Evaluation of DNA damage in lymphocytes of radiology personnel by comet assay

Muhammad Khisroon[1,†], Ajmal Khan[1,†], Maryam Naseem[1,†], Naheed Ali[1], Sardar Khan[2] and Syed Basit Rasheed[1]

[1]Department of Zoology, University of Peshawar, Pakistan and [2]Department of Environmental Sciences, University of Peshawar, Pakistan

Abstract: Evaluation of DNA damage in lymphocytes of radiology personnel by comet assay: Muhammad Khisroon, *et al*. **Department of Zoology, University of Peshawar, Pakistan—Objectives:** The importance of X-rays as a diagnostic medical tool cannot be denied. However, continuous exposure to X-rays can cause DNA damage. This study aimed to use the comet assay technique to investigate the level of DNA damage in lymphocytes due to X-rays in occupationally exposed personnel. **Methods:** Blood samples were collected from 74 exposed and 70 control subjects for analysis. A total of 100 randomly captured cells from each slide were examined using an epifluorescent microscope. The comets were analyzed by a visual scoring method according to comet tail length. **Results:** The results indicated a significant increase ($p<0.05$) in DNA damage in X-rays technicians (129.8 ± 17.2) as compared with the control group (53.0 ± 25.0). A significant increase ($p<0.02$) in DNA damage was also observed with an increase in exposure duration of technicians because of their service length. **Conclusions:** The present study suggests that the exposed radiology personnel should carefully comply with radiation protection procedures such as wearing of lead apron during diagnostic procedures and minimize radiation exposure where possible to avoid potential genotoxic effects due to X-rays. (J Occup Health 2015; 57: 268−274)

Key words: Blood lymphocytes, Comet assay, DNA damage, X-rays

The X-ray is one of the most important components of medical technology. In the past few decades, X-radiation has been put into use for many purposes in medical science such as for therapy and diagnosis of human ailments[1]. Diagnostic radiology, a field of physical medicine uses X-rays to obtain functional and anatomical information about an individual's body[2]. X-rays have been classified as carcinogenic by the International Agency for Research on Cancer (IARC) and Government of the United States[3]. Medical X-rays account for 0.6 to 3% of all types of cancers worldwide and at least 20% of cancer in developed countries[4,5]. Radiation in heavy doses has been proved to be lethal for cells, while in mild doses have been proved to damage the DNA of the exposed cells[6]. The DNA molecule may be damaged directly by interaction with ionizing radiation or indirectly by interaction with reactive products of the degradation of water by ionizing radiation[7]. Damage to DNA is regarded as the most important initiating step in the development of cancer and genetic diseases after exposure[8].

Previously, numerous studies have focused on radiation-induced genetic effects using animal models[9]. Radiation has been proved to be carcinogenic in all types of experimental animals (mice, rats and monkeys for X-radiation and mice, rats, rabbits and dogs for gamma radiation) and has been shown to induce tumors in at least 17 different tissue sites, that is, sites at which tumors have been observed in humans (i.e., leukemia, thyroid gland, breast and lung)[10].

Radiation technicians are qualified and authorized personnel performing different radiation- related examinations in hospitals and private clinics. They

are occupationally exposed to radiation emitted from X-ray devices for a long time during handling of patients for diagnostic and therapeutic purposes[11]. However, the level of exposure depends on the working area, exposure time, job rotation and protective measures adopted by the individual workers[12]. Trends in radiation exposure for both patients and staff are affected not only by advances in radiation protection but also by the level of doses used in the practice of medicine[13].

Several research methods including sister chromatid exchange, chromosomal aberration and the micronucleus assay are normally used for investigating genetic damage. However, these methods are economically costly, time-consuming and require proliferating cells. Therefore, the use of single cell gel electrophoresis (SCGE) or/and the comet assay for genotoxicity studies have greatly increased during the past few decades[14–16].

The comet assay (CA) was first introduced by Ostling and Johanson (1984)[17] as a technique to determine DNA single-strand breaks that caused relaxation of DNA supercoils. This technique was further modified by Singh *et al.* (1988)[18]. The CA or SCGE is a precise, simple and fast test that has been extensively used to calculate both in-vitro DNA damage and repair following exposure to a variety of genotoxic agents and even for human biomonitoring[19,20]. Different possible modifications of the comet assay have helped in the detection of single strand breaks (SSBs), double-strand breaks (DSBs), alkali-labile sites, incomplete excision repair sites, inter-strand cross linkages and cell death or apoptosis[21].

Considering the hazardous effects of X-rays and lack of awareness among radiation personnel, this study was conducted to assess the level of DNA damages in local radiology technicians using the CA technique.

Materials and Methods

Study population

A total of 74 radiology personnel employed in various government and private hospitals of Peshawar, Pakistan, were included in this study (Table 1). Their average age ranged from 20 to 52, with mean age of 35.2 ± 8.5 years and a median age of 37.5 years. Among the exposed group, 11 subjects were smokers and 63 subjects were nonsmokers. The control population comprised 70 healthy individuals selected from students and staff of the University of Peshawar with ages ranging from 18 to 62, a mean age of 36.2 ± 13.2 years and a median age of 32.5 years (Table 1). The control group had no previous history of occupational exposure to X-rays or known genotoxic chemicals and none reported medicine intake, presence of chronic diseases or any known inherited genetic disorders. Prior to the start of the study informed consent was obtained from all participants.

Questionnaire

A questionnaire regarding personal data, type and duration of occupational exposure, working activities and information on exposure to possible confounding factors (smoking habits, medicine intake, viral diseases, presence of known inherited genetic disorders, recent vaccinations, chronic disease, family history of cancer and radiodiagnostic examinations) was completed by each individual before collection of blood samples.

Blood sample collection and lymphocyte separation

Peripheral blood was collected by venipuncture

Table 1. Distribution of the main characteristics of the radiology Personnel and control group

Main characteristics	Radiology personnel	Control group
N	74	70
Mean Age (years)	35.2 ± 8.5	36.2 ± 13.2
Mean Duration of Job (years)	7.8 ± 5.3	—
Nature of Job		
X-ray Technician	74 (100%)	—
Occupation of Control group		
University students	—	28 (40%)
University employees	—	22 (31.4%)
Unemployed	—	20 (28.6%)
Smoking		
Yes	11 (14.8%)	—
No	63 (85.1%)	70

N: number.

into EDTA tubes. Blood samples from both exposed and control subjects were handled in the same manner. After collection, all blood samples were coded, refrigerated at 4ºC, transported to the laboratory and processed within 2 to 3 hours. Lymphocytes were separated from the blood by Histopaque 1,077 density gradient centrifugation and washed in phosphate buffered saline (PBS). The viability of the cells was tested by trypan blue exclusion. The number of dye-excluding cells was kept greater than 90%.

Alkaline comet assay (CA)

The alkaline CA was performed according to the protocol described by Singh *et al.* (1988)[18] with some modifications. Cleaned conventional microscopic slides were dipped into 0.7% normal melting agarose (NMA) solution. Then the slides were gently removed, their undersides were wiped to remove extra agarose, and they were then stored at room temperature (25°C) until needed. First layer slides were generally prepared one day before use. The cell suspension (15 μl) was mixed with 70 μl of low melting point agarose (LMPA) (0.7%) and spread on top of first layer slide. The slides were kept at 0°C for 5 minutes and then a second layer of 85 μl LMPA was added to fill any residual holes; the slides were kept at 0°C for 5 minutes to solidify. After solidification, slides were gently immersed in freshly prepared cold-lysing solution [(2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris, pH 10) with 1% Triton X-100 and 10% DMSO added just before use] overnight at 4°C.

Electrophoresis and neutralization

The slides were then removed from lysing solution, immersed in freshly made electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13) and left for 20 minutes to allow the unwinding of DNA and the expression of alkali-labile sites. The slides were then subjected to electrophoresis for 30 minutes at 300 mA and 25 V. All the steps were conducted in the dark at 4ºC. After electrophoresis, the slides were neutralized by washing three times with neutralization buffer (400 mM Tris, pH 7.5) for 5 minutes each.

Staining, scoring and visualization of slides

For slide staining, 70 μl acridine orange (20 μg/ml) was applied to each slide for 5 minutes and then covered with a cover slip before observation under a fluorescent microscope. Images of 100 randomly selected cells were taken at 400x- magnification using a fluorescent microscope (Nikon Eclipse 80 i) equipped with a 450-490 nm excitation filter. Comet tail lengths (consisting of the nuclear region and tail) were scored visually as suggested by Collins[20] into 5 comet classes (Fig. 1): a) comet class 0 (no damage,
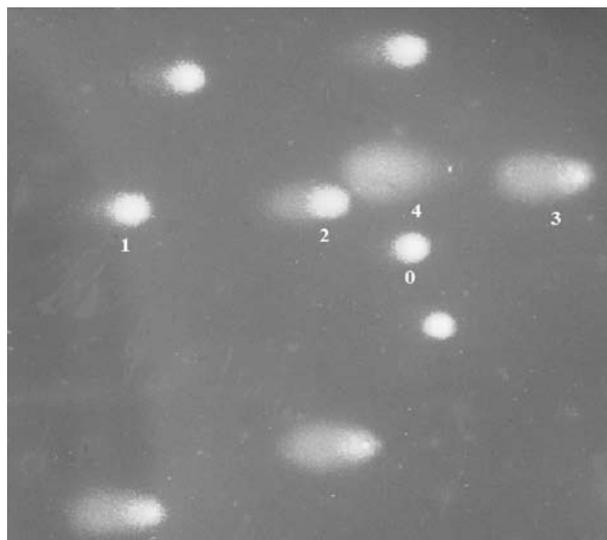

Fig. 1. Acridine orange stained images (400x magnification) of lymphocytes of radiology personnel subjected to single cell gel electrophoresis. Numbers indicate classes, i.e., 0, 1, 2, 3 and 4, of comets according to the visual scoring method.

hence no tail), b) comet class 1 (tail up to 1.5 times the diameter of the comet nucleus), c) comet class 2 (tail 1.5−2.0 times the diameter of the comet nucleus), d) comet class 3 (tail 2.0−2.5 times the diameter of the comet nucleus) and e) comet class 4 (maximally damaged with total DNA in its tail). A final overall total comet score for all 100 cells was obtained by summing up the number of cells in each class times the class number, giving a rating between 0 (completely undamaged) and 400 (maximum damaged) Collins[20] i.e. total comet score TCS=0(n) + 1(n) + 2(n) + 3(n) + 4(n), where (n) indicates the number of cells in each class.

Statistical analysis

Mean and standard deviations of TCS and differences among the means of X-rays exposed and control subjects were calculated using the Student's t-test with the SPSS (V.16.0) for Window software. Correlation was calculated for the duration of occupational exposure and TCS using online software (Wessa, 2011)[21]. The *p* value was kept at 0.05 for statistical significance.

Results

Table 1 summarizes the main characteristics of the studied population including age, tobacco consumption, duration and nature of occupation (Table 1).

Undamaged cells had an intact nucleus without a tail and damaged cells had the appearance of a comet (Fig. 1). Significantly greater DNA damage was

Table 2. Mean frequency of each comet class per 100 cells (± standard deviation) and overall mean of total comet score (± standard deviation) of the radiology personnel and control group

Comet Class	0	1	2	3	4	TCS
Radiology Personnel (n=74)	41.9 ± 5.8	20.4 ± 3.5	13.6 ± 3	13.8 ± 3	10.2 ± 3	129.8 ± 17.2
Control (n= 70)	72.4 ± 11.4	13.6 ± 6	6.3 ± 4.3	4.0 ± 3.9	3.7 ± 3.2	53.0 ± 25.0

p=0.001. TCS: total comet score.

observed in the radiology personnel (TCS=129.8 ± 17.2) compared with that observed in the control group (TCS=53.0 ± 25.0, p<0.001) (Table 2). In addition, comet class 3 (13.9 ± 3.0 cells) and class 4 (10.2 ± 3 cells) were observed more frequently in radiology personnel than in the control group (comet class 3=4.0 ± 3.9 and comet class 4=3.7 ± 3.2 cells) (Table 2). The opposite results were observed with undamaged cells; comet class 0 was observed more frequently in the control group (72.4 ± 11.4) as compared with the radiology personnel (41.9 ± 5.8 cells) (Table 2).

A strong positive correlation (r=0.62, p<0.001) was observed between the duration of occupational exposure and TCS values. Among the technicians, the lowest TCS value (119.6 ± 14.1) was observed in those working for less than one year, while the highest TCS value (136.6 ± 18.3) was observed in those working for more than 11 years (Table 3). The results indicate that the TCS values increased with the increase in duration of exposure of technicians to X-rays. In the exposed group, a very weak and nonsignificant (r=0.31, p>0.05) positive correlation was observed between age and TCS. Furthermore, TCS values were also closely connected with use of a lead apron by the technicians. The TCS values of the radiology technicians (132.0 ± 16.7) who had not used a lead apron for protection were significantly higher (p<0.01) than those who had used a lead apron (111.0 ± 5.6) while performing their duties (Table 4). DNA damage in the X-ray technicians who were smokers (Table 5) was slightly lower (128.8 ± 18.8) than in X-ray technicians who were nonsmoker (129.9 ± 17.0) but this difference was not significant (p>0.05).

Discussion

This study showed using the CA technique that DNA damage occurred in personnel (radiology technicians) occupationally exposed to X-rays. The DNA damage in the radiation technicians was significantly higher (p=0.001) compared with that in controls. This increase in DNA damage may be associated with the professional conditions to which radiology personnel were exposed. The radiation workers were exposed to more X-rays; therefore, they are at higher

Table 3. Comet score distributed according to duration of occupational exposure

Duration in years	TCS subject
< 1	119.6 ± 14.1
2 to 5	126.1 ± 15.1
6 to 10	127.1 ± 13.0
>11	136.6 ± 18.3

r=0.96, p=0.02

Table 4. Protective measures and total comet score.

Subject	TCS
Lead apron users	111.0 ± 5.6
Nonusers of lead apron	132.0 ± 16.7

Table 5. Total comet scores of smokers and non-smokers

Subject	TCS
Smokers	128.82 ± 18.80
Nonsmokers	129.92 ± 17.03

p>0.05.

risk of developing detrimental effects than the general public[22].

Characteristically, X-rays have been thought to be responsible for various cytogenetic defects. An increase in chromosomal aberrations has been observed among the X-rays workers, and even at low levels of X-rays the same consequences along with dicentrics chromosomes have been observed among the hospital workers[23,24]. High frequencies of centromere-positive and centromere-negative micronuclei have been reported in the peripheral lymphocytes of hospital workers occupationally exposed to X-rays[25]. Our research findings are in agreement with these studies and showed a significant increase in DNA damage in X-ray technicians as compared with the control group (Table 2).

X-radiation has been proved to be carcinogenic in different animals. This radiation has been found to induce genetic defects and tumors in various tissues[26]. The first cases of leukemia were reported in 1911 in radiation workers[27]. It has been reported that skin cancer and leukemia in radiology technicians and their occurrence rates increased with increasing service duration[28]. The present study also showed a significant increase in DNA damage with increasing service duration of radiology personnel, which may lead to the most important initiating step in the development of cancers and genetic diseases.

Exposure to X-rays may cause deterministic and stochastic effects in living organisms and a lead apron can be used to protect personnel from this radiation[29]. The findings of this study indicated a significant decrease in DNA damage resulting from use of protective lead aprons by the radiology personnel. However, a lead apron could not ensure full protection against DNA damage. This may be due to improper use and handling of the aprons by the technicians. It has been reported that the quality of aprons and improper storage and use can decrease their protection efficiency/level and make them more radioparent than the defined limit. This may be the reason why significant DNA damage was still observed in the radiation technicians who used lead operon as compared with the control group.

A previous study related to DNA damage in workers occupationally exposed to X-rays showed a significant correlation between age and DNA damage index in the comet assay[31]. In the present study, no correlation was observed between age and the DNA damage index in the exposed group, which is in agreement with other studies showing no correlation between age and the DNA damage index[34,35]. However, in X-ray-exposed personnel, the DNA damage index was significantly correlated with time of exposure or experience as reported by various studies[29–31].

In the past, several studies on monitoring DNA damage caused by occupational exposure have demonstrated the damage caused by smoking[30,31]. Tobacco smoke contains a high number of mutagenic and carcinogenic substances; hence, smoking is one of the important variables and therefore should be considered in biomonitoring studies. Cigarette smoke extract has been shown to have carcinogenic and mutagenic activities in rodents and human cells during *in vitro* studies[32]. Smoking has been associated with increased DNA damage in a study conducted both on young and old individuals[33]. Furthermore, an increase in DNA damage has also been reported in the workers of a cigarette factory[34]. The level of DNA damage is closely related to the intensity of smoking; therefore, the level of DNA damage in individuals who quit

smoking has been shown to decrease over the course of 1 year[35]. In contrast, some studies have reported no difference in terms of DNA damage between smokers and nonsmokers[36,37]. In the present study, no correlation was established between the smoking and nonsmoking radiation technicians. Certain studies have reported that cigarette smoking is not a significant confounding factor for the comet score[42–45] and this may be the possible reason for nonsignificant correlation of the DNA damage index observed between smokers and nonsmokers.

The results of the present study confirmed the harmful effects of X-rays on human DNA. These radiations-imposed genotoxic effects can cause significant DNA damage in radiology personnel during their occupational exposure. The increase in DNA damage may be attributed to handling of X-ray examinations without the use of any protective measures; therefore, the DNA damage in the workers using a lead apron was lower than in those who did not use a lead apron. Thus radiation personnel need to be aware about the hazards associated with excessive exposure to radiation.

This study recommends reduction of the exposure duration in any radiation area because the radiation dose received by a person is closely related to the time spent in the radiation area. The current study emphasizes proper use and storage of good quality lead aprons to minimize X-ray penetration and it should be made compulsory for hospital administrations to take proper steps to reduce exposure to X-rays in the workplace. Individual biomonitoring should be conducted regularly, and new radioprotection policies should be introduced on the basis of genetic studies like the present one.

Conflict of interest: We declare that there is no conflict of interest.

References

1) Padmaja T, Maddileti U, Hema Prasad, et al. Reproductive epidemiology in radiographers exposed to diagnostic X-rays. Hum Ecol 2006; 20: 233–5.
2) De Souza E, Soares PDM. Occupational and technical correlations of interventional radiology. Vascular Brasileior 2008; 7: 341–50.
3) Roobottom CA, Mitchell G, Morgan-HughesG. Radiation-reduction strategies in cardiac computed tomographic angiography. Cli Radiol 2010; 65: 859–67.

4) Berrington A, de Gonzalez A, Darby S. Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. Lancet 2004; 363: 345–51.

5) Picano E. Risk of Cancer from diagnostic X-rays: impressive but underestimated. Lancet 2004; 363: 1909–10.

6) Andreassi MG. The biological effects of diagnostic cardiac imaging on chronically exposed physicians: the importance of being non-ionizing. Cardiovascular Ultrasound 2004; 22: 2–25.

7) IARC. X-Radiation and γ radiation. In Ionizing Radiation, Part 1: X- and Gamma Radiation, and Neutrons. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans.vol 75. Lyon (France): International Agency for Research on Cancer; 2000. p.121–359.

8) Pavanello S, Clonfero E. Biological indicators of genotoxic risk and metabolic polymorphisms. Mutat Res 2000; 463: 285–308.

9) Dubrova YE. Long-term genetic effects of radiation exposure. Mutat Res 2003; 544: 433–9.

10) Bushong SC. Radiology. In: Radiologic science for technologists: physics, biology and protection. 9th ed. Missouri. Elsevier 2008; 500–2.

11) Faust F, Kassie F, Knasmullar S, Boedecker RH, Mann M, Mersch-Sundarmann V. The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. Mutat Res 2004; 566: 209–29.

12) Hendee WR, Edwards FM. Trends in radiation protection of medical workers. Health Phys 1990; 58: 251–7.

13) Singh NP, McCoy MT, Tice RR, Schneider LL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175: 184–91.

14) Tice RR, Andrews PW, Hirai O, Singh NP. The single cell gel (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells, In Witmer CM, Snyder RR, Hollow DJ, Kalf GF, Kocsis JJ, Sipes JG (eds). Biological Reactive Intermediates. IV. Molecular and Cellular Effects and their impact on Human Health. New York: Plenum Press; 1991. p.157–64.

15) Olive PL. DNA damage and repair in individual cells: applications of the comet assay in radiobiology. Int J Radiat Biol 1999; 75: 395–405.

16) Ostling Johanson KJ. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Bioche Biophys Res Commun 1984; 123: 291–8.

17) Collins A, Dusinska M, Franklin M, et al.. Comet assay in human biomonitoring studies – reliability, validation and applications. Environ Mol Mutagen 1997; 30: 139–46.

18) Kopjar N, Garaj-Vrhovac V. Application of the alkaline comet assay in human biomonitoring for genotoxicity: a study on Croatian medical personnel handling antineoplastic drugs. Mutagenesis 2001; 16: 71–8.

19) Piperakis SM, Visvardis EE, Tassiou AM. Comet assay for nuclear DNA damage. Methods Enzymol 1999; 300: 184–94.

20) Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular. Biotechnology 2004; 26: 249–61.

21) Wessa P. Free statistical software. Office for Research Developmental Education. 2011, Version 1.1.23-r7. [Online]. 2011 [cited 2014 Mar 22]; Available from URL: http://www.wessa.net/rankcorr. wasp

22) Streffer C, Müller WU, Kryscio A, Böcker W. Micronuclei—biological indicator for retrospective dosimetry after exposure to ionizing radiation. Mutat Res 1998; 404: 101–5.

23) Maddileti U, Padmajra T, Parsad MH, Reddy PP. Analysis of chromosomal abberrations in the peripheral lymphocytes of workers exposed to diagnostic x-rays. Int J Hum Genet 2002; 2: 265–8.

24) Bigatti P, Lamberti L, Ardito G, Armellino F. Cytogenetic monitoring of hospital workers exposed to low-level ionizing radiation. Mutat Res 1988; 204: 343–7.

25) Thierens H, Vral A, Morthier R, Aoufalah B, DeRiddert L. Cytogenetic monitoring of hospital workers occupationally exposed to ionizing radiation using the micronucleus centromere assay. Mutagenesis 2000; 15: 245–9.

26) Upton AC, Albert RE, Burns FJ, Shore RE (eds). Historical perspectives on radiation carcinogenesis. In: Radiation. Carcinogenesis. New York: Elsevier; 1986. p.1–10.

27) Wang JX, Zhang LA, Li BX, Zhao YF, Aoyama T. Cancer incidence and risk estimation among medical x-ray workers in China, 1950–1995. Health Phys 2002; 82: 455–66.

28) Oyar O. Kişlalioğlu A. How protective are lead aprons we use against ionizing radiations? Diagn Interv Radiol 2012; 18: 147–52.

29) Betti C, Davini T, Gianessi L, Loprieno N, Barale R. Microgel electrophoresis assay (comet assay) and SCE analysis in human lymphocytes from 100 normal subjects. Mutat Res 1994; 307: 323–33.

30) Frenzili G, Betti C, Davini T, et al. Evaluation of DNA damage in leukocytes of ex-smokers by single cell gel electrophoresis. Mutat Res 1997; 375: 117–23.

31) Maluf SW, Passos FD, Bacelar A, Speit G, Erdtmann B. Assessment of DNA damage in lymphocytes of workers exposed to X-radiation using the micronucleus test and the comet assay. Environ Mol Mutagen 2001; 38: 311–5.

32) McCurdy D, Tai LQ, Frias S, Wang Z. Delayed repair of DNA damage by ionizing radiation in cells from patients with juvenile systemic lupus erythematosus and rheumatoid arthritis. Radiat Res 1997; 147: 48–54.

33) Barquinero JF, Barrios L, Caballin MR, et al. Cytogenetic analysis of lymphocytes from hospital workers occupationally exposed to low levels of

ionizing radiation. Mutat Res 1993; 286: 275–9.

34) Cardoso RS, Takahashi-Hyodo S, Peitl P, Ghilardi-Neto T, Sakamoto-Hojo ET. Evaluation of chromosomal aberrations, micronuclei, and sister chromatid exchanges in hospital workers chronically exposed to ionizing radiation. Teratog Carcinog Mutagen 2001; 21: 431–9.

35) Dhawan A, Mathur N, Seth PK. The effect of smoking and eating habits on DNA damage in Indian population as measured in the Comet assay. Mutat Res 2001; 474: 121–8.

36) Sardas S, Aygun N, Karakaya AE. Genotoxicity studies on professional hair colorists exposed to oxidation hair dyes. Mutat Res 1997; 394: 153–61.

37) Palus J, Dziubaltowska E, Rydzynski K. DNA damage detected by the comet assay in the white blood cells of workers in a wooden furniture plant. Mutat Res 1999; 444: 61–74.

38) Bonassi S, Neri M, Lando C, et al. Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human Micro Nucleus project. Mutat Res 2003; 543: 155–66.

39) Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 1983; 221: 1256–64.

40) Piperakis SM, Visvardis EE, Sagnou M, Tassiou AM. Effects of smoking and aging on oxidative DNA damage of human lymphocytes. Carcinogenesis 1998; 19: 695–8.

41) Zhu CQ, Lam TH, Jiang CQ, et al. Lymphocytes DNA damage in cigarette factory workers measured by the comet assay. Mutat Res 1999; 444: 1–6.

42) Hartmann A, Pfuhler S, Dennog C, Germadnik D, Pilger A, Speit G. Exercise-induced DNA effects in human leukocytes are not accompanied by increased formation of 8-hydroxy-2'-deoxyguanosine or induction of micronuclei. Free. Radic Biol Med 1998; 24: 245–51.

43) Wojewodzka M, Kruszewsky M, Iwanenko T, Collins AR, Szumiel I. Lack of adverse effect of smoking habit on DNA strand break and base damage, as revealed by the alkaline comet assay. Mutat Res 1999; 440: 19–25.

44) Hellman B, Friis L, Vaghef H, Edling C. Alkaline single cell gel electrophoresis and human biomonitoring for genotoxicity: a study on subjects with residental exposure to radon. Mutat Res 1999; 442: 121–32.

45) Garaj-Vrhovac V, Kopjar N. The alkaline Comet assay as biomarker in assessment of DNA damage in medical personnel occupationally exposed to ionizing radiation. Mutagenesis 2003; 18: 265–71.