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GENETIC BIOMONITORING IN WORKERS OF THE RADIOLOGY SERVICE OF PNP NATIONAL HOSPITAL LUIS N. SAÉNZ

BIOMONITOREO GENÉTICO EN TRABAJADORES DEL SERVICIO DE RADIOLOGÍA DEL HOSPITAL NACIONAL POLICIAL LUIS N. SÁENZ

Walter Iván Infantes-Vizcarra[1,2,a.]

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ABSTRACT

Objective: To evaluate the genotoxic effect on workers exposed to X-rays in the radiology service of the Luis N. Sáenz national hospital PNP. **Methods:** The type of study was observational, prospective, analytical, using the comet assay as an analysis technique. The study population was 20 workers exposed to X-rays and 20 people without exposure. **Results:** The mean length of migration of damaged DNA in the control group was 1.28 ± 0.38 µm compared to 10.39 ± 9.44 µm for the exposed group (p=0.001). DNA damage was significantly correlated with years of exposure and dose received (p <0.05) but not with age. **Conclusion:** X-rays at low permissible doses can cause damage to DNA integrity, correlating with the years of exposure and total dose exposure of personnel working in the radiology service.

Key words: Genotoxic; Exposure; X-ray; Comet test; Occupational health (source: MeSH NLM).

RESUMEN

Objetivo: Evaluar el efecto genotóxico en trabajadores expuestos a rayos X en el servicio de radiología del Hospital Nacional Luis N. Sáenz PNP. **Métodos:** Estudio observacional, prospectivo, analítico, utilizando el ensayo cometa como técnica de análisis. La población de estudio fue de 20 trabajadores expuestos a los rayos X y 20 personas sin exposición. **Resultados:** La media de longitud de migración de ADN dañado en el grupo control fue de 1,28±0,38 µm en comparación con 10,39±9,44 µm para el grupo expuesto (p=0,001). El daño de ADN se correlacionó significativamente con los años de exposición y dosis recibida pero no con la edad. **Conclusión:** Los rayos X a dosis bajas consideradas como permisibles pueden causar daño en la integridad del ADN, teniendo correlación con los años de exposición en el personal que trabaja en el servicio de radiología.

Palabras clave: Genotoxicidad; Rayos X; Ensayo cometa; Salud laboral (fuente: DeCS BIREME).

INTRODUCTION

The integrity of the genetic material is compromised with exposure to ionizing radiation, giving rise to genetic changes such as deletions and rupture of one or both strands of DNA[1]. The biological effects of ionizing radiation (X-rays) vary according to the exposure time and intensity, and the consequences are manifested in many organs, people exposed to high doses of radiation, induces cell death causing loss of functionality of one or more various tissues[2,3]. Currently, the effects of ionizing radiation at low doses and even more at doses less than 20-250mSv / year, the limit established by international organizations (IAEA) for the work-exposed population as the value below which the risk is minimal but not non-existent[4,5]. That is why research in this regard has been increased in vitro and in vivo, from the analysis of the mechanisms of molecular toxicity, genotoxicity, to the search for tools of genetic biomonitoring and biodosimetry that allow an approach to damage and better evaluation of short, medium and long term risk, allowing the

[1] Radiology Service, National Hospital PNP "Luis N. Sáenz", Lima-Peru.
[2] Faculty of Biological Sciences, Ricardo Palma University, Lima-Peru.
[a] Biologist.

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implementation of control measures[6]. Single chain ruptures and the formation of alkali labile sites in DNA are widely used parameters for genotoxicity detection. The comet test is very versatile and adaptable, capable of giving information about the different types of DNA damage present in a cell, and also about the cellular capacity to repair the damage[7].

In Peru, few statistics talk about occupational risks or accidents and injuries, to which radiology professionals are exposed[8]. The usefulness of the genotoxic study lies in demonstrating and determining the relationship between genetic damage and ionizing radiation. It also allows recommendations to be made to prevent diseases[9,10]. The purpose of this research was to determine the genotoxic effect of X-rays on the occupationally exposed personnel of the Radiology Service of the Luis N. Sáenz National PNP of Lima.

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METHODS

The type of study was observational, prospective, cross-sectional, analytical, during the period September-October 2013. The study population was 40 people divided into 2 groups, the first group is of the 20 workers exposed to the X-rays of the Service of Radiology of the National Hospital PNP LUIS N. SAENZ of Lima, with 6 hours of daily exposure for 5 days a week and a working time of exposure of at least 2 years and age of 20 years or more. The 2nd group was the control, formed by people from the same work center without exposure to X-rays. Participation in the study was voluntarily signing an informed consent letter to authorize the blood sample previously approved by the ethics committee of the hospital. The samples were sent to the Toxicogenetics laboratory of the National Center for Occupational Health and Environmental Protection for Health (CENSOPAS – INS, acronym in Spanish) for processing.

The comet test was performed under alkaline conditions, according to the method of Singh et al. (1988) with some minor modifications[11,12].

Blood samples were obtained by venous puncture with heparin tubes and the lymphocyte was obtained by a Percoll gradient that was centrifuged at 2200 rpm for 10 minutes. Subsequently, 25 ul aliquots of the lymphocyte samples were prepared and suspended in 75 µL of low melting point agarose (0.5%) and added to two sheets previously prepared with agarose. Subsequently, these were submerged in lysis solution (NaCl 2.5 mol / L, EDTA 0.1 mol / L and Tris 0.01 mol / L, 1% Triton, 10% DMSO, pH 10) for 1 h at 4° C and subjected to 20 min of unwinding in electrophoresis regulatory solution (3% NaOH 10 mol / L, 0.5% EDTA 0.2 mol / L, pH 13). The electrophoresis was performed at 300 mA and 1.25 V / cm for 20 min, all in darkness. The sheets were washed with neutralizing regulatory solution (Tris 0.4 mol / L, pH 7.5) and then the sheets were dehydrated with ethanol for 3 minutes. The preparations were stained with ethidium bromide[11,12]. Visualization was performed using an epifluorescence microscope. The image of each cell was captured using the Komet 4 program. To quantify DNA damage, the distance of tail length migration in µm was considered, considering the following levels: level 0, no damage (0-5 µm); level 1, low damage (6-20 µm); level 2, medium damage (21-40 µm); level 3, high damage (41-80 µm), and level 4, very damaged (> 80 µm) (table 1)[12,16.17].

The statistical analysis was carried out through the use of the SPSS v 21.0 software, the Mann Whitney U test for the contrast of groups, Chi - square for comparison of damage levels, Correlation of Spearman for the contrast of the variables and a significance level of $\alpha = 0.05$ was established.

RESULTS

The total number of individuals analyzed was 40 people, of which 5 women and 15 men were from the exposed group and 7 women and 13 men from the control group. The average age of the exposed group was 43.2 ± 9.31 years, while the control group was 45.5 ± 5.92 years. The average dose values of the ionizing radiation of the exposed group were 0.64 ± 0.58 mSv (V.N 20-50 mSv). The working time of the exposed personnel is 4-30 years with an average exposure of 12 ± 8.98 years.

Table 1, summarizes the values obtained from the comet test, between the control and exposed groups. The Mann Whitney U comparison statistic for the 2 groups was $p = 0.001$, so the difference found is significant. The X^2 test to compare the levels of damage between the groups also presented a significant value ($p < 0.05$).

Table 1. Comparison of DNA damage by X-rays between the exposed and control group.

Group	Damaged DNA Migration	DNA damage level				
	(µm)	0 (0-5 µm)	1 (6-20 µm)	2 (21-40 µm)	3 (41-80 µm)	4 (>80µm)
Control	1.26±0.38	1.26±0.38	0	0	0	0
Exposed	10.39±9.44	4.85±2.28	16.43±1.44	26.77±3.97	0	0

U Test of Mann Whitney p= 0,001 (p<0,05).
Test X^2 p=0,01 (p<0,05) Damage level : 0= without damage, 1=low damage, 2=medium
Showed values refer to the average±D.E. of the studied groups.

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Table 2, shows the Spearman coefficients for the correlation of age, exposure year, dose received (mSv) and DNA damage. Statistically significant positive correlations (p <0.05) were found between the parameters of exposure year, dose received and DNA damage. No significant correlation was found with age (p> 0.05).

Table 2. Spearman's correlation coefficient between age, exposure years and mSv.

Study Variables						
	Age		Years Exhibition		mSv	
	r	p	r	p	r	p
DNA tail damage	-0.237	0.141	0.720	0.003	0.218	0.001

DISCUSSION

The data obtained indicate that occupational exposure in the staff of the Radiology Service presents a statistically significant increase p = 0.001 (p <0.05). The comet test showed that the mean value of migration of DNA damage in the control group was only 1.28 ± 0.38 µm and the exposure was 10.39 ± 9.44 µm. This finding is in accordance with what was described by other authors such as Baquero et.al. (2004), Muñoz et.al (2008) and Ramírez (2002), where the cumulative effective doses showed that the exposed cohort has higher values compared to the unexposed, confirming its exposure to the emitting source, that is to say the intensifier of images[3,5,8]. The damage levels found in the control group were level 0 (no damage) concerning the exposed group that showed levels 1 and 2 (low and medium damage) presenting a significant statistic of p <0.05. These results are related to the studies by Guerci and Grillo (2007) where they point out that the lesions observed in cells exposed to ionizing radiation correspond to mild damage, which could be attributed mainly to single-chain lesions and base damage[15].

Spearman's correlation study between age and DNA damage in personnel exposed to X-rays was p> 0.05, with no statistical significance. These results agree with the studies of Gadhia et.al. (2004) and Martínez et.al. (2010), which did not show a direct relationship of age in individuals exposed with radiation[4,14].

The correlation of DNA damage and the years of exposure presented a p = 0.003 and a significant r = 0.720, indicating that there is greater damage in the first years of radiation exposure. The group exposed to X-rays showed an average exposure of 12 ± 8.98 years, presenting a range of exposure at work of 4-30 years. The results found are related to the studies carried out by Baquero et.al. (2004) and Díaz-Valecillos et.al. (2004) on chromosomal aberrations and years of exposure to X-rays at work, they agreed that the increase in genetic damage was between 1-10 years of exposure. This can be interpreted as an increase in chromosomal damage at the beginning of exposure[3,13].

The correlation between the received dose of mSv and DNA damage showed a p = 0.001 and a low significant r = 0.218. The average dose value of the ionizing radiation of the exposed group was 0.64 ± 0.58 mSv, which is below the annual values (V.N 20-50 mSv)[8,19]. The results suggest that DNA can be damaged with low values of exposure to X-rays. The results obtained are consistent with the findings of Díaz-Valecillos et.al (2004), Muñoz et.al (2008) and Fuentes Puebla et.al (2015) who concluded that chronic exposure to low doses of ionizing radiation is cumulative in the long

term and can induce the appearance of chromosomal alterations by keeping direct proportion to the total amount of radiation absorbed over time[3,5,18].

CONCLUSION

X-rays at low permissible doses can cause damage to the integrity of the DNA, having a correlation with the first years of exposure in personnel working in the radiology service of the Hospital Nacional Luis N. Sáenz PNP. The findings suggest the usefulness of the comet test as a useful tool to assess occupational health and prevent the early onset of pathologies.

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Correspondence: *Walter Iván Infantes Vizcarra.*

Address: *Laboratorio de Biotecnología animal. Facultad de Ciencias Biológicas. Universidad Ricardo Palma. Av. Alfredo Benavides 5440, Santiago de Surco, Lima-Perú.*

Telephone: *(01) 708-0000*

E-mail: *ivan.infantes.vizcarra@gmail.com*

BIBLIOGRAPHIC REFERENCES

1. Del Cura J, Pedraza S, Gayete A. RADIOLOGIA ESENCIAL. 4ta Ed. Buenos Aires. Argentina. Editorial. Panamericana. 2010. Vol. 1. p. 1-27. Disponible en: https://www.medicapanamericana.com/Libros/Libro/6361/Radiologia-Esencial-incluye-version-digital.html.

2. Munar Casas CR, Rios Mora YM. Análisis del programa de vigilancia epidemiológica de trabajadores con exposición a radiaciones ionizantes en una IPS de Colombia [Internet] [Salud Ocupacional]. [Colombia]: Universidad del Rosario; 2011 [citado 9 de noviembre de 2019]. Disponible en: https://repository.urosario.edu.co/handle/10336/2272

3. Baquero Pulido H, Guevara Pardo G, Giraldo Suárez M, Osorio Soto LM. Aberraciones cromosómicas en trabajadores expuestos a radiaciones ionizantes. Rev Cienc Salud [Internet]. 2004 [citado 9 de noviembre de 2019];2(1). Disponible en: https://revistas.urosario.edu.co/index.php/revsalud/article/view/591

4. Gadhia PK, Nehal S, Sweta N, Swati P, Krupa P, Meonis P. Cytogenetic Analysis of Radiotherapeutic and Diagnostic Workers Occupationally Exposed to Radiations: Int J Hum Genet. 2004;4(1):65-9.Disponible en: https://doi.org/10.1080/09723757.2004.11885872

5. Muñoz J, López A, Sarmiento I, Herrera C, Sánchez M. Biomonitoreo genético de individuos expuestos a radiaciones ionizantes y su relación con el cáncer. Lab. Genética molecular y Citogenética. UCE 2008; 18 (1): 75-82. Disponible en: https://issuu.com/udlaecuador/docs/librogeneticamolecular

6. Prieto M, Moreno M, Nava P, Zapata. Investigación, mediante técnicas de dosimetría biológica, de los efectos sobre la salud por causa de las radiaciones ionizantes de profesionales de líneas aéreas. Hospital General Gregorio Marañón. [Tesis de Grado]. Universidad Autónoma de Madrid. España. 2006. Disponlibe en: https://www.semae.es/wp-content/uploads/2011/11/Radiaciones-y-L%C3%ADneas-A%C3%A9reas.pdf

7. Garaj-Vrhovac V, Zeljezic D. Comet assay in the assessment of the human genome damage induced by γ-radiation in vitro. Radiol Oncol. 1 de marzo de 2004;38(1):43-7. Disponible en : https://www.researchgate.net/publication/288582942_Comet_assay_in_the_assessment_of_the_human_genome_damage_induced_by_g-radiation_in_vitro

8. Ramirez A. Tercerización del trabajo y sobreexposición a radiación ionizante en postulantes a services de minería. An Fac Med. 2002;63(4):291-300. Disponible en: http://sisbib.unmsm.edu.pe/BVRevistas/anales/v63_n4/pdf/tercerizacion_trabajo.pdf

9. Arencibia D, Rosario L., Rodríguez Y. Evaluación del daño basal e inducido en el ADN de linfocitos de tres líneas de ratones, mediante el ensayo cometa alcalino. Biotecnología Aplicada 2011; 28 (2): 101-105. Disponible en: https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=36410

10. Collins AR., Oscoz A., Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, et al. The comet assay: topical issues. Mutagénesis. 2008; 23(3):51-143. Disponible en: https://www.ncbi.nlm.nih.gov/pubmed/18283046

11. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res. marzo de 1988;175(1):184-91. Disponible en: https://www.ncbi.nlm.nih.gov/pubmed/3345800

12. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen. 2000;35(3):206-21. Disponible en: https://www.ncbi.nlm.nih.gov/pubmed/10737956

13. Díaz-Valecillos M, Fernández J, Rojas A, Valecillos J, Cañizales J. Alteraciones cromosómicas en trabajadores expuestos a radiaciones ionizantes. Invest. clín [Internet]. 2004 Sep [citado 2019 Nov 09] ; 45(3): 197-211. Disponible en: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0535-51332004000300002&lng=es.

14. Martínez A, Coleman M, Romero-Talamás CA, Frias S. An assessment of immediate DNA damage to occupationally exposed workers to low dose ionizing radiation by using the comet assay. Rev. Invest. Clin. 2010; 62 (1): 23-30. Disponible en: https://www.ncbi.nlm.nih.gov/pubmed/20415056

15. Güerci A., Grillo C. Evaluación del efecto genotóxico por exposición crónica a dosis bajas de radiación ionizante a través de un modelo in vitro. Revista Radiobiología 2007; 7: 166 – 173. Disponible en: https://pdfs.semanticscholar.org/a237/93f92621da78242c457ffb5be3fb8f6b72c3.pdf

16. Güerci A, Zúñiga L, Marcos R. El Valor Predictivo del Ensayo Cometa en la Evaluación de la Radiosensibilidad Individual en Sangre Periférica. Theoria, 2006; 15(2): 41-52. Disponible en: https://www.redalyc.org/pdf/299/29915205.pdf

17. Ravi K, Santosh M, Ajay K. Assessment of DNA Damage by Comet Assay in Lymphocytes of Workers Occupationally Exposed to Petroleum fumes. International Journal of Genetics 2010; 2 (1): 18-22. DOI: 10.9735/0975-2862.2.1.18-22 Disponible en: https://bioinfopublication.org/files/articles/2_1_3_IJG.pdf

18. Fuentes Puebla L, Felipe Torres S, Valencia Fernández V. Efectos biológicos de los Rayo-X en la práctica de Estomatología. Rev haban cienc méd [Internet]. 2015 Jun [citado 2019 Nov 09] ; 14(3): 337-347. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1729-519X2015000300011&lng=es.

19. Montoya M. La Ley N° 28028 y su aporte trascendental para mejorar la seguridad radiológica y física en el Perú. 2014. Disponible en : https://alicia.concytec.gob.pe/vufind/Record/IPEN_b77cbc881e20f5b2e13cc2bc21b91b62