

A Preliminary Study on DNA Damage in Peripheral Blood of Medical Personnel Occupationally Exposed to Ionizing Radiation Using Comet Assay

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Abstract. *The aim of this study is to estimate DNA strand breaks in lymphocytes of peripheral blood of medical personnel that occupationally exposed and unexposed to radiation. Methods: The exposed group consisted of 29 volunteers and the control group consisted of 4 volunteers. Samples consisting of 3 ml whole blood were collected by venepuncture. DNA damage in lymphocytes was detected by alkaline comet assay. The frequency of DNA damaged cells were evaluated by counting a total number of 50 cells per slide and determining DNA damage. The measurements of comets parameter for determining DNA damage was done using the semi-automatic software CASP. The results showed that value of the tail length, tail DNA, and the tail moment of medical workers exposed to radiation are higher than that of the control group but not significant. The similar results were also found based on gender consideration where female response were higher than of male but not significant. The conclusion that occupational exposure to ionizing radiation leads to an enhanced level of DNA damage in peripheral blood lymphocytes of nuclear medicine employees. This preliminary study results show that the comet assay can be used to detect DNA damage in radiation workers for the continuous efforts made in maintaining health and safety in the use of ionizing radiation. Further research needed to optimized established the method to be used as a biomarker of molecular biology in the study of radiation effects.*

Key words: comet assay, DNA damage, lymphocyte, radiation, occupational exposure.

Introduction

Application of ionizing radiation in many different fields is continuously increasing, including the use for medical purposes. Medical radiation workers are employees of hospitals, clinics and private offices where radiation is used in the process of delivering health care to humans. It has been shown that workers engaged in operational radiology (Zakeri F., 2010), and Nuclear Medicine is chronically exposed to low-level ionizing radiation (Bouraoui S et al, 2013).

The exposure of patients and workers to radiation in medicine is a direct consequence of the use of radiation to improve the health of individuals. Trends in radiation exposure of both patients and workers are effected not only by developments in radiation protection, but also by the doses used in the practice of medicine (Krajewska G, 2013). It is very important to estimate absorbed doses from individuals occupationally exposed to ionizing radiation in order to carry out radioprotection procedures and restrict the hazards to human health (Eken A, 2010). Proteins, cell membranes and DNA was subjected to radiation exposure, and the effect can be direct and indirect damages. Direct damage to the structure due to free radicals generated of ionization processes which interact directly with DNA. DNA damage caused by ionizing/excitation of DNA or the surrounding material, most of the water within the radical-diffusion 4 nm of DNA in a mobile environment is indirect damage (Morgan WF, 2006, Brooks, 2014).

The lymphocytes are the major and most important cells that are used as the bio indicators for the effect of ionizing radiation. The lymphocyte has

long been recognized as one of the most radiation-sensitive cells in the body. Lymphocytes located elsewhere in the body, such as in the lymph nodes, the thymus, the bone marrow and the spleen, have also been shown to be highly radiation sensitive although not all to the same degree (Muhammad, 2015). In other words, the time between irradiation and manifestation of the effect is considerably shorter in the case of peripheral lymphocytes. Of course, this difference may be due to the different methods of assessing radiation damage, or it may also be due to a difference in the immediate reaction of the cells to a radiation exposure (Muhammad et. al 2015, Małgorzata et. al 2014).

Another straight forward technique for the quantification of DNA damage in a cell-by-cell approach is the single cell gel electrophoresis or Comet assay (Nandhakumar S et al 2011). Its application under alkaline conditions as a sensitive technique in the regular health screening of workers who are occupationally exposed to genotoxic environmental agents to assess the possibility of different types of DNA damage [DNA strand breaks and alkali-labile sites (ALS)] is well documented (Collins A et al 2014). The comet assay is relatively sensitive for detection limit of γ -rays in peripheral blood lymphocytes (PBLs) and the number of cells needed for this analysis were, respectively, 0.6 cGy and 50 cells (Wang Y et al 2014). In this study we have done on the assessment of lymphocytes by using single cell gel electrophoresis technique (Comet) for detection of single DNA strand breaks mediated by quantifying the DNA damage that described the comet parameters, tail length (TL), tail DNA (%T), and tail moment (TM) was carried out

as an indicator of DNA damage among exposed hospital workers to radiation.

Materials and Methods

Subjects of Study

The population studied comprised 33 volunteer blood donors: 29 of them had been occupationally exposed to ionizing radiation and 4 were unexposed control subjects. Each person completed a standardized questionnaire which included items concerning personal data (age and health status) and occupational exposure to ionizing radiation at the time of the study. The questionnaire also included items concerning non-occupational exposure to potential mutagenic hazards, such as smoking, alcohol and drug consumption, viral diseases, recent vaccinations and radiodiagnostic examinations.

The exposed group consisted of 29 working in the radiology and surgery units (5 were physicians, 24 were radiological technicians). Their mean duration of occupational exposure was 17,5 years (range 5–48 years). Control subjects were administration hospitals employees (3 female and 1 male), The mean age of the control group was 41,75 (range 29–47 years). None of them had ever had any contact with sources of ionizing radiation or been occupationally exposed to known genotoxic agents.

Blood Sampling

Peripheral blood samples of the exposed and control subjects were collected once into heparinized tubes. Blood sampling and processing of exposed and control donors were carried out simultaneously. All blood samples were coded, cooled and processed within a maximum 2 h period after collection. The alkaline Comet assay in whole blood samples was performed immediately after blood transportation.

Lymphocyte Isolation

Lymphocytes are separated from it using Histopaque-1077. Briefly, blood is diluted 1:1 with PBS and layered over 3 ml Histopaque and centrifuged at 1500 RPM for 30 minutes. The 'buffy' coat is aspirated into 3-5 ml of PBS and centrifuged at 1000 RPM for 15 minutes to pellet the lymphocytes. The pellet is resuspended in ~1 ml of IRPMI and counted over a Haemocytometer. Nearly 2×10^4 cells per 100 μL of medium are taken for each dose of the test material (Singh et al 1988).

The Comet Assay

The Comet assay was carried out under alkaline conditions, basically as described by. Fully frosted slides were covered with 1% normal melting point agarose (Sigma). After solidification, the slides were then coated with 0.5% low melting point agarose (Sigma). When this layer had solidified, a second layer containing the whole blood sample

mixed with 0.5% low melting point (LMP) agarose (Sigma) was placed on the slides. After 10 min solidification on 4^oC. Afterwards the slides were immersed for 1 h in 4^oC cold freshly prepared lysis solution [2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris-HCl, 1% Na-sarcosinate (Sigma), pH 10] with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Sigma) added fresh to lyse cells and allow DNA unfolding. The slides were then placed in a horizontal gel electrophoresis tank, facing the anode. The unit was filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM Na₂EDTA, pH 13.0) and the slides were set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4^oC under dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis the slides were rinsed gently three times with a neutralization buffer (0.4 M Tris-HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 $\mu\text{g}/\text{ml}$) and covered with a coverslip. Slides were stored at 4^oC in sealed boxes until analysis (Tice RR, 2000).

Staining and Microscope Analysis

The air dried slides were stained with ethidium bromide solution (Merck, 20 $\mu\text{g}/\text{ml}$) and covered with cover slips. The slides were analyzed in a two sided blind manner under a Fluorescent microscope (Nicon) equipped with video camera (Sony). The frequency of DNA damaged cells were evaluated by counting a total number of 50 cells per slide and determining DNA damage. The measurements of comets parameter for determining DNA damage using The semi-automatic software CASP (E. Gonzalez 2012).

Statistical Analysis

Statistical analysis was carried out using Excell program. Student's *t-test* along with repeated measurement ANOVA were used to evaluate differences between exposed control populations, either male or female. Value of less than 0.05 was regarded as a significant level.

Results and Discussion

In the studies conducted on 33 workers aged between 24-71 year, from hospitals with a range of tenure 1-48 years. Medical workers evaluated include nurses, radiologists and physicians who working at the radiodiagnostic examination.

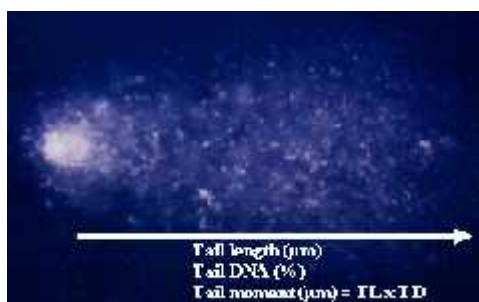


Figure 1. Comet areas included in comet tail moment calculation (1.3)

The comet preparats visualized by fluorescent microscopy and using CASPLab software to evaluated parameters for measuring DNA damage including tail length (TL), tail DNA (%T), and tail moment (TM) are used (Figure 1). The result of analysis the alkaline comet assay in medical workers presented in Figure 2.

There were significant differences between the damage to genetic material of lymphocytes in the control and exposed groups; however, there was variability between individuals in both groups (Fig. 2). The range values for the comet tail lengths in the control group varied from 12,45 to 15,06, whereas in exposed group varied from 9,36 to 21,94, % tail DNA varied in the control group from 8,69 to 9,92 and in the exposed group from 18 to 18,74, similarly tail moments varied in the control group from 0,98 to 5,5 µm and in the exposed group from 1,81 to 2,23 µm. (Fig. 2).

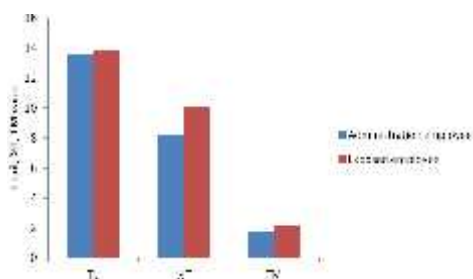


Figure 2. DNA damage in lymphocytes in the control and exposed subjects that analysis with the comet assay.

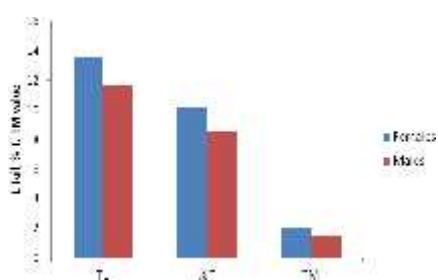


Figure 3. DNA damage in lymphocytes of females and males among exposed subjects that analysis with the comet assay.

DNA damage in lymphocytes of females and males among exposed subjects (Figure 3). The mean values for the comet tail lengths in Females $13,6 \pm 4,5$, whereas males in exposed group $11,6 \pm 3,1$, The mean value of % tail DNA in females $10,2 \pm 3,2$, whereas males $8,5 \pm 2,2$, the mean values of tail moments in females $2,1 \pm 1,2$ and in males $1,5 \pm 0,5$. The level of DNA damage in white blood cells of exposed females was higher compared with the exposed males, whereas the differences were not significant.

In our study, there were no significant differences between damage to DNA in the control and exposed groups, females compared with males in the exposed groups, both similiar.

The same study conducted by Vrhovac and Kopjar of Croatia doing research to see DNA damage due to radiation exposure at medical workers with using the alkaline comet test. The population studied comprised 40 volunteer blood donors: 25 of them had been occupationally exposed to ionizing radiation and 15 were unexposed control subjects. The results were the mean values of comet tail lengths measured in exposed subjects of $17,49 \pm 0,13$ µm, % tail DNA which were in the range of $14,85 \pm 0,21$ %. The mean values of tail length measured in control subjects were $13,56 \pm 3,4$ µm, % tail DNA were $8,14 \pm 2,3$ %. TL and % T workers comet assay results are significantly different than the control ($p < 0,05$, Mann-Whitney *U* test) (Kopjar 2005).

The results of our study of DNA damage caused by radiation in medical Hospitals as well as Vrhovac and Kopjar research shows the parameter results of the comet assay of radiation workers more higher than control. Both these two studies showed the influence of radiation on DNA damage in peripheral blood lymphocytes exposed workers.

Ionizing radiation induces DNA damage directly (as result of deposition of energy in cells) or indirectly (as result of free radicals formation and oxidative damage). It has been long known that physicochemical interactions between ionizing radiation and DNA produce a broad spectrum of DNA lesions including damage to nucleotide bases, DNA-DNA and DNA-protein cross-links, alkali labile sites as well as DNA single-strand and double-strand breaks (DSBs). Lesions induced by ionizing radiation in DNA can be detected by the alkaline single cell gel electrophoresis or comet assay (Azqueta 2013, Georgakilas 2008, Santos 2010).

Comet assay is based on the assumption that DNA migrating from the nucleus within the gel after electrophoresis is the result of genotoxic damage that is converted to DNA single- or double-strand breaks. Many studies have found that the exposed worker increased DNA migration (Liao et al 2009) and our results are consistent with the findings (Figure 3). The increased comet values in peripheral

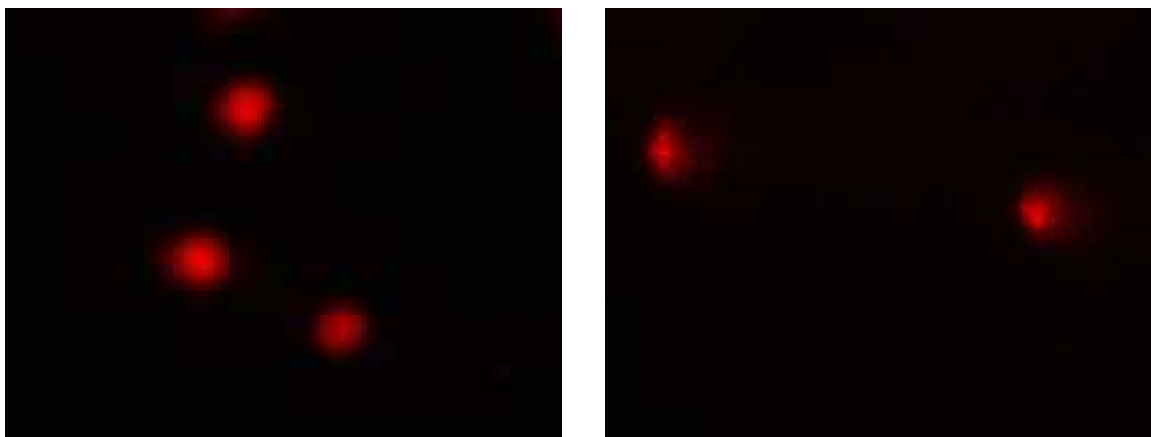


Figure 3. The results of the test visualization comet Lymphocyte cell of administration employee (left) and exposed employee (right).

blood leukocytes of radiological workers exposed to ionizing radiation in the present study indicate a higher level of radiation-induced primary DNA damage (Martínez 2010).

On the results of the initial study on DNA damage test with the comet assay abroad range of data obtained show high variation between individuals comet data. Comet measurements may reflect both individual repair ability and A damage level. Because the measured damage level is a result of equilibrium between damage infliction and repair, a low damage level as assessed experimentally in an individual may be the result of an actual low number of lesions or of a high efficiency of repair (Georgakilas et al 2008).

Ionizing radiation is a ubiquitous environmental physical agent whose DNA damaging effects are fairly well established. It induces DNA damage directly (as result of deposition of energy in cells) or indirectly (as result of free radicals formation and oxidative damage). It has been long known that physicochemical interactions between ionizing radiation and DNA produce a broad spectrum of DNA lesions including damage to nucleotide bases, DNA-DNA and DNA-protein cross-links, alkali labile sites as well as DNA single- and double-strand breaks (DSBs). Lesions induced by ionizing radiation in DNA can be detected by the alkaline single cell gel electrophoresis or comet assay (Collins et al 2014). The same method was evaluated in the present study on occupationally exposed medical personnel. The comet assay is an easy, quick and accurate test that has been widely applied to measure both *in vitro* DNA damage and repair following exposure to various genotoxic agents and for human biomonitoring (E. Gonzalez 2012). Weakness in the comet assay is a variation on the results of the comet assay is an important issue

that must be considered variations can occur between some of the experiments in the laboratory even used the same method, because of the need to use the reference standard is very useful when performing on going research to determine the consistency of the methods used (Collins 2014). In present study the alkaline comet assay revealed the heterogeneity in the level of DNA breakage induced in human lymphocytes by occupational exposure to ionizing radiation.

The results of the evaluation of the comet assay studies, the value of the tail length, tail DNA, and the tail moment of medical workers exposed to radiation are higher than the control group but not significant. The similar results were also found based on gender consideration where female response were higher that of male but not significant.

Conclusion

The conclusion that occupational exposure to ionizing radiation leads to an enhanced level of DNA damage in peripheral blood lymphocytes of nuclear medicine employees. The preliminary studied results show that the comet assay can be used to detect DNA damage in radiation workers for the continuous efforts made in maintaining health and safety in the use of ionizing radiation. Further research needed to optimized established the method to be used as a biomarker of molecular biology in the study of radiation effects.

References

- Azqueta A, Collins AR. (2013). The essential comet assay: a comprehensive guide to measuring DNA damage and repair. *Arch Toxicol.*, 87(6), 949–68.
- Bouraoui S, Mougou S, Drira A, et al. (2013). A cytogenetic approach to effects of low levels of ionizing radiation (IR) on the exposed Tunisian hospital workers. *Int J Occup Med Environ Health*; 26: 144–54
- Brooks, A.; Lawrence, D. (2014). Radiation Dosimetry and Risk in the Practice of Nuclear Medicine. *Advances in Radiation Biology: Effect on Nuclear Medicine*, 44, 179 – 186,
- Collins A, Koppen G, Valdiglesias V, Dusinska M, Kruszewski M, Møller P, et al. (2014). The comet assay as a tool for human biomonitoring studies: The ComNet Project. *Mutat Res Rev Mutat Res.*, 759, 27-39.
- Collins AR, Yamani NE, Lorenzo Y, Shaposhnikov S, Brunborg G, Azqueta A. (2014). Controlling variation in the comet assay. *Frontiers in Genetic* , 5 (359), 1-6.
- E. Gonzalez, I. Romero, J.F. Barquinerob, O. Garciaa. (2012). A Laboratorio de Radiobiología, Centro de Protección e Higiene de las Radiaciones, La Habana, Cuba, Automatic analysis of silver-stained comets by CellProfiler software *J. Mutation Research*, 748, 60– 64.
- Eken A, Aydın A, Erdem Q, Akey C, Sanal H. T, Soykut B, Somuncu I. (2010). Cytogenetic analysis of peripheral blood lymphocytes of hospital staff occupationally exposed to low doses of ionizing radiation. *Toxicology and Industrial Health*, 26:,273-80.
- Georgakilas, A.G., (2008): Processing of DNA damage clusters in human cells: current status of knowledge, *Mol. BioSyst.*, 4 ,30–35.
- Krajewska G, Pachocki KA. (2013). Assessment of exposure of workers to ionizing radiation from radioiodine and technetium in nuclear medicine departmental facilities. *Med Pr*; 64 (in press).
- Kopjar N and Garaj-Vrhovac V (2005) Assessment of DNA damage in nuclear medicine personnel comparative study with the alkaline comet assay and the chromosome aberration test. *International Journal of Hygiene and Environmental Health*, 208: 179 - 191.
- Liao, W.; McNutt, M.A.; Zhu, w.g. (2009). The comet assay: a sensitive method for detecting DNA damage in individual cells. *Methods*, 48, 46 – 53, 2009.
- Małgorzata M. Dobrzyńska, Krzysztof A. Pachock, Aneta Gajowik, Joanna Radzikowska and Agata Sackiewicz. (2014). The Effect Occupational Exposure to Ionizing Radiation on the DNA Damage in Peripheral Blood Leukocytes of Nuclear Medicine Personnel, , *J Occup Health*, 56, 379–386
- Martínez, A.; Coleman, M.; Romero-Talamás, C.A., Frías, S. (2010). An assessment of immediate DNA damage to occupationally exposed workers to low dose ionizing radiation by using the comet assay. *Rev Invest Clin*, 62, 23– 0.
- Morgan WF. (2006). Will radiation-induced bystander effects or adaptive responses impact on the shape of the dose response relationships at low doses of ionizing radiation? *Dose Response*, 4, 257 - 262.
- Muhammad Khisroon1, Ajmal Khan1, Maryam Naseem1, Naheed Ali1, Sardar Khan2 and Syed Basit Rasheed1. (2015). Evaluation of DNA damage in lymphocytes of radiology personnel by comet assay, *J Occup Health*, 57, 268–274
- Nandhakumar S, Parasuraman, Shanmugam MM, Ramachandra Rao K, Parkash Chand and Vishnu Bhat B. (2011). Evaluation of DNA damage using single cell gel electrophoresis(Comet Assay). *J pharmacol pharmacother*, 2(2),107-11.
- Santos RA, Teixeira AC, Mayorano MB, Carrara HHA, Andrade JM, Takahashi CS. (2010). Basal levels of DNA damage detected by micronuclei and comet assays in untreated breast cancer patients and healthy women. *Clin Exp Med.* ,10 (2), 87–92.
- Singh NP, Mc Coy, Tice RR, Schneider EL. (1988). A simple technique for quantization of low level of DNA damage in individual cells. *Exp Cell Res.*, 175, 184–91.
- Tice RR, Agurell E, Anderson D, et al. (2000). Single cell gel/comet assay guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen*, 35, 206–21
- Wang Y, Xu C, Du L, Cao J, Liu J, Su X, et al. (2013). Evaluation of the Comet Assay for Assessing the Dose-Response Relationship of DNA Damage Induced by Ionizing Radiation. *Int J Mol Sci.*, 14(11), 22449-61.
- Zakeri F, Hirobe T. (2010). A cytogenetic approach to the effects of low levels of ionizing radiations on occupationally exposed individuals. *Eur J Radiol*, 73, 191–5.

Discussion

Q : Cameron Jeffries

What dose of employees received ?.

A : Darlina

Unfortunately, until now the hospital have not give us the data of employees dose of radiation they received.