# THE ASSESSMENT OF PRIMARY DNA DAMAGE IN MEDICAL PERSONNEL OCCUPATIONALLY EXPOSED TO IONIZING RADIATION

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## INTRODUCTION

In physico-chemical interaction with cellular DNA ionizing radiation produces a variety of primary lesions, such as single-strand breaks (SSB), alkali-labile sites, double-strand breaks (DSB), DNA-DNA and DNA-protein crosslinks, and damage to purine and pyrimidine bases. The effects of low-level exposure to ionising radiation are of concern to large number of people, including workers receiving radiation exposure on the job. It is very important to estimate absorbed doses from individuals occupationally exposed to ionising radiation for carrying out radioprotection procedures and restrict the hazards to human health. A wide range of methods is presently used for the detection of early biological effects of DNA-damaging agents in environmental and occupational settings. Currently, unstable chromosomal aberrations in peripheral blood lymphocytes, in particularly dicentrics, are the most fully developed biological indicators of ionizing radiation exposure [1,2]. This methodology usually complements data obtained by physical dosimetry. As a routine, it is used whenever the individual dosimeter shows an exposure to penetrating radiation above its limit of detection. One of the advantages of cytogenetic dosimetry is that this biological dosimeter can be assessed at any moment whereas physical dosimeters are not always present in the subject. During the last years, the single cell gel electrophoresis (SCGE) or comet assay has gained widespread acceptance for genotoxicity testing [3,4]. In molecular epidemiology studies DNA damage evaluated by the comet assay is utilized as a biomarker of exposure [5,6]. The comet assay permits the detection of primary DNA damage and the study of repair kinetics at the level of single cells [4].

The aim of the present study was to assess and quantificate the levels of DNA damage in peripheral blood leukocytes of medical workers

occupationally exposed to ionizing radiation and corresponding unexposed control subjects. As a sensitive biomarker of exposure the alkaline comet assay was selected.

## SUBJECTS AND METHODS

The population studied comprised 100 volunteer blood donors: 50 of them had been occupationally exposed to ionizing radiation and 50 were unexposed control subjects. Each person completed a standardised questionnaire which included items concerning personal data (age, health status) and occupational exposure to ionizing radiation at the time of the study. The exposed group consisted of 16 female and 34 male subjects aged 22-62 years (mean age 43.3 years) working in the radiology and surgery units of six Croatian hospitals. Ten of them were nurses, 15 physicians and 25 radiological technicians. Their mean duration of occupational exposure was 17.8 years (range 2-37 years). During their work they all wore individual dosimeters (film badges). The range of dosimeter readings for exposed group during one year prior the study was 0-8548 μSv. Fifteen exposed subjects were smokers (8 female and 7 male), and 35 non-smokers (8 female and 27 male). One year prior to the beginning of the present study exposed subjects had not been subjected to diagnostic X-ray examinations.
Control subjects were healthy students and office employees (20 female and 30 male), chosen from the general Croatian population. 29 of them were non-smokers (12 female and 17 male), and 21 of them were smokers (8 female and 13 male). The mean age of the control group was 42.1 (range 22-60 years). None of them had ever had any contact with sources of ionizing radiation. They also had not been occupationally exposed to known genotoxic agents. Peripheral blood samples of the exposed and control subjects were collected by venipuncture into heparinised tubes. The alkaline comet assay was performed immediately after blood transportation. The comet assay was carried out under alkaline conditions, basically as described by Singh [3]. A total of 100 randomly captured comets from each slide were examined using an epifluorescent microscope connected through a black and white camera to an image analysis system (Comet Assay II, Perceptive Instruments Ltd., U.K.). To quantify the DNA damage tail length (TL) and tail moment (TM) were evaluated. Both comet parameters measured in the exposed and control groups were evaluated by using non-parametric Mann-Whitney U test and MANOVA on LOG transformed data. The level of significance was set at 5%.

## RESULTS

Results of the alkaline comet assay have been summarised in Table 1. Among radiologists tail length measures were in range: technicians > nurses > phisicians (Table 1), while the highest individual level of DNA migration was recorded in exposed subject No. 3 (a nurse employed at radiology unit). Among surgeryst tail length measures were also in range: technicians > nurses > physicians (Table 1). Although mean values of comet parameters measured in peripheral blood leukocytes of medical personnel employed at surgery unit were increased compared to those recorded in subjects employed at radiology, observed differences were not statistically significant (MANOVA, $p>0.05$). No statistically significant differences were found between the mean values of comet tail parameters recorded in exposed smokers and non-smokers, independently on their gender and occupation (Mann-Whitney *U* test; MANOVA, $p>0.05$). It was also observed that age did not significantly influence the levels of DNA damage among exposed subjects under 30 and above 50 years. However, in exposed subjects aged between 30 and 50 years increased DNA migration compared both to younger and older exposed age groups was noticed (MANOVA, $p<0.05$).

On the other hand, distribution of comet tail parameters in control subjects was characterised by nuclei with smaller comets. Although some unexposed smokers had increased levels of DNA damage compared to non-smokers, the mean values of both comet parameters evaluated did not differ significantly. The same was observed for the differences recorded between unexposed female and male subjects (Mann-Whitney *U* test and MANOVA) (Table *1*).

*Table 1.* Results of the comet assay on exposed personnel and control.

| No. | Age / Sex Smoking | Exposure (years) | Dose* (μSv) | Comet parameters evaluated | |
|---|---|---|---|---|---|
| | | | | TL ± SE (μm) | TM ± SE |
| **RADIOLOGY** | | | | | |
| **1** | 41; $F^N$ | 13 | 1160 | 16.14 ± 0.26 | 13.67 ± 0.26 |
| **2** | 43; $F^S$ | 8 | 720 | 15.48 ± 0.23 | 13.02 ± 0.23 |
| **3** | 41; $F^N$ | 20 | 617 | 22.56 ± 0.47 | 19.67 ± 0.44 |
| **4** | 47; $F^S$ | 8 | 410 | 16.35 ± 0.29 | 13.79 ± 0.28 |
| **5** | 43; $F^S$ | 16 | 220 | 16.49 ± 0.46 | 13.90 ± 0.44 |
| *NURSES* | | | **Mean ± SE** | **17.40 ± 1.30** | **14.81 ± 1.23** |
| **6** | 53; $M^N$ | 28 | 2210 | 15.33 ± 0.27 | 12.82 ± 0.26 |
| **7** | 42; $M^N$ | 15 | 1740 | 15.26 ± 0.20 | 12.84 ± 0.19 |
| **8** | 55; $M^S$ | 29 | 1119 | 16.81 ± 0.35 | 14.27 ± 0.33 |
| **9** | 42; $M^N$ | 10 | 790 | 18.52 ± 0.32 | 15.79 ± 0.31 |
| **10** | 43; $M^N$ | 15 | 250 | 18.23 ± 0.25 | 15.60 ± 0.24 |
| **11** | 55; $F^S$ | 23 | 203 | 17.81 ± 0.31 | 15.17 ± 0.29 |
| **12** | 62; $M^N$ | 34 | 200 | 18.03 ± 0.35 | 15.45 ± 0.33 |
| **13** | 62; $M^S$ | 32 | 150 | 18.06 ± 0.37 | 15.39 ± 0.35 |
| **14** | 45; $M^N$ | 16 | 150 | 15.53 ± 0.27 | 12.72 ± 0.26 |
| **15** | 49; $M^N$ | 13 | 50 | 16.61 ± 0.21 | 13.98 ± 0.20 |
| **16** | 40; $M^N$ | 10 | 0 | 16.39 ± 0.32 | 13.84 ± 0.31 |
| *PHYSICIANS* | | | **Mean ± SE** | **16.96 ± 0.37** | **14.35 ± 0.36** |
| **17** | 60; $M^N$ | 31 | 8548 | 16.12 ± 0.31 | 13.69 ± 0.31 |
| **18** | 47; $F^N$ | 23 | 4090 | 18.20 ± 0.51 | 15.53 ± 0.48 |
| **19** | 38; $M^N$ | 9 | 3340 | 21.65 ± 0.40 | 18.82 ± 0.39 |
| **20** | 39; $M^N$ | 5 | 1153 | 18.92 ± 0.30 | 16.16 ± 0.30 |
| **21** | 43; $M^N$ | 22 | 780 | 18.64 ± 0.55 | 15.85 ± 0.49 |
| **22** | 49; $M^N$ | 29 | 470 | 18.46 ± 0.51 | 15.72 ± 0.46 |
| **23** | 42; $M^N$ | 19 | 380 | 18.88 ± 0.66 | 16.06 ± 0.57 |
| **24** | 28; $M^N$ | 3 | 290 | 16.51 ± 0.27 | 13.92 ± 0.26 |
| **25** | 45; $M^N$ | 22 | 200 | 17.67 ± 0.30 | 15.08 ± 0.30 |
| **26** | 38; $M^N$ | 15 | 170 | 18.10 ± 0.33 | 15.42 ± 0.31 |
| **27** | 38; $M^N$ | 15 | 110 | 17.83 ± 0.43 | 15.24 ± 0.39 |
| **28** | 42; $F^N$ | 20 | 110 | 15.83 ± 0.20 | 13.11 ± 0.20 |
| **29** | 44; $M^N$ | 22 | 100 | 17.78 ± 0.34 | 15.16 ± 0.33 |
| **30** | 38; $M^S$ | 13 | 100 | 16.18 ± 0.19 | 13.59 ± 0.19 |
| **31** | 58; $M^N$ | 32 | 50 | 16.87 ± 0.25 | 14.25 ± 0.25 |

**Table 1. continued**

| | | | | | |
|---|---|---|---|---|---|
| **32** | 51; $F^N$ | 26 | 50 | 16.70 ± 0.30 | 14.02 ± 0.29 |
| **33** | 51; $M^N$ | 32 | 0 | 16.41 ± 0.33 | 14.06 ± 0.32 |
| **34** | 43; $M^N$ | 24 | 0 | 17.18 ± 0.27 | 14.44 ± 0.25 |
| **35** | 44; $M^S$ | 17 | 0 | 16.91 ± 0.31 | 14.37 ± 0.30 |
| **36** | 45; $F^S$ | 25 | 0 | 16.17 ± 0.23 | 13.53 ± 0.23 |
| **37** | 31; $M^S$ | 5 | 0 | 19.52 ± 0.68 | 16.63 ± 0.63 |
| **38** | 60; $M^N$ | 37 | 0 | 18.16 ± 0.35 | 15.51 ± 0.34 |
| **39** | 37; $M^N$ | 17 | 0 | 18.09 ± 0.37 | 15.53 ± 0.36 |
| *TECHNICIANS* | | | **Mean ± SE** | **17,69 ± 0.28** | **15.03 ± 0.27** |
| **RADIOLOGY** | | | **Mean ± SE** | **17.45 ± 0.25** | **14.81 ± 0.24** |
| **SURGERY** | | | | | |
| **40** | 42; $F^S$ | 24 | 450 | 17.56 ± 0.35 | 14.87 ± 0.32 |
| **41** | 43; $F^S$ | 24 | 220 | 17.93 ± 0.32 | 15.19 ± 0.31 |
| **42** | 22; $F^N$ | 2 | 150 | 20.23 ± 0.31 | 17.35 ± 0.30 |
| **43** | 36; $F^N$ | 12 | 120 | 17.10 ± 0.32 | 14.41 ± 0.30 |
| **44** | 29; $F^S$ | 9 | 0 | 15.12 ± 0.21 | 12.59 ± 0.20 |
| *NURSES* | | | **Mean ± SE** | **17,59 ± 0.82** | **14,88 ± 0.76** |
| **45** | 39; $F^N$ | 14 | 187 | 17.33 ± 0.55 | 14.63 ± 0.50 |
| **46** | 33; $M^N$ | 5 | 180 | 19.26 ± 0.46 | 16.43 ± 0.42 |
| **47** | 29; $M^N$ | 3 | 160 | 17.01 ± 0.46 | 14.41 ± 0.44 |
| **48** | 39; $M^N$ | 15 | 60 | 15.92 ± 0.24 | 13.30 ± 0.22 |
| *PHYSICIANS* | | | **Mean ± SE** | **17,38 ± 0.69** | **14,69 ± 0.65** |
| **49** | 30; $M^S$ | 8 | 140 | 16.23 ± 0.24 | 13.74 ± 0.24 |
| **50** | 47; $M^S$ | 22 | 116 | 20.69 ± 0.33 | 17.74 ± 0.32 |
| *TECHNICIANS* | | | **Mean ± SE** | **18,46 ± 2.23** | **15,74 ± 2.00** |
| **SURGERY** | | | **Mean ± SE** | **17.67 ± 0.53** | **14.97 ± 0.49** |
| **SMOKERS** | | | **Mean ± SE** | **17.15 ± 0.38** | **14.52 ± 0.35** |
| **NON-SMOKERS** | | | **Mean ± SE** | **17.64 ± 0.28** | **14.99 ± 0.26** |
| **EXPOSED GROUP** | | | **Mean ± SE** | **17.49 ± 0.23↑** | **14.85 ± 0.21↑** |
| **CONTROL GROUP** | | | | | |
| **SMOKERS** | | | **Mean ± SE** | **14.31 ± 0.12** | **11.76 ± 0.12** |
| **NON-SMOKERS** | | | **Mean ± SE** | **13.86 ± 0.20** | **11.24 ± 0.23** |
| **CONTROL GROUP** | | | **Mean ± SE** | **14.05 ± 0.13** | **11.46 ± 0.15** |

**M**-male; **F**-female; **S**-smoker; **N**-non-smoker; * - cummulative dose measured in the last one year before blood sampling; **TL** – tail length; **TM** – tail moment; ↑ $p<0.05$ compared to control (Mann-Whitney *U* test).

## CONCLUSION

Working in the presence of ionizing radiation is one of many managed risks within a hospital. Among individuals occupationally exposed to ionizing radiation different cytogenetic changes, for example increased frequencies of chromosome aberrations and micronuclei are well known [1,2]. Lesions induced by ionizing radiation in DNA can be also detected by comet assay [7]. The increased comet values measured in peripheral blood leukocytes of exposed subjects indicate highly significant levels of primary radiation-induced DNA damage compared to control. However, the influences of the different occupational settings and doses absorbed on the levels of DNA damage in majority of subjects might be excluded. In the present study smoking habit did not significantly increased the levels of primary DNA damage both in control and exposed subjects. Cigarette smoking was not a very potent confounding factor on the comet parameters measured. The results indicate that the alkaline comet assay might be useful additional complement to standard biodosimetric methods. By detection of momentary DNA damage and/or repair activity, it reflects the concurrent exposure and the actual levels of DNA damage present in peripheral blood leukocytes of the radiological workers at the moment of blood sampling.

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