

## High background radiation areas of Ramsar in Iran: evaluation of DNA damage by alkaline single cell gel electrophoresis (SCGE)

J.R. Masoomi <sup>a</sup>, Sh. Mohammadi <sup>b</sup>, M. Amini <sup>c</sup>, M. Ghiassi-Nejad <sup>a,\*</sup>

<sup>a</sup> *Biophysics Department, College of Sciences, Tarbiat Modarres University, Tehran, Iran*

<sup>b</sup> *Radiation Molecular Genetic Laboratory, National Radiation Protection Department (NRPD), Iranian Nuclear Regulatory Authority (INRA), P.O. Box 14155-4494, Tehran, Iran*

<sup>c</sup> *Faculty of Pharmacy, Azad University of Tehran, Iran*

Received 1 January 2005; received in revised form 1 July 2005; accepted 1 August 2005

---

### Abstract

The hot springs in special areas in Ramsar, a northern coastal town in Iran, contain <sup>226</sup>Ra and <sup>222</sup>Rn. The natural radiation effects, radiosensitivity or adaptive responses, on the inhabitants of high natural radiation in Ramsar were studied. The single cell gel electrophoresis was used to monitor DNA damages. Three groups of volunteers were selected, one from high natural background radiation areas as the *case group* and two from normal background radiation areas as controls (*control 1* and *control 2*). The latter one had the similar living situation to *case group* while the other (*control 2*) had different living situation from the other groups. Peripheral blood mononuclear cells (PMNCs) were separated and irradiated by <sup>60</sup>Co source at five different gamma doses. It was found that the spontaneous level of DNA damage and the induced DNA damage in all challenging doses in *case group* was considerably higher than *control groups* ( $p < 0.05$ ). On the other hand, the repair rate in those volunteers, who received less than 10.2 mSv/y was significantly more than the control groups. In the contrary, individuals who live in homes with more than 10.2 mSv/y had incomplete repair. Additionally the plasma and urinary levels of vitamin C were measured spectrophotometrically. Although the concentration of vitamin C of plasma was equal in *case and control 1* groups, the urinary level of vitamin C was found to be lower in the *case group*.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** High natural radiation areas; Ramsar; Gamma radiation; Comet assay; Adaptation response; Vitamin C

---

\* Corresponding author. Tel.: +98 21 80110013494.

E-mail address: [ghiassi@mailcity.com](mailto:ghiassi@mailcity.com) (M. Ghiassi-Nejad).

## 1. Introduction

Health effects of low dose ionizing radiation are the subject of long-term debate and a problem of public concern. To elucidate these effects, the epidemiological works and radiobiological studies are needed. The great interest expressed worldwide for the study of naturally occurring radiation and environmental radioactivity has led to the performance of extensive investigations in many countries (Mohanty et al., 2004). Such investigations can be useful for the assessment of public dose rates, keeping reference-data records and ascertaining possible changes in the environmental radioactivity due to nuclear, industrial and other human activities. Epidemiological and radiobiological studies in high background radiation areas (HBRAs) have provided the opportunity to obtain direct observation of long-term radiation on human beings. Naturally ionizing radiation is the largest contributor to the collective dose received by the world population (Lin et al., 1996; Masse, 2000; UNSCEAR, 2000; Tzortzis et al., 2004). Natural radionuclides in soil generate an important component of the background radiation exposure of the population (Karahan and Bayulken, 2000; Tzortzis and Tsertos, 2004). In several high background radiation areas in the world, such as Brazil, India, China and Iran, the radiation levels that the local inhabitants are exposed to are similar to or above those of the workers of nuclear industry or medical centers (Mishra, 1993; Sohrabi, 1997; Nair et al., 1999; Ishima et al., 2000; Juliao et al., 2003; Mohanty et al., 2004). Among them, Ramsar has been given special consideration. Due to the presence of natural radionuclides, especially  $^{226}\text{Ra}$ , in hot springs, the inhabitants in some areas of Ramsar receive an annual effective dose that is up to 260 mSv/y, substantially higher than the 20 mSv/y that is permitted for radiation workers (Sohrabi, 1998; Ghiassi-Nejad et al., 2002, 2003). Radioactivity is due to  $^{226}\text{Ra}$  and its decay products which have been brought up to the earth's surface by the water of more than 9 hot springs (Aghamiri et al., 2000; Ghiassi-Nejad et al., 2003). The mean effective dose resulting from  $^{226}\text{Ra}$  due to consumption of vegetables from this area is reported to be 12 times greater than the average effective dose resulting from this radionuclide due to foods and drinking water in normal area (Ghiassi-Nejad et al., 2003).

A number of studies on HBRAs of Yangjiang in China have not shown any evidence of increased cancer mortality (Tao et al., 2000; Wei and Sugahara, 2000). The cytogenetic analysis has shown higher incidence of stable and unstable chromosomal aberrations in the HBRA group compared to the control group (Ghiassi-Nejad et al., 2004). Limited studies on the effects of high level natural radiation on some cytogenetical parameters in the inhabitants of HBRAs of Ramsar have been reported. DNA is considered to be the primary target for cell killing by ionizing radiation. Gamma irradiation is able to break the DNA directed by deposition of energy in the deoxyribose phosphate backbone (Olive et al., 1990). As a consequence, single strand breaks, double strand breaks, inter-strand protein cross-links, and damage to the DNA bases and sugars are produced (Visvardis et al., 1997). In addition, ionizing radiation also causes a variety of cellular responses. Chronic low-level irradiation from different isotopes or to a low dose of X-rays is known to induce the adaptive response (Sasaki, 1996; Konopacka et al., 1998; Ghiassi-Nejad et al., 2002) and clastogenic factor (Frenzilli et al., 1998, 2001). Irradiation with 0.05–0.1 Gy of X-rays diminishes the bone marrow death after mid-lethal irradiation in ICR male mice two months after the priming irradiation (Yonezawa et al., 1996). A small dose (generally below 30 cGy) may protect against a subsequent exposure to radiation that may be substantially larger than the initial dose (Friedmann et al., 1990; Joiner et al., 1996). On the other hand many studies have indicated that some antioxidants such as vitamin C, E and  $\beta$ -carotene enhance the resistance to radiation-induced DNA damage (Cooke et al., 1998; Konopacka et al., 1998; Noroozi et al., 1998; Collins

and Horvathova, 2000; Konopacka and Rzeszowska-Wolny, 2001; Anderson, 2001). The level of DNA damage was modulated by treating animals or cultured cells with antioxidants such as naturally occurring compounds of plants or vitamins (Anderson et al., 1994; Konopacka et al., 1998). According to the habitual diet in Ramsar, people generally consume more fresh vegetable and fruits, especially citreous, which are rich in antioxidants. Accordingly the presence of an important amount of antioxidants such as vitamin C in the population's nutrition may have a potential role to modulate the effect of radiation.

A wide variety of methods are presently used for the detection of early biological effects of DNA-damaging agents in environmental and occupational settings. These include well-established biomarkers for chromosome damage measured by chromosome aberration (CA) and sister chromatid exchange (SCE). However, both the methods are laborious and time consuming. The micronucleus assay (MN) has found its place in biomonitoring as an assay that offers an easier technical procedure than the CA and SCE assays, but the sensitivity of comet assay was found higher than that of MN (He et al., 2000). The comet assay introduced by Östling and Johanson (1984), has proven to be both sensitive and rapid in the detection of DNA strand breaks in individual cells. The alkaline comet assay has been proven useful for several years as an alternative method for the quantization of oxidative damage to DNA (Kassie et al., 2000; Møller et al., 2000; Menke et al., 2001; Faust et al., 2004; Lee et al., 2004; Pool-Zobel et al., 2004). The advantage of studying DNA strand breaks in single cells is that it allows the assessment of the heterogeneity of this phenomenon within a cell population.

In this work the effect of chronic irradiation and the potential role of antioxidant level in inhabitants of HBRA were studied. The DNA damage and DNA repair were studied by single cell gel electrophoresis or comet assay method.

## 2. Materials and methods

### 2.1. Chemicals

The used chemicals were purchased from the following suppliers: RPMI 1640 from Gibco and FCS was from sigma. Agarose (NMP & LMP) and ethidium bromide (EtBr) were obtained from Boehringer Mannheim. All other chemicals were purchased from Merck.

### 2.2. Population and sampling

The study was carried out in Ramsar. As the case group, 33 inhabitants of the HBRA of Ramsar were chosen (from Talesh Mahaleh, Chaparsar, and Abe-siah) with their full informed consent (their age range was 14–65). Radiation dose at five or six corners of each house was measured directly (using a Multi-Purpose detector Rados RDS-110, manufactured by RADOS Technology GmbH Hamburg, Germany) and the average value was calculated as indoor dose. All individuals filled in a detailed questionnaire covering the occupational, medical and family history and habitual behavior of food consuming, drugs, tobacco, alcohol, etc. In assessment of questionnaire, it was found that the radiation doses in the high background dwellings varied from home to home with a mean of 10.2 mSv/y. Consequently according to the annual receiving dose, the volunteers with  $\leq 10.2$  mSv (ranged from 0.53 to 7.62 mSv/y), were put into a subgroup named *LD* (containing 28 individuals). In our case group there were five individuals who were receiving an annual dose of more than 10.2 mSv (ranged from 13.23 to 61.67 mSv/y), were put into the *HD* group. Additionally, two control groups which were matched for age, sex and smoking habits were chosen from normal background radiation areas (NBRAs). One group (control 1) contained 24 healthy

volunteers (ranged in age 15–59) was from some areas in Ramsar with normal background radiation and their social and economic status, including life style and living standards was similar to the HBRAs. Another 16 healthy volunteers (control 2) were chosen from Tehran (ranged in age 19–49) had different life style from the other two. All individuals were categorized in terms of their age, smoking habit, work-related exposure to hazardous agents, previous exposures to diagnostic X-rays and nuclear medicine.

A 5 mL aliquot of heparinized peripheral blood was collected via venipuncture from each volunteer. Peripheral blood mononuclear cells (PMNCs) were isolated from the heparinized blood sample by centrifugation at  $800 \times g$  for 30 min at 20 °C on lymphoprep (AXIS – SHIELD PoC As, Oslo, Norway) then resuspended in RPMI 1640 with 10% FCS.

### 2.3. Cell treatments

The cells were irradiated with  $^{60}\text{Co}$  gamma ray (gamma beam 150 Atomic Energy Canada) at a dose rate of 0.5 Gy/min for 0, 0.3, 1, 2 and 4 Gy doses. In order to stop DNA repair, the cells were kept in ice.

### 2.4. Initial and residual damages

Immediately after all mentioned treatments, an aliquot of the cell suspension was processed to evaluate the initial damage. Strand rejoining was allowed to proceed by incubating another aliquot of treated cells for 2 h in RPMI 1640 with 10% FCS containing penicillin/streptomycin 100 U/100  $\mu\text{g}/\text{mL}$  at 37 °C in 95% humidified air and 5%  $\text{CO}_2$ .

### 2.5. Comet assay

Alkaline comet assay was used to estimate the radiosensitivity of PMNCs. The protocol was performed according to Singh et al. (1988). Immediately after treatment, slide preparation as sandwich types was done by embedding 40000 PBMCs. The slides were transferred onto a chilled lysing solution (pH = 10) of 2.5 M NaCl, 100 mM  $\text{Na}_2\text{EDTA}$ , 10 mM Tris, 10% DMSO and 1% Triton X-100 and kept at 4 °C in reduced light for 60 min. Afterward they were placed on a horizontal gel electrophoresis unit and covered with fresh buffer (1 mM  $\text{Na}_2\text{EDTA}$ , 300 mM NaOH, pH > 13) and were left in this buffer for 20 min and then the electrophoresis at 0.56 V/cm and  $\sim 290$  mA was done for 20 min. They were then rinsed with neutralization buffer (0.4 M Tris, pH = 7.5) for 15 min, fixed by methanol and stained later with 20  $\mu\text{g}/\text{mL}$  ethidium bromide.

### 2.6. Evaluation of DNA damages

The slides were coded and examined at  $200\times$  magnification using a fluorescence microscope (Nikon Eclipse E600). For each case, as the slides were remained coded, about 100–150 cells were analyzed and the result was normalized to 100. The extent of DNA migration was evaluated by visual analysis according to the criteria established by Anderson et al. (1994). The comets were classified into five categories (0–4) according to the DNA damage. Comets with bright head and no tail were classified as class 0 and comets with a small head and long diffuse tails, as class 4, i.e. highly damaged cells. Comets with intermediate characteristics were classified as classes 1, 2 or 3. Quantitative DNA damage (DD) was estimated by the formula described by Jalszynski et al. (1997):

$$\text{DD} = (n_1 + 2n_2 + 3n_3 + 4n_4) / \Sigma \quad (1)$$

where DD is the DNA damage in arbitrary units (au),  $n_1$ – $n_4$  the number of classes 1–4 comets, and  $\Sigma$  the total number of scored comets, including class 0. To validate this classification, Noroozi et al. (1998) had compared this method with the results obtained by image analyzer. There was a close relation between the

subjective visual score and the measurements of the percentage of DNA in the tail by image analysis. The amount of repair was calculated using the following formula:

$$R_{\gamma} = (D_{\gamma-0} - D_{\gamma-2}) / D_{\gamma-0} \quad (2)$$

where  $R_{\gamma}$  is the DNA repaired,  $D_{\gamma-0}$  and  $D_{\gamma-2}$  are the initial and residual damages at corresponding  $\gamma$ -radiation dose.

### 2.7. Statistical analyses

Data were analyzed using the statistical analysis system software packages of Microcal Origin and Excel. Analysis of *t*-student test with two-sample unequal variance was used for analyzing. The level of signification was set as 5%.

### 2.8. Evaluation of plasma and urinary ascorbic acid

Within 1 h of blood collection, plasma was prepared from heparinized whole blood (5 mL) by centrifuging (800 × g, 20 min, and 4 °C) and was stored in the liquid nitrogen.

One milliliter of urine was collected from each individual. In order to increase the stability of vitamin C, 20  $\mu$ L of HCl 1 M was added to samples (Young et al., 1999) and the samples were placed in liquid nitrogen immediately. Before the measurements, samples were thawed and diluted with distilled water (200  $\mu$ L urine + 800  $\mu$ L distilled water). The dilution is required to decrease the interfering substance e.g. sugar, amino acids, thiosulfates, glucuronic acid and reductones (Washko et al., 1992). One milliliter of trichloroacetic acid 10% (TCA) was added to 1 mL of sample and centrifuged for 30 min (1300 × g) at 20 °C. Subsequently 400  $\mu$ L of 2,4-dinitrophenylhydrazin (2,4-DNPH) was added to 1 mL of supernatant. The samples were placed in a bath at 56 °C for 1 h and then were transferred to the refrigerator for cooling. Two milliliters of sulfuric acid 85% was added to each sample that dissolves the complex (2,4-dinitrophenylhydrazin). The effect of specimen dilution, by TCA and water, was corrected.

Vitamin C level in plasma and urine was determined photometrically as described by McCormick and Green (1994) and Mirskandari et al. (1999). A standard curve with the concentrations 0.1, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/dL was used to evaluate the concentration of vitamin C in plasma and urine (with a spectrophotometer of Perkin Elmer Model 2 Lambda).

## 3. Results

Fig. 1 shows the initial DNA damage for all groups. It is remarkable that the spontaneous level of DNA damage in *case* group is significantly elevated over that of control groups. Moreover, applying the challenging doses induced higher damages in *case* group. There were meaningful discrepancies in all doses between case and controls ( $p < 0.05$ ). It is noticeable that the initial damage in *control 2* was higher than *control 1* (at least 32%). To evaluate the DNA damage which is induced by radiation, the damage at 0 Gy was subtracted from the subsequent damage induced by irradiation (Fig. 2). Results also showed that for all doses, the induced damage related to *control 1* was lower than that of the high background *case* group and *control 2*, where the difference was meaningful at higher challenging radiations ( $p < 0.05$ ). Fig. 3 shows the resultant damages after classifying the *case* into subgroups i.e. *LD* and *HD*. In *HD* group, the induction of DNA damage was higher than in the *LD*. *Control 1* had less damage when compared to both the case groups. After 2 h of incubation, most of damage was repaired (Fig. 3). The amount of repair in both controls and case subgroups was calculated according to Eq. (2). The results are presented in Table 1. The weakest DNA repair ability is considered for the *HD*

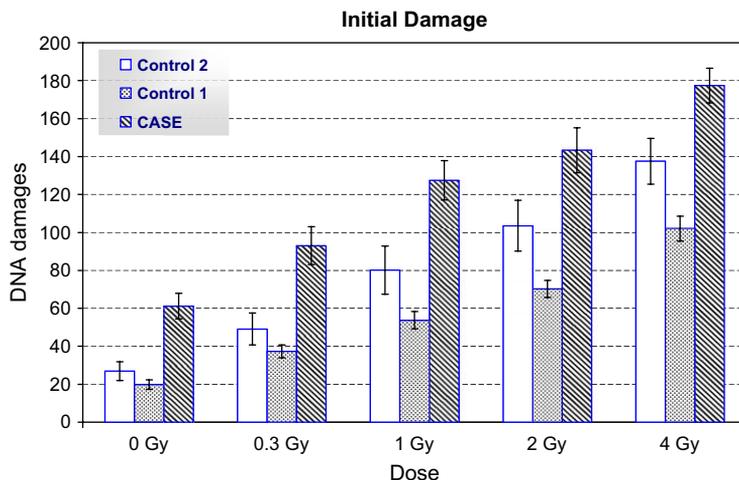


Fig. 1. The initial DNA damage of blood mononuclear cells of all groups at all applied doses of gamma irradiation (0, 0.3, 1, 2 and 4 Gy). In case group (open bars), the initial damage was higher ( $p < 0.05$ ) than the control groups (stippled and cross hatched bars).

group. Both control groups had similar repair rate and the discrepancies in all doses were not significantly meaningful. It is remarkable that in the *LD* group the repair ability was much higher than control groups in lower irradiation doses (0.3 and 1 Gy).

Fig. 4 shows the results of analysis of plasma and urinary vitamin C measurements. Plasma vitamin C for both of the Ramsar volunteers (case and *control 1*) were similar but they were significantly higher than the level for *control 2* ( $p < 0.05$ ). The evaluation of the urinary

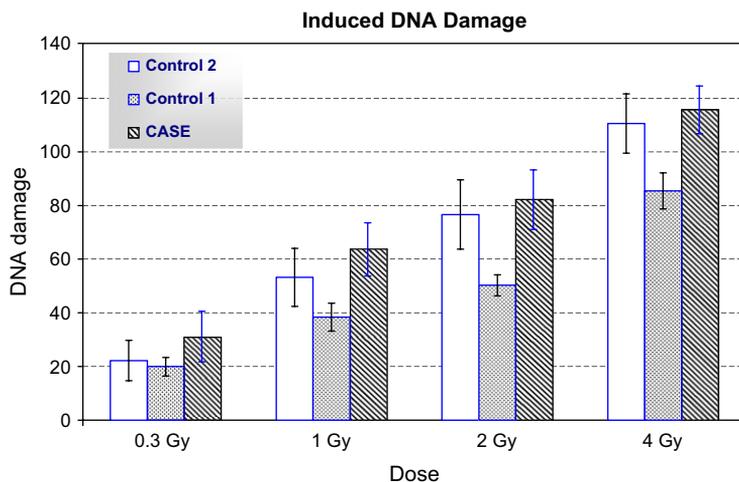


Fig. 2. The induced DNA damage by different challenging doses (see Section 2). For all doses, the induced damage in control 1 (stippled bars), social and economic status was similar to the case group, was lower than the case (cross hatched bars) and control 2 (open bars), which had the different life style from the other two. The difference was meaningful at higher challenging radiations ( $p < 0.05$ ).

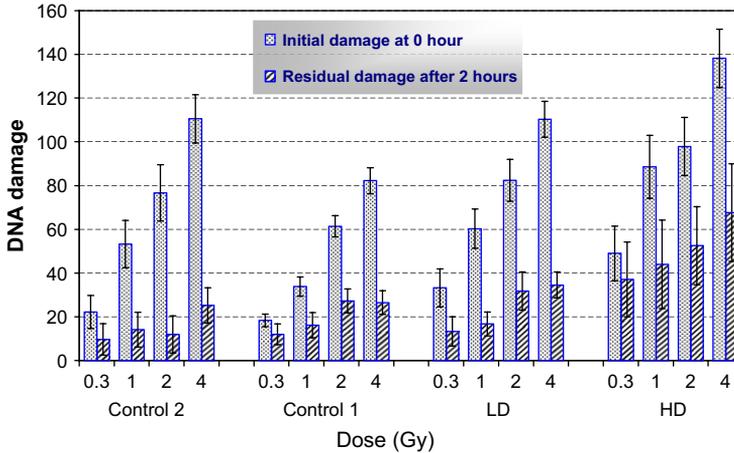


Fig. 3. The induced (dotted bars) and residual DNA damages (cross hatched bars) at 0 and 2 h after incubation, at different doses of gamma irradiation. After 2 h of incubation, main part of damages was repaired. According to the annual receiving dose, the volunteers were divided into two subgroups, i.e. an HD group (annual received dose  $> 10.2$  mSv) and an LD group (annual received dose  $\leq 10.2$  mSv). In HD group, the induction of acute damage was higher than in the LD. This difference at lower challenging doses was more evident (48 and 47% at 0.3 and 1 Gy, respectively), while at 2 and 4 Gy this difference was about 19 and 25%.

vitamin C showed that the extracted vitamin C was higher in *control 1* but did not differ in the *control 2* and *case* groups.

#### 4. Discussion

Natural ionizing radiation is the largest contributor to the collective dose received by the world population (Lin et al., 1996; Masse, 2000; Tzortzis et al., 2004). The cytogenetic factors of inhabitants in HBRAs of Ramsar have shown the higher incidence of stable and unstable chromosomal aberrations in the HBRAs group compared to the control group (Ghiassi-Nejad et al., 2004). In this work the effect of chronic irradiation and the level of vitamin C, which could have an antioxidant role, on residents of HBRAs were studied. The DNA damage and DNA repair were studied by comet assay.

The results of this study have shown that the spontaneous level of DNA damage in *case group* was significantly higher than control groups. In addition the damage induced by challenging doses in the population who live in high background areas was higher than the populations who live in normal background areas. Besides, certain differences between the people who live in *LD* areas and *HD* areas in HBRAs were found: more severe damage and an

Table 1  
The amount of DNA repair, calculated by formula (2)

Group	Challenging dose (Gy)			
	0.3	1	2	4
<i>Control 1</i>	0.35	0.52	0.56	0.68
<i>LD group</i>	0.5	0.72	0.61	0.71
<i>HD group</i>	0.24	0.50	0.38	0.51
<i>Control 2</i>	0.37	0.54	0.53	0.67

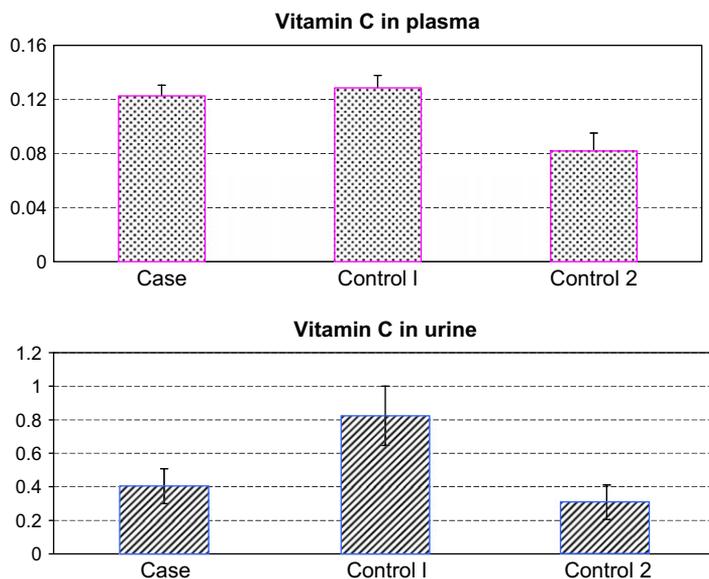


Fig. 4. Concentration of extracted plasma and urinary vitamin C in two control groups and *case* group. The evaluation of plasma vitamin C showed that this vitamin was found with similar levels in *case* and *control 1* and was higher than *control 2*. But the urinary vitamin C showed that this vitamin was higher in *control 1* but did not differ in the *control 2* and *case* groups.

incomplete repair system were observed in *HD* group, the ability of repair in low-level groups was substantially higher than the repair potential of both control groups (especially at lower challenging doses). These results revealed that the lymphocytes of residents in high background radiation are more radiosensitive. However, comparing the repair suggests that it is likely that an adaptive response is induced in the population who live in *LD* areas and less radio-protectivity might have been induced in the *HD* group. We consider that the higher irradiation dose at *HD* areas was detrimental and may have lead to stable damage in DNA. At higher challenging doses, more radiation sensitivity was also observed in *LD* group. It could be suggested that when the challenging dose is not elevated, the volunteers of this group are probably adapted to radio-stress and are able to repair induced lesion. In comparing of control groups, less damage was observed in *control 1*, while any differentiations were observed in their repair capability. The differences between living situations such as diet; air pollution, etc. could account for these differences. As our assessment of vitamin C has shown, lower concentration of this vitamin was found in plasma of *control 2*. Of course antioxidants are proving to have an important role in diminishing the harmful effect of ionizing radiation and decrease the degree of DNA damage (Anderson et al., 1994; Noroozi et al., 1998; Pflam et al., 1998). According to the questionnaire results and our knowledge, inhabitants of Ramsar habitually consume fresh vegetable and fruits, especially citreous, olive, carrot, garlic, etc. which are rich in antioxidants. In this study, as was expected, the plasma level of vitamin C in *case* and *control 1* was similar and was higher than *control 2*. However, in *case* group in spite of equal level of vitamin C in plasma, more radiosensitivity was observed. Accordingly, *control 1* whose habitual diet contained large amount of vitamin C, was less sensitive and more protective to oxidative DNA damage than *control 2*. In comparing with *control 1*, in spite of similar habitual diet, extracted urinary vitamin C was found lower in the *case* group than in *control 1*. This result suggests that the

stored vitamin may possibly have used against chronic radiation and repair of DNA damages in the *case* group.

## 5. Conclusion

Present study has shown that the radiosensitivity is highly dependent on the received dose. Less radiosensitivity and more repair rate were observed in the subgroup of cases who had received a lower chronic dose. The other subgroup which was exposed to higher chronic doses of ionizing radiation showed more radiosensitivity, repair deficiency and no adaptive response. On the other hand, the induced damage in *control 2* was more than *control 1*, which showed that the radio-protectivity in *control 1* was higher. We conclude that probably the consumption of more antioxidants by *control 1* is an important factor in their radio-protectivity. Despite the fact that the *control 1* and *case* volunteers have similar habitual diet and the plasma level was similar in both groups, the level of urinary vitamin C was higher in *control 1*. These results could suggest that in the *case* volunteers their storage vitamin C is probably consumed and is used as antioxidants.

## Acknowledgements

We would like to thank all the volunteers especially kind inhabitants of Talesh Mahaleh, Chaparsar, and Abe-siah of Ramsar for their helpful co-operation. We would like to acknowledge the health center of Ramsar for their assistance in blood sampling.

## References

- Aghamiri, S.M.R., Seaward, M.R.D., Beitolahi, M., 2000. Soil-to-plant  $^{226}\text{Ra}$  concentration ratio in elevated areas in Iran. In: WONUC. (Ed.), Proceedings of the Effects of Low and Very Low Doses of Ionizing Radiation on Human Health. 17 and 18 June 1999, Versailles, France. Elsevier, Amsterdam, pp. 193–202.
- Anderson, D., Yu, T.W., Phillips, B.J., Schmezer, P., 1994. The effect of various antioxidants and other modifying agents on oxygen-radical-generated DNA damage in human lymphocytes in the comet assay. *Mutat. Res.* 307, 261–271.
- Anderson, D., 2001. Factors that contribute to biomarker responses in humans including a study in individuals taking vitamin C supplementation. *Mutat. Res.* 480–481, 337–347.
- Collins, A.R., Horvathova, E., 2000. Oxidative DNA damage, antioxidants and DNA repair: applications of the comet assay. *Biochem. Soc. Trans.* (Pt 2), 337–341.
- Cooke, M.S., Evans, M.D., Podmore, I.D., Herbert, K.E., Mistry, N., Mistry, P., Hickenbotham, P.T., Hussieni, A., Griffiths, H.R., Lunec, J., 1998. Novel repair action of vitamin C upon in vivo oxidative DNA damage. *FEBS Lett.* 439, 363–367.
- Faust, F., Kassie, F., Knasmüller, S., Kevekordes, S., Mersch-Sundermann, V., 2004. Use of primary blood cells for the assessment of exposure to occupational genotoxicants in human biomonitoring studies. *Toxicology* 198, 341–350.
- Frenzilli, G., Lori, A., Panasiuk, G., Ferdeghini, M., Barale, R., 1998. Comet assay on children's leukocytes 8 years after the Chernobyl disaster. *Mutat. Res.* 415, 151–158.
- Frenzilli, G., Bosco, E., Antonelli, A., Panasiuk, G., Barale, R., 2001. DNA damage evaluated by alkaline single cell gel electrophoresis (SCGE) in children of Chernobyl, 10 years after the disaster. *Mutat. Res.* 491, 139–149.
- Friedmann, P.S., Rees, J., White, S.I., Matthews, J.N., 1990. Low-dose exposure to antigen induces sub-clinical sensitization. *Clin. Exp. Immunol.* 81 (Suppl.), 507–509.
- Ghiassi-Nejad, M., Mortazavi, S.M.J., Cameron, J.R., Niroomand-rad, A., Karam, P.A., 2002. Very high background radiation areas of Ramsar, Iran: preliminary biological studies. *Health Phys.* 82, 87–93.
- Ghiassi-Nejad, M., Beitollahi, M.M., Asefi, M., Reza-Nejad, F., 2003. Exposure to  $^{226}\text{Ra}$  from consumption of vegetables in the high level natural radiation area of Ramsar–Iran. *J. Environ. Radioact.* 66, 215–225.
- Ghiassi-Nejad, M., Zakeri, F., Assaei, R.G., Kariminia, A., 2004. Long-term immune and cytogenetic effects of high level natural radiation on Ramsar inhabitants in Iran. *J. Environ. Radioact.* 74, 107–116.

- He, J.L., Chen, W.L., Jin, L.F., Jin, H.Y., 2000. Comparative evaluation of the in vitro micronucleus test and the comet assay for the detection of genotoxic effects of X-ray radiation. *Mutat. Res.* 469, 223–231.
- Ishima, H., Koga, T., Tatsumi, K., Nakai, S., Sugahara, T., Yuan, Y., Wei, L., 2000. Dose measurement, its distribution and individual external dose assessments of inhabitants in the high background radiation areas in China. *J. Radiat. Res. (Tokyo)* 41 (Suppl.), 9–23.
- Jaloszynski, P., Kujawski, M., Czub-Swierczek, M., Markowska, J., Szyfter, K., 1997. Bleomycin-induced DNA damage and its removal in lymphocytes of breast cancer patients studied by comet assay. *Mutat. Res.* 385, 223–233.
- Joiner, M.C., Lambin, P., Malaise, E.P., Robson, T., Arrand, J.E., Skov, K.A., Marples, B., 1996. Hypersensitivity to very-low single radiation doses: its relationship to the adaptive response and induced radio resistance. *Mutat. Res.* 358, 171–183.
- Juliao, L.M., Sousa, W.O., Santos, M.S., Fernandes, P.C., 2003. Determination of  $^{238}\text{U}$ ,  $^{234}\text{U}$ ,  $^{232}\text{Th}$ ,  $^{228}\text{Th}$ ,  $^{228}\text{Ra}$ ,  $^{226}\text{Ra}$  and  $^{210}\text{Pb}$  concentration in excreta samples of inhabitants of a high natural background area. *Radiat. Prot. Dosimetry* 105, 379–382.
- Karahan, G., Bayulken, A., 2000. Assessment of gamma dose rates around Istanbul (Turkey). *J. Environ. Radioact.* 47, 213–221.
- Kassie, F., Parzefall, W., Knasmuller, S., 2000. Single cell gel electrophoresis assay: a new technique for human bio-monitoring studies. *Mutat. Res.* 463, 13–31.
- Konopacka, M., Widel, M., Rzeszowska-Wolny, J., 1998. Modifying effect of vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in mouse cells. *Mutat. Res.* 417, 85–94.
- Konopacka, M., Rzeszowska-Wolny, J., 2001. Antioxidant vitamins C, E and beta-carotene reduce DNA damage before as well as after gamma-ray irradiation of human lymphocytes in vitro. *Mutat. Res.* 491, 1–7.
- Lee, E., Oh, E., Lee, J., Sul, D., Lee, J., 2004. Use of the tail moment of the lymphocytes to evaluate DNA damage in human biomonitoring studies. *Toxicol. Sci.* 81, 121–132.
- Lin, Y.-M., Chen, C.-J., Lin, P.-H., 1996. Natural background radiation doses assessment in Taiwan. *Environ. Int.* 22 (Suppl.), S45–S48.
- Masse, R., 2000. Ionizing radiation. *C. R. Acad. Sci. III.* 323, 633–640.
- McCromick, D.B., Green, H.L., 1994. Vitamins. In: Burtis, C.A., Ashwood, E.R. (Eds.), *Fundamentals of Clinical Chemistry*, second ed. W.B. Saunders, Philadelphia.
- Menke, M., Chen, I., Angelis, K.J., Schubert, I., 2001. DNA damage and repair in *Arabidopsis thaliana* as measured by the comet assay after treatment with different classes of genotoxins. *Mutat. Res.* 493, 87–93.
- Mirskandari, L., Ziai, S.A., Salehi, P., Noormohammadi, I., Mahmoudi, M., 1999. Serum ascorbic acid, vitamin A and beta-carotene levels in Iranian patients with cancer. *Arch. Iran. Med.* 2, 195–197.
- Mishra, U.C., 1993. Exposure due to high natural radiation areas background and radioactive springs around the world. In: Sohrabi, M., Ahmed, J.U., Durrani, S.A. (Eds.), *Proceedings of an International Conference on High Levels of Natural Radiation*. 3–7 November 1990, Ramsar, Iran. IAEA, Vienna, pp. 29–35.
- Mohanty, A.K., Sengupta, D., Das, S.K., Saha, S.K., Van, K.V., 2004. Natural radioactivity and radiation exposure in the high background area at Chhatrapur beach placer deposit of Orissa, India. *J. Environ. Radioact.* 75, 15–33.
- Møller, P., Knudsen, L.E., Loft, S., Wallin, H., 2000. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. *Cancer Epidemiol. Biomarkers Prev.* 9, 1005–1015.
- Nair, M.K., Nambi, K.S., Amma, N.S., Gangadharan, P., Jayalekshmi, P., Jayadevan, S., Cherian, V., Reghuram, K.N., 1999. Population study in the high natural background radiation area in Kerala. *India Radiat. Res.* 152 (Suppl.), S145–S148.
- Noroozi, M., Angerson, W.J., Lean, M.E., 1998. Effect of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. *Am. J. Clin. Nutr.* 67, 1210–1218.
- Olive, P.L., Banath, J.P., Durand, R.E., 1990. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the comet assay. *Radiat. Res.* 122, 86–94.
- Östling, O., Johanson, K.J., 1984. Microelectrophoresis study of radiation-induced DNA damages in individual mammalian cells. *Biochem. Biophys. Res. Commun.* 123, 291–298.
- Pflam, M., Kielbassa, C., Garmyn, M., Epe, B., 1998. Oxidative DNA damage induced by visible light in mammalian cells: extent, inhibition by antioxidants and genotoxic effect. *Mutat. Res.* 408, 137–146.
- Pool-Zobel, B.L., Dornacher, I., Lambert, R., Knoll, M., Seitz, H.K., 2004. Genetic damage and repair in human rectal cells for biomonitoring: sex differences, effects of alcohol exposure, and susceptibilities in comparison to peripheral blood lymphocytes. *Mutat. Res.* 551, 127–134.
- Sasaki, M.S., 1996. Radioadaptive response: an implication for the biological consequences of low dose-rate exposure to radiations. *Mutat. Res.* 358, 207–213.

- Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A single technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175, 184–191.
- Sohrabi, M., 1997. World high level natural radiation and/or radon-prone area with special regard to dwellings. In: Luxin, W., Tsutomu, S., Zufan, T. (Eds.), *Proceedings of the Fourth International Conference on High Levels of Natural Radiation*. 21–25 October 1996, Beijing, China. Elsevier Sciences, Amsterdam, pp. 57–68.
- Sohrabi, M., 1998. The state-of-the-art on worldwide studies in some environments with elevated naturally occurring radioactive materials (NORM). *Appl. Radiat. Isot.* 49, 169–188.
- Tao, Z., Zha, Y., Akiba, S., Sun, Q., Zou, J., Li, J., Liu, Y., Kato, H., Sugahara, T., Wei, L., 2000. Cancer mortality in the high background radiation areas of Yangjiang, China during the period between 1979 and 1995. *J. Radiat. Res. (Tokyo)* 41 (Suppl.), 31–41.
- Tzortzis, M., Svoukis, E., Tsertos, H., 2004. A comprehensive study of natural gamma radioactivity levels and associated dose rates from surface soils in Cyprus. *Radiat. Prot. Dosimetry* 109, 217–224.
- Tzortzis, M., Tsertos, H., 2004. Determination of thorium, uranium and potassium elemental concentrations in surface soils in Cyprus. *J. Environ. Radioact.* 77, 325–338.
- UNSCEAR, 2000. Sources and Effects of Ionizing Radiation Report to the General Assembly, with annexes. United Nations Scientific Committee on the effects of Atomic Radiation. United Nations, New York.
- Visvardis, E.E., Tassiou, A.M., Piperakis, S.M., 1997. Study of DNA damage induction and repair capacity of fresh and cryopreserved lymphocytes exposed to H<sub>2</sub>O<sub>2</sub> and gamma irradiation with the alkaline comet assay. *Mutat. Res.* 383, 71–80.
- Washko, P.W., Welch, R.W., Dhariwal, K.R., Wang, Y., Levine, M., 1992. Ascorbic acid and dehydroascorbic acid analysis in biological sample. *Anal. Biochem.* 204, 1–14.
- Wei, L., Sugahara, T., 2000. An introductory overview of the epidemiological study on the population at the high background radiation areas in Yangjiang, China. *J. Radiat. Res. (Tokyo)* 41 (Suppl.), 1–7.
- Yonezawa, M., Misonoh, J., Hosokawa, Y., 1996. Two type of X-ray-induced radioresistance in mice: presence of 4 dose ranges with distinct biological effects. *Mutat. Res.* 358, 237–243.
- Young, J.F., Nielsen, S.E., Haraldsdottir, J., Daneshvar, B., Lauridsen, S.T., Knuthsen, P., Crozier, A., Sandstrom, B., Dragsted, L.O., 1999. Effect of fruit juice intake on urinary quercetin excretion and biomarkers of antioxidative status. *Am. J. Clin. Nutr.* 69, 87–94.