	6.67	4.97	5.27	13.27	9.43	2.13	3.47
		desv. típ.	5.287	4.65	4.712	12.736	7.44	2.403	4.066

**BN = bi nucleate.*

Table 4. Comparison of mean frequencies of MN and nuclear abnormalities in the exposed and non-exposed (control) populations.

	Non exposed population	Exposed population
Micronuclei/2000 cells	1,68	6,67
Binucleate cells	0,39	4,97
Karyolysis	0,61	5,27
Karyorrhexis	1,42	13,27
Broken eggs	2,06	9,43
Condensed chromatin	0,37	2,13
Pyknosis	0,48	3,47

Value of P < 0.006 for frequency of MN and pyknosis.

Figures 1 and 2 show the mean micronuclei frequency in the exposed and non exposed populations, respectively, broken down by age; the readings are higher in the exposed population.

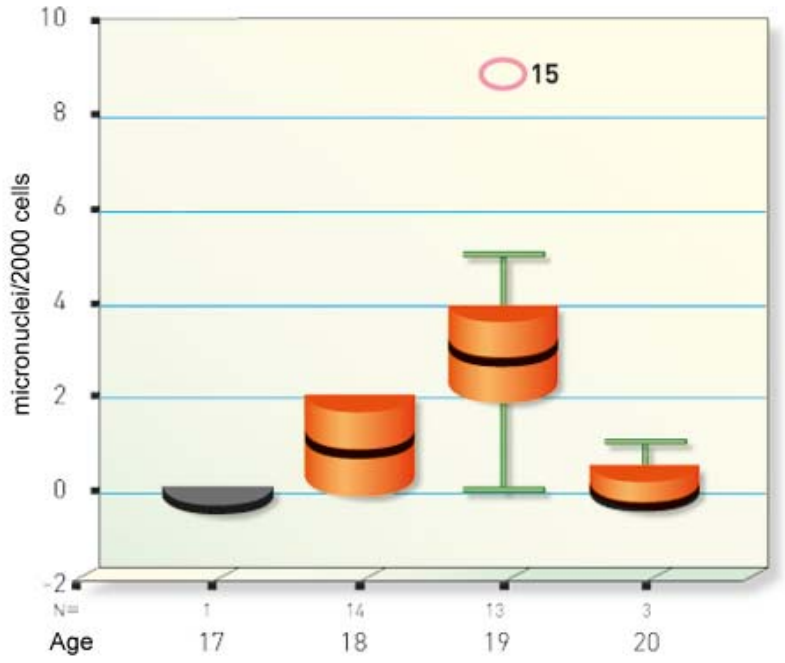

Figure 1. Boxplot of micronuclei per 2000 cells of the control (non-exposed) population, broken down by ages

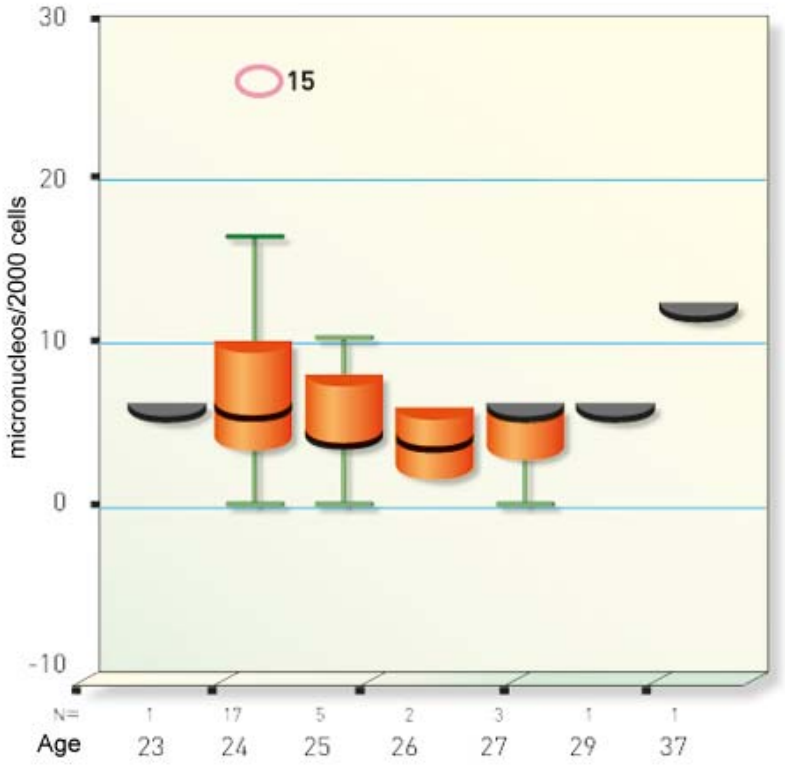

Figure 2. Boxplot of micronuclei frequency in cells of the exposed population, broken down by ages

The exposed population was made up by students in the final year of their degree course, who have been taking patient X rays for a chronic exposure period of three years. The control group is made up by students in the first years of their degree course, not exposed to X rays

Figure 3 compares the mean nuclear abnormality rate between the exposed and non-exposed population. This shows an increase in the mean figures of the exposed population, taking pyknosis, karyorrhexis and condensed chromatin to be characteristic indicators of cells in apoptosis, and binucleate cells and broken eggs to be characteristic of the genotoxic effect. Figures 4 and 5 show Feulgen stained buccal mucous at 1000 x magnification, with micronucleus and without respectively. Figures 6 and 7 show nuclear abnormalities such as binucleate cells and apoptotic cells with karyorrhexis.

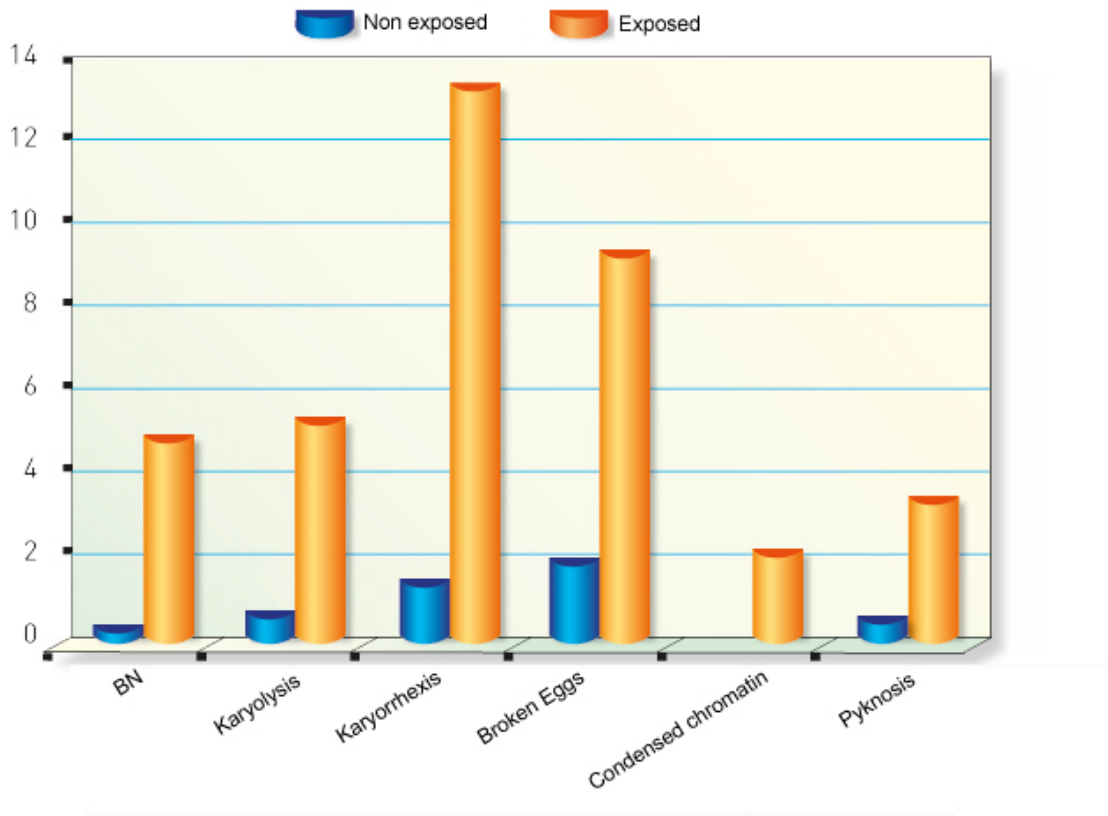

Figure 3. Nuclear abnormalities in exposed and non-exposed populations

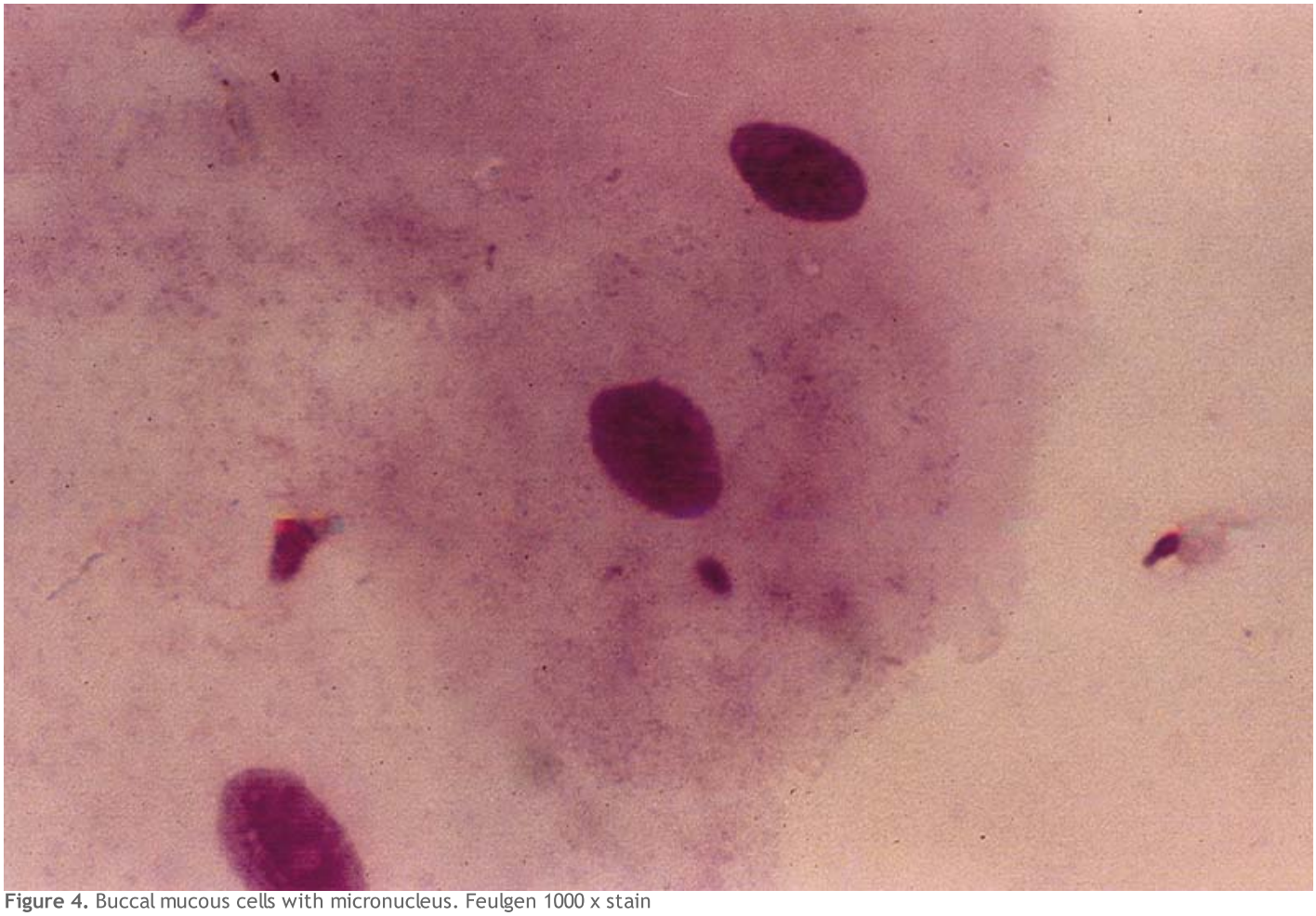

Figure 4. Buccal mucous cells with micronucleus. Feulgen 1000 x stain

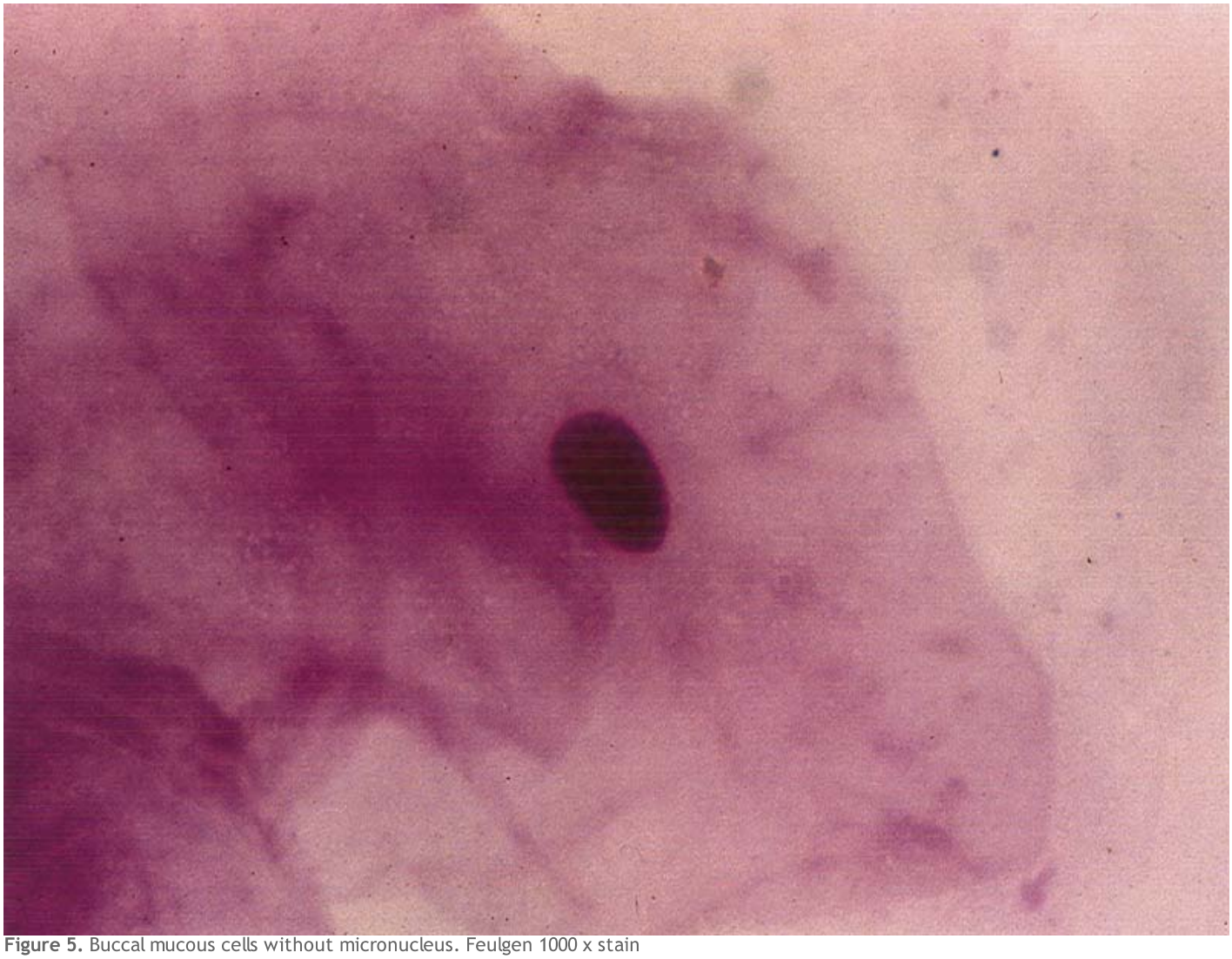

Figure 5. Buccal mucous cells without micronucleus. Feulgen 1000 x stain

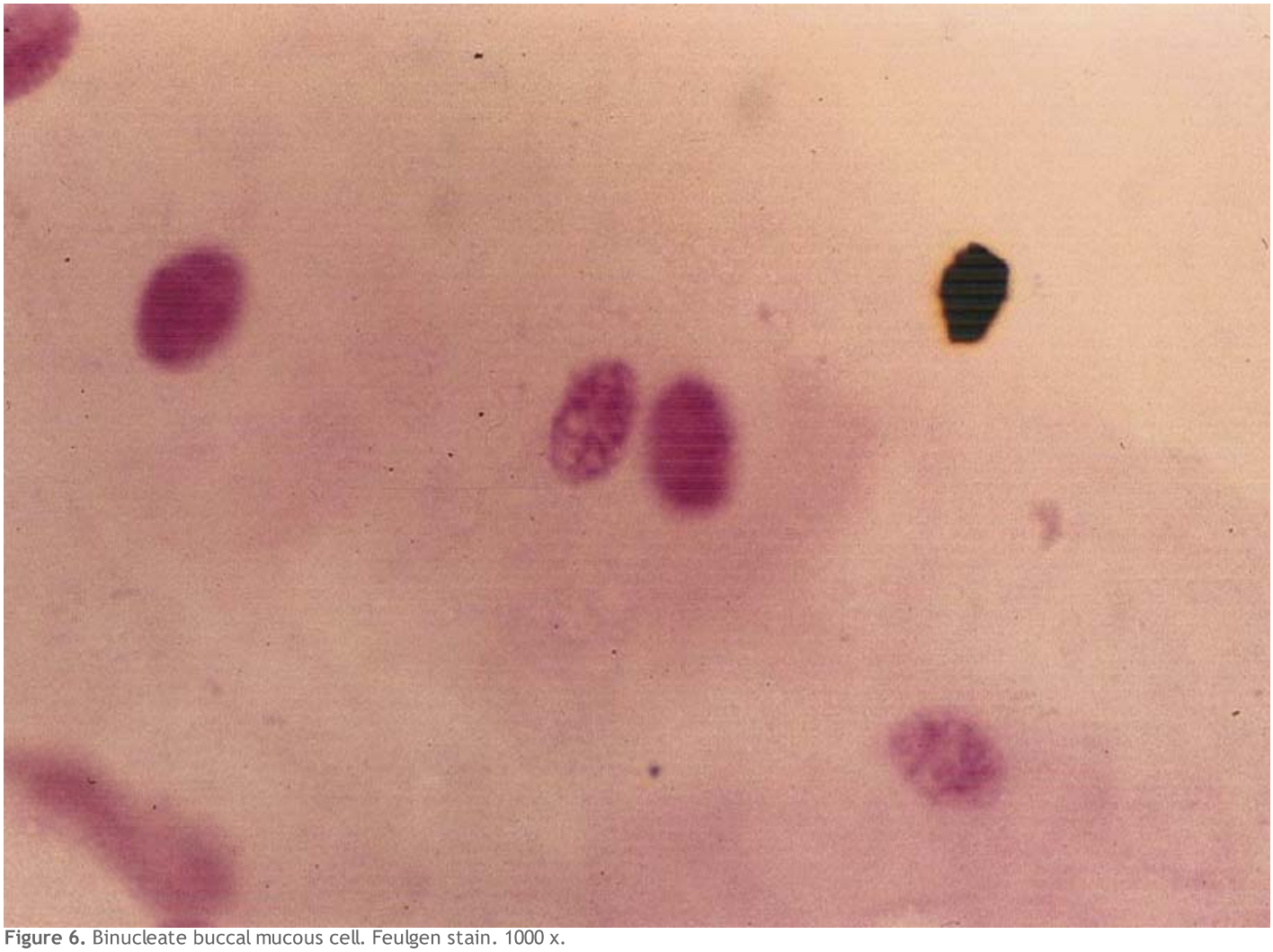

Figure 6. Binucleate buccal mucous cell. Feulgen stain. 1000 x.

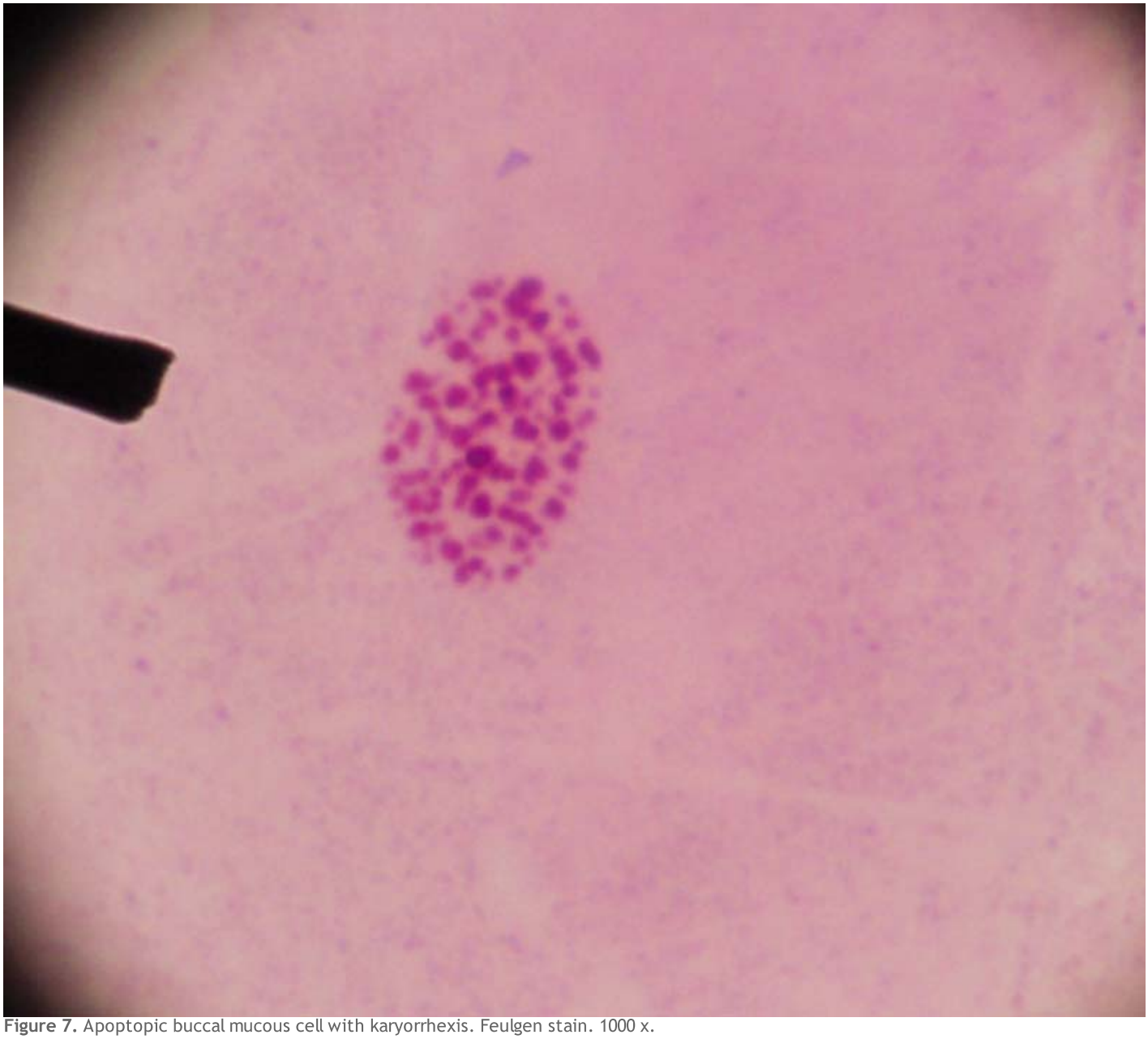

Figure 7. Apoptopic buccal mucous cell with karyorrhexis. Feulgen stain. 1000 x.

Comet assay evaluation of the genotoxic effect

By visual check

The results show a significant increase in the frequency of micronuclei (p< 0.05) and cells in apoptosis and other abnormalities of the nucleus like karyorrhexis, condensed chromatin, broken eggs and pyknosis in students chronically exposed to X rays

Table 5 shows percentage readings with different levels of cell damage according to the criterion of Speit (1995) and Collins (2004). This table shows a level I increase in the exposed population as compared with the non exposed population. The readings for levels II, III and IV, however, are not significant according to the comparison of student's T readings and the non parametric Mann Whitney U test for independent samples, throwing up a value of p =0.006 less than 0.05 for level I, and ipso facto considered to be statistically significant, and values higher than 0.05 in the other categories. Figure 11 shows the values for levels I, II, III and IV in both populations, with an obvious increase in level damage in the exposed population. The damage index (N= number of cells in category I + 2 X number of cells in category II + 3 X number of cells in category III + 4 X number of cells in category IV) was 37.8 for the exposed population and 3.4 for the non-exposed population, with a significant difference (p< 0.05) according to the Mann Whitney U test. These values show that the cell damage index in the exposed population is low, given the predominance of cells with level I damage. Figures 8, 9 and 10 show undamaged cells and cells with damage levels I and II, respectively.

Table 5. Cell damage levels according to the criterion of Speit (1995) and Collins (2004).

Groups	N	Mean	Standard deviation

Level 0	control group	31	74,52	15,558
	exposed group	29	64,38	16,003
Level I	control group	31	,55*	,888
	exposed group	29	6,41*	10,527
Level II	control group	31	,03	,180
	exposed group	29	,66	1,542
Level III	grupo control	31	,00	
	grupo expuestos	29	,31	
Level IV	Control group	31	,00	,000
	Exposed group	29	,31	1,339
Total damage	control group	31	3,4*	
	exposed group	29	37,8*	

**Student's T test p< 0,05*

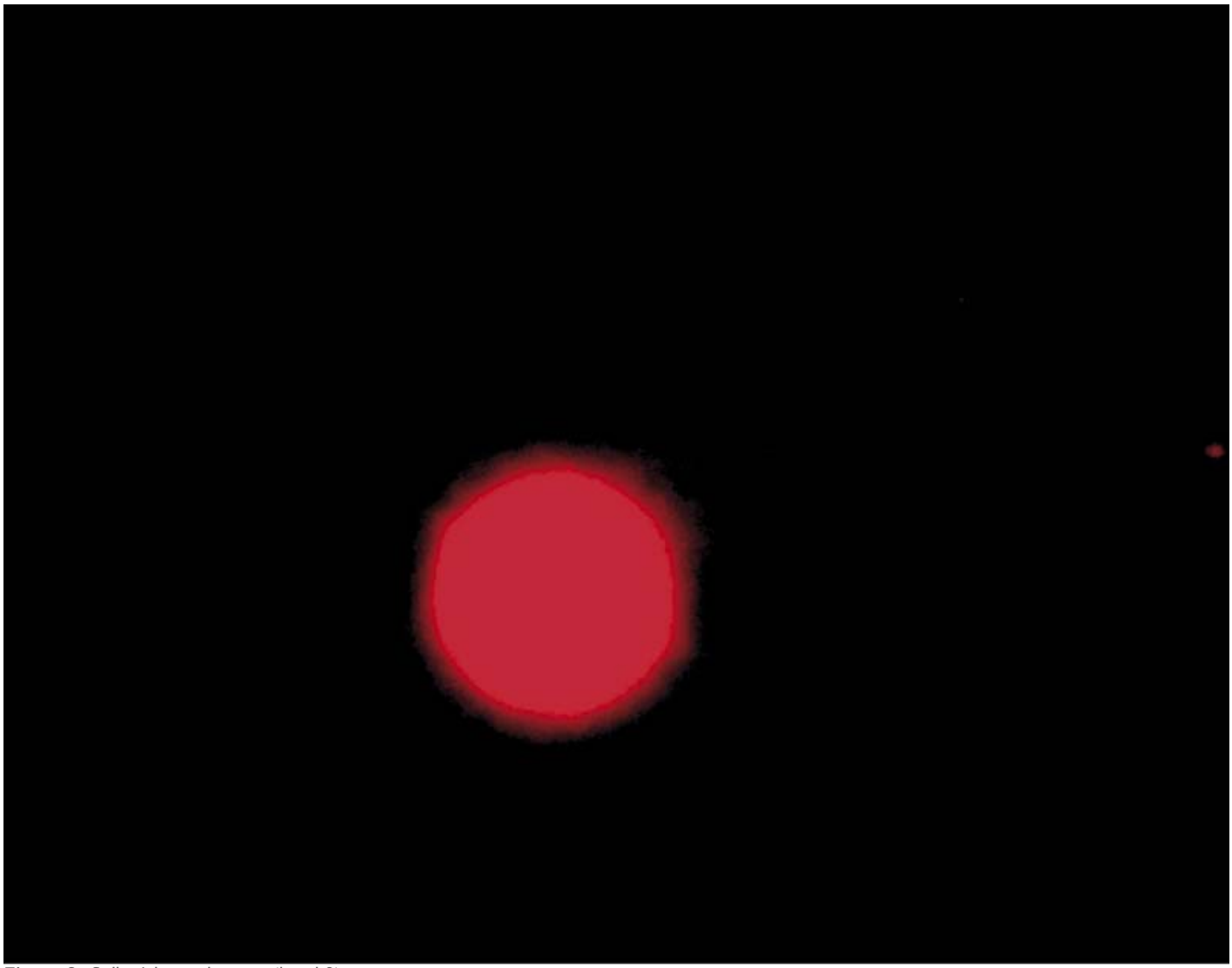

Figure 8. Cell without damage (level 0).

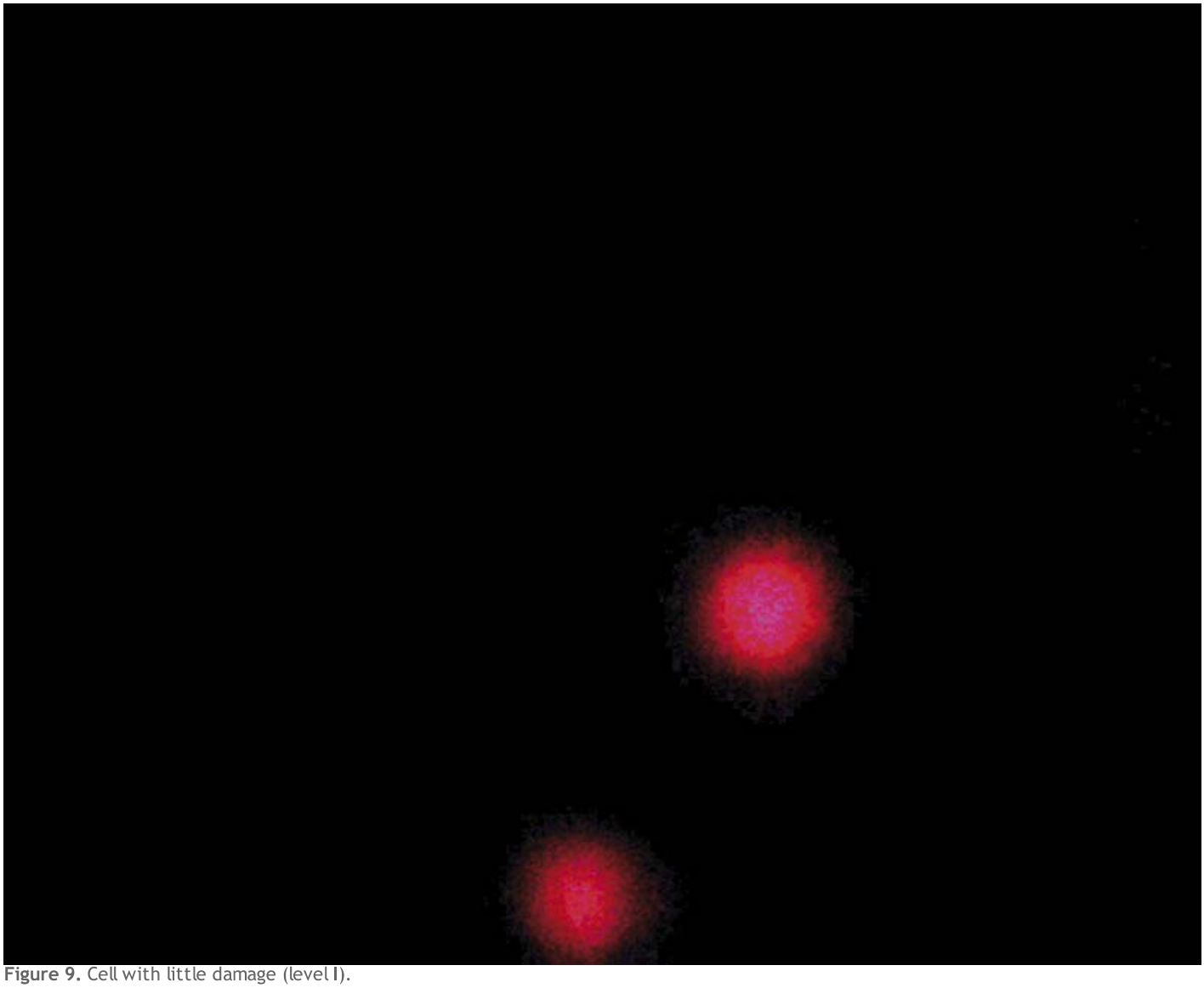

Figure 9. Cell with little damage (level **I**).

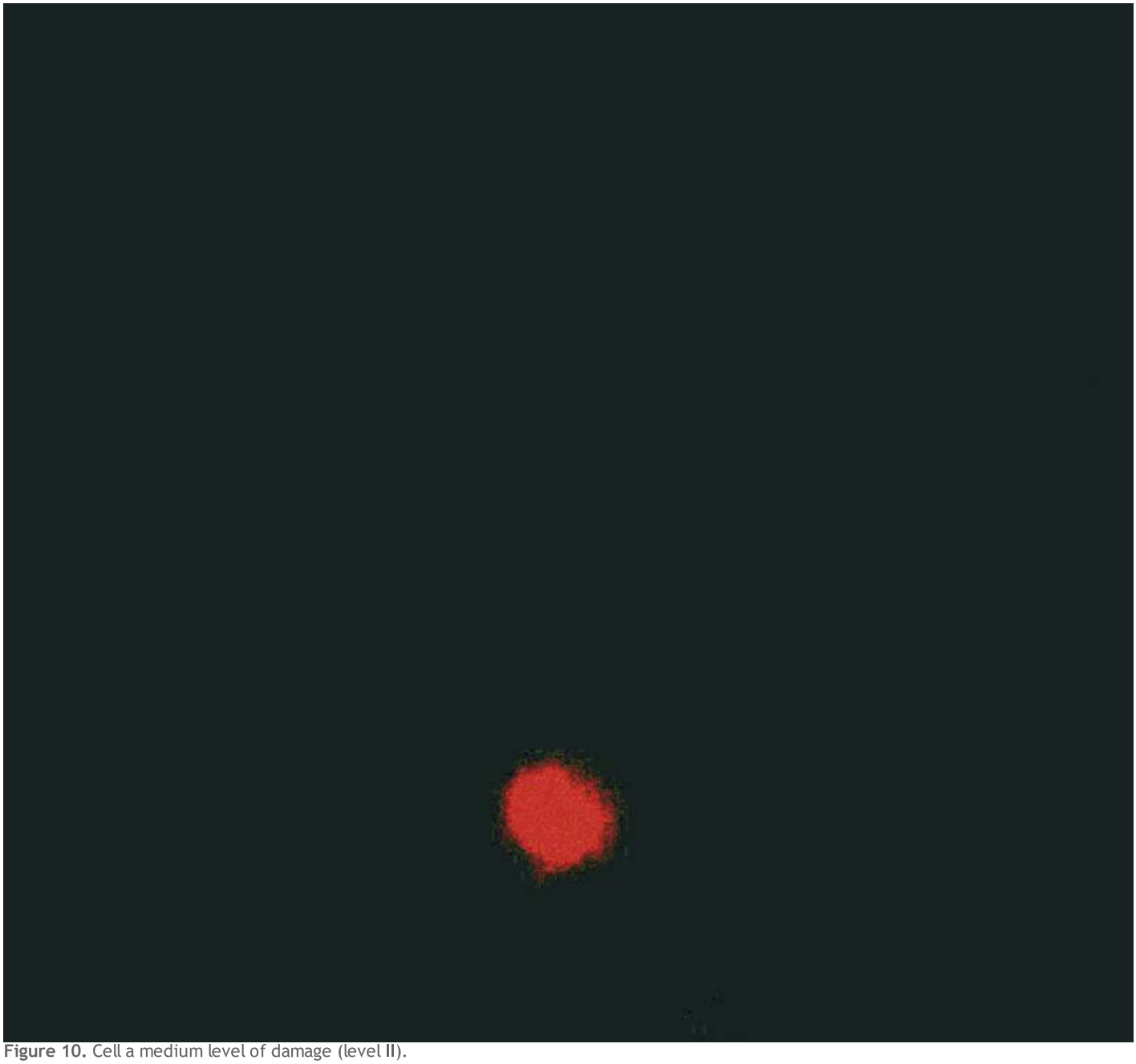

Figure 10. Cell a medium level of damage (level **II**).

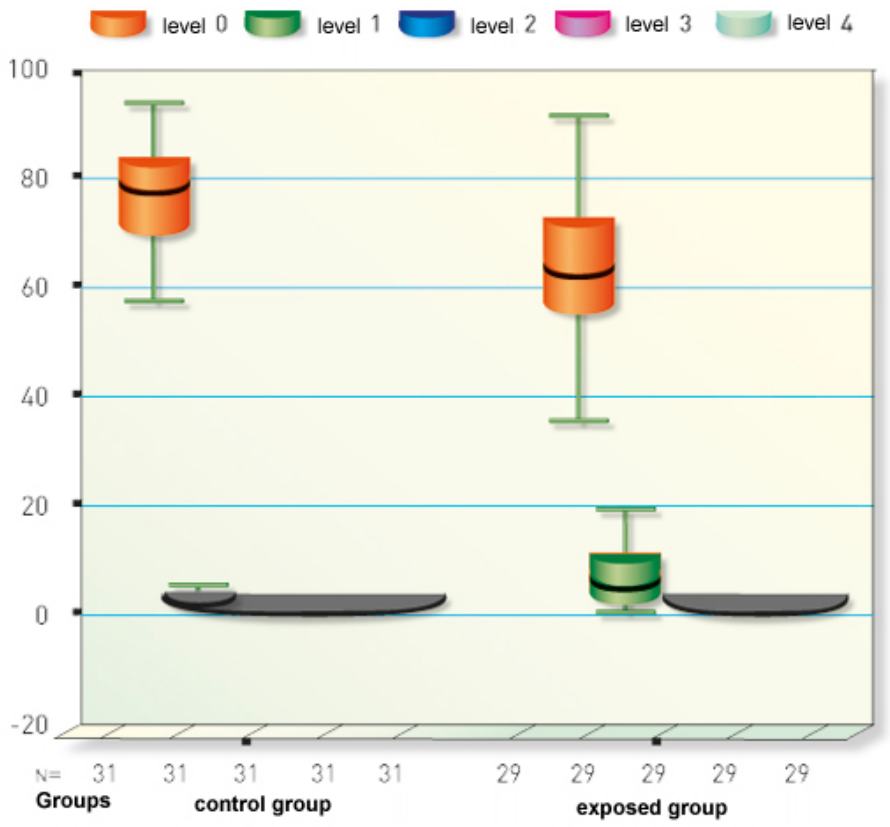

Figure 11. Boxplot of genotoxic damage by level in the control and exposed groups

The comet assay assesses percentage cell viability, ascertaining the rate of cells in apoptosis (figure 12). Table 6 shows the mean values obtained of cells in apoptosis for non exposed populations (22.6%) and exposed (47.9%) by means of a visua determination. Figure 13 reflect the values of cells in apoptosis for both populations.

Table 6. Mean apoptosis readings in exposed and non-exposed groups.

	Groups	N	Mean	Standard deviation	Typical mean error
Apoptosis	Grupo control	31	22,68	13,029	2,340
	Grupo expuestos	29	47,93	12,473	2,316

**Prueba de t p< 0,05*

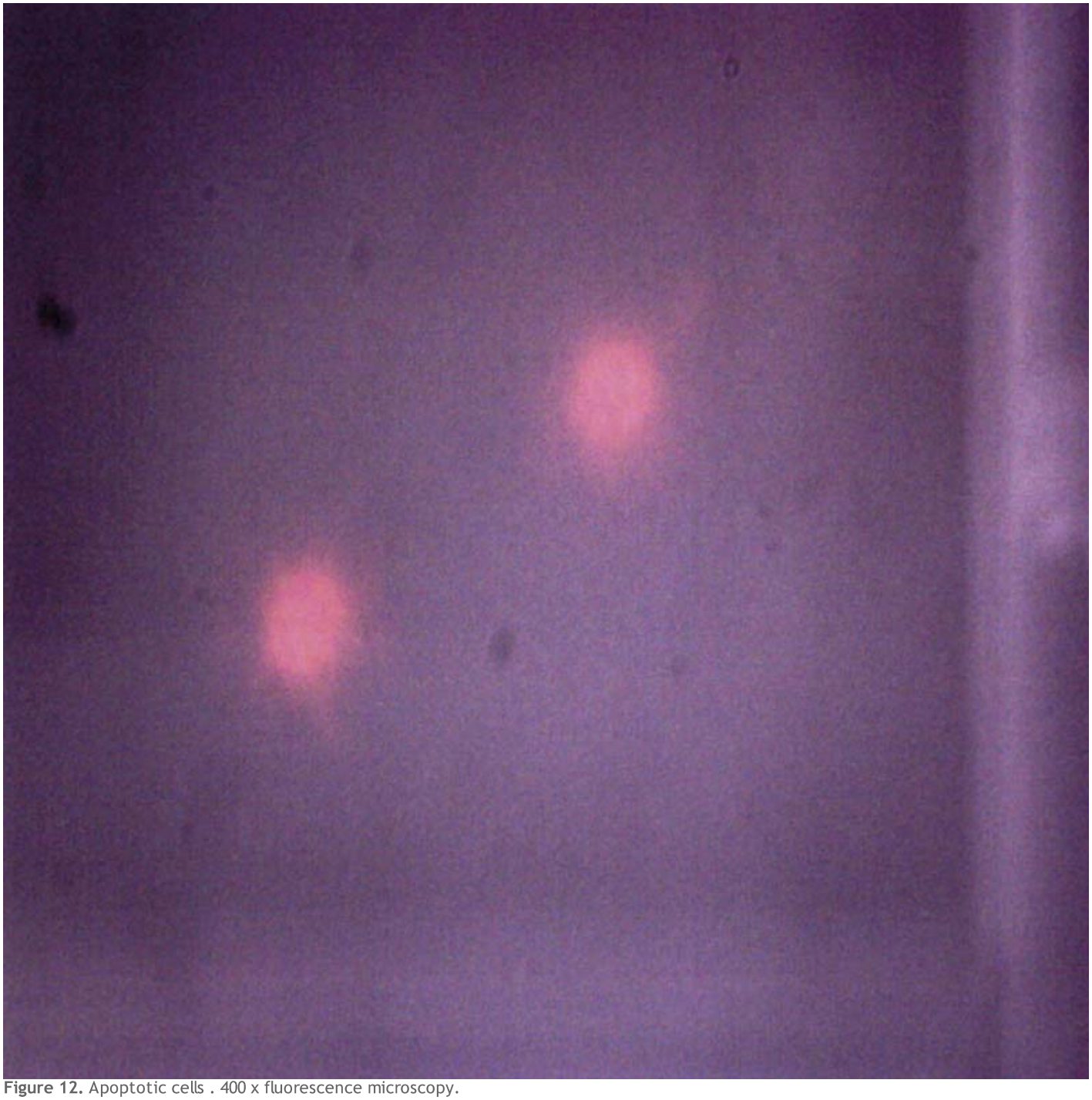

Figure 12. Apoptotic cells . 400 x fluorescence microscopy.

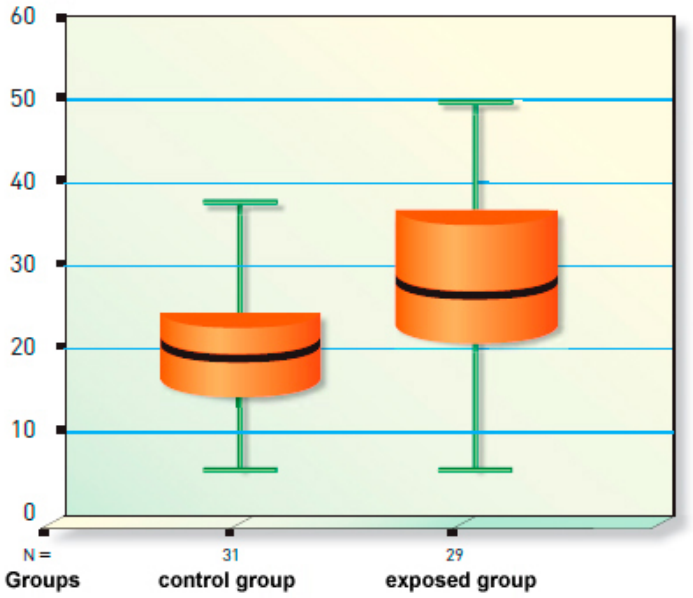

Figure 13. Apoptotic cell readings for both populations.

Evaluation by the software Casp Lab Comet Assay

No significant differences were found in DNA damage as measured by the comet assay and in comparison with the control population, indicating a cytotoxic rather than genotoxic effect

Table 7 shows the mean comet assay values for tail moment and tail momen olive of the exposed and control populations. Note the slight increase in reading for the exposed population; according to the Student's T test, however, these differences are not statistically significant . Figures 14 and 15, for their part show the mean boxplot values of both populations for the tail moment and tai moment olive, respectively.

Table 7. Comet assay tail moment and tail moment olive readings.

	Exposed	Control
Tail moment	1,34± 2,12	0,73±0,15
Tail moment olive	0,93±0,49	0,51±0,24

p>0.05 for Student's T test t.

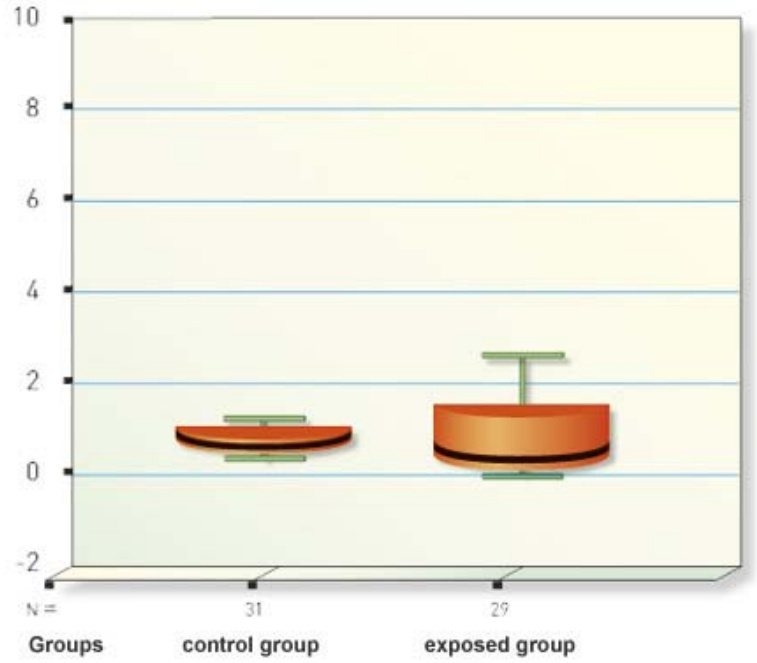

Figure 14. Mean boxplot tail moment readings for the exposed and non exposed populations.

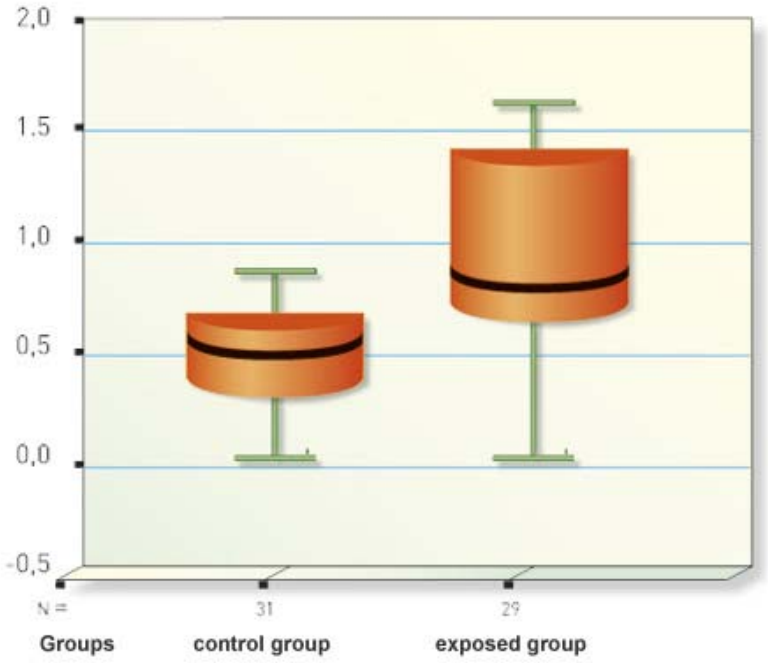

Figure 15. Mean boxplot tail moment readings for the exposed and non exposed populations

Conclusions

The results show the need for biomonitoring in other populations exposed chronically to subtoxic X ray doses

Study results show that the X ray dose and exposure time to which X-ray taking dentistry students are exposed produces a low level of damage (level I) to the DNA molecule in peripheral blood cells; the effects are rather cytotoxic than genotoxic. This finding chimes in with the opinion of other authors, who report an increase in cytotoxicity in buccal mucous in adult patients and children after panoramic X rays[14, 15,16], but not a significant increase in the frequency of micronuclei. The significant micronuclei-frequency values found could be due to exposure time (over three years) and the dose received, since the frequency of micronuclei increases with the dose and exposure to X rays (Ribeiro, 2008). These values might stem from failures in the cell division mechanism rather than chromosome breakage[16].

These findings suggest that exposure should be kept down to the strictly necessary and always with due radioprotection precautions.

BY WAY OF A GLOSSARY

Broken eggs. Nuclear abnormality characterised by the presence of a protuberance of variable size in the cell nucleus. It is related to DNA damage.
Pyknosis. Nuclear abnormality involving a nucleus of greatly reduced size, generally in response to a cellular lesion. It is characteristic of cells in apoptosis or necrosis.
Karyorrhexis. Disintegration of the nucleus and nuclear membrane. The chromatin is condensed in small groups, in apoptotic cells.
Karyolysis. A colourless look to the nucleus due to the dissolution of the chromatin. It occurs as a result of necrosis.
Condensed chromatin. The chromatin appears greatly condensed within the nuclear membrane, in response to high levels of cellular lesion.

ACKNOWLEDGEMENTS

To FUNDACIÓN MAPFRE, through its research aid programme (2010).

To the Centro para el Desarrollo de la Ciencia (CEDIC), for allowing us to use their labs.

To Dr. Enrique Zamorano Ponce, of the Universidad del Bío Bío de Chillán (Chile), for his constant assistance and advice and the training received.

To Dr. José Corvalán Amigo, Dean of the Facultad de Ciencias de la Salud of the Universidad Católica, for his help in carrying out the project and offering lab facilities.

To the managers of the Facultad de Odontología Pierre Fauchard, for authorising the participation of the students in this research.

To the dentistry students who took part in the research.

To the medical students of the Universidad Católica «Nuestra Señora de la Asunción» Juan Segovia, Luis Gatti and Daniela Solís for their help in carrying out the micronuclei bioassay.

TO FIND OUT MORE

1. Mi-Young A y Tae- Hwan K. Frequencies of micronuclei in peripheral lynphocytes in Korean population after chronic low dose radiation expousure. Veterinary Science 2002; 3:213-218
2. Cerqueira EMM. y Meireles JRC. Genotoxic effects of X-rays on keratinized mucosa cells during panoramic dental radiography. Dentomaxillofacial Radiology 2008; 37, 398-403.
3. Antonio E. L. Genotoxicidade e citoxicidade dos raios X no epitelio da mucosa oralnde criancas submetidas a radiografias panoramica (Tesis de maestría). Curitiba, Universidad Federal do Paraná, 2010.
4. Serto F, Finottus S, Glaconelli L, Mazzotti D, Tamanin R. The miconucleous assay in exfoliated cells of the human buccal mucosa mutagenesis 1987; 2: 11-17.
5. Bonassi S, Fenech M, Lando C. Yiping L, Ceppi M, Wushou et al. Human MicroNucleus Project: International database comparison for results with the Cytokinesis- Block micronucleous assay in human lymphocytes: I. Effect of Laboratory Protocol, Scoring Criteria, and Host Factors on the Frequency of Micronuclei. Mutat Res. 1999; 428: 271-283.
6. Mudry M. y Carballo, A. Genética toxicológica. De los Cuatro Vientos. Bs AS 2006.
7. Tolbert P, Shy C, Allen J. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat Res 1992; 271: 69-77.
8. Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S. The Human Micronucleus Project: an international collaborative study on the use of micronucleus technique for measuring DNA damage in humans. Mut Res. 1999; 428: 271- 283.
9. Singh N, McCoy M, Tice R, Schneider E. A simple technique for quantitation of low levels of DNAdamage in individual cells. EExp Cell Res 1988; 175: 184 -191
10. Singh NN, Stephens R, Schneider E. Modofocationns of alkaline microgel electrophoresis for sensitive detection of DNA damage. Inst Radiat Biol 1994; 66:23-28
11. Zúñiga Venegas L. Optimizaciones metodológicas del ensayo del cometa y su aplicación en biomonitorización humana

(tesis doctoral). Universidad Autónoma de Barcelona. Barcelona, 2009.

12. Speit G, Hartman A. The comet assay (single-cell gel test). A sensitive genotoxicity test for detection of DNA damage and repair. Iotwa N.J. 1995; 113: 20- 212
13. Colins A. The Comet assay for DNA damage and repair molecular. Biotechnology 2004; 26:249 - 261
14. Ribeiro L, Salvadori D, Marques E. Mutageneses ambiental. Ulbra, Sao Paulo 2003.
15. Cerqueira EMM, Gomes-Filho IS, Trindade S, Lopes MA, Passos JS, Machado-Santelli GM. Genetic damage in exfoliated cells from oral mucosa of individuals exposed to X-rays during panoramic dental radiographies. Mutat Res 2004; 562 111-117
16. Ribeiro D y Angelieri F. Cytogenetic biomonitoring of oral mucosa cells from adults exposed to dental X rays. Radiat Med 2008;26: 325 – 30.