

Radioadaptive Responses Induced in Human Lymphocytes of the Inhabitants of High Level Natural Radiation Areas in Ramsar, Iran

SMJ. Mortazavi

Medical Physics Department, School of Medicine,
Rafsanjan University of Medical Sciences (RUMS), Rafsanjan, Iran

National Radiation Protection Department (NRPD),
Iranian Nuclear Regulatory Authority (INRA),
P.O. Box 14155-4494, Tehran, Iran

A. Shabestani-Monfared

Medical Physics Department, School of Medicine,
Babol University of Medical Sciences, Babol, Iran

M. Ghiassi-Nejad

National Radiation Protection Department (NRPD),
Iranian Nuclear Regulatory Authority (INRA),
P.O. Box 14155-4494, Tehran, Iran

H. Mozdarani

Medical Genetics Department, School of Medical Sciences,
Tarbiat Modarres University, Tehran, Iran.

Abstract : Ramsar, a city in northern Iran has among the highest levels of natural radiation known to exist in an inhabited area. The high levels of natural radiation in Ramsar prompted us to assess the radioadaptive response of some of the residents with high cumulative doses. We report the effect of cumulative lifetime dose on the coefficient of radioadaptive response.

Twenty two residents of high level natural radiation areas and thirty three residents from an adjacent normal level natural radiation area participated in this study. In the 1st phase of the experiment 15 healthy residents from high level natural radiation areas and 30 healthy inhabitants of a nearby normal level natural radiation area were studied. After description of the study and its objectives to the participants, they were asked to complete questionnaires, participate in interviews, allow radiation measurements of their homes, and to submit blood samples. In the 2nd phase, seven healthy residents with cumulative lifetime doses of up to 10 Sv were studied for assessing the induction of adaptive response in each study participant and obtaining complementary data. Cultured cells were given a challenge dose of either 2 Gy (1st phase of the study) or 1.5 Gy (2nd phase of the study) of Co-60 gamma radiation.

Overall data showed a significant radioadaptive response in the residents of high level natural radiation areas. Results obtained in the 2nd phase of the study, showed that five out of seven inhabitants exhibited a reduction in induced chromosomal aberrations following exposure to a 1.5 Gy challenge dose of gamma radiation.

However, the response in 2 residents with much higher cumulative doses than the others was not statistically different than that of the control population. Regression analysis suggests a linear relationship between the radioadaptive response and cumulative gamma radiation doses up to 1 Gy.

High levels of natural radiation may induce significant adaptive responses in the inhabitants. As the cumulative dose increased from a few hundred mGy to 1 Gy, the magnitude of the induced adaptive response increased linearly. Further research is needed to clarify if decreased radiation susceptibility in the residents of high level natural radiation areas can influence radiogenic risk and this phenomenon may be considered as a potential beneficial effect of high levels of natural radiation.

Keywords : Adaptive response; chromosomal aberrations; background radiation; cumulative dose.

Introduction

Animals and plants have been exposed to natural radiation since the evolution of life on Earth. Life evolved in a radiation field that was much more intense than today (Jaworowski 1997, Karam and Leslie, 1999) and levels of natural radiation vary greatly over the Earth. Ramsar, a northern coastal city in Iran (Fig. 1), has areas with some of the highest levels of natural radiation measured to date. Extensive radiological studies have been carried out in Ramsar since 15 years ago (Sohrabi 1990). The annual effective dose (excluding radon progeny contributions) in high level natural radiation areas (HLNRAs) of Ramsar is a few times higher than the ICRP-recommended annual effective dose limit for radiation workers. Some inhabitants receive annual doses over 130 mGy yr^{-1} from external terrestrial sources. The HLNRAs of Ramsar are due to ^{226}Ra and its decay products, which have been brought to the surface by the waters of hot springs. There are at least 9 hot springs with different concentrations of radium in Ramsar that are used as spas by both tourists and residents. Due to levels of natural radiation in these areas, up to 200 times higher than normal level natural radiation areas (NLNRAs), some radiation experts have suggested that dwellings having such high levels of natural radiation need remedial actions (Sohrabi 1997). In spite of this, nearly all inhabitants still live in their unaltered paternal dwellings.

Many radiation advisory bodies and regulatory agencies assume that any exposure to radiation carries some degree of risk. This assumption has

been contested by many radiation scientists. This paper reports on evidence for a protective radioadaptive response caused by the high natural radiation doses to some residents of Ramsar, Iran. The induction of adaptive response was first reported by Samson and Cairns (1977) in *Escherichia coli*. Chromosome aberrations caused in peripheral blood lymphocytes serve as the best biological indicator of the exposure to ionizing radiation (Hayata *et al.* 1992). The induction of the cytogenetic radioadaptive response in human lymphocytes by low doses of ionizing radiation was first reported by Olivieri *et al.* (1984). They reported that the frequency of chromatid aberrations was down to 50% less than they expected after exposure to 1.5 Gy of x-rays.

Many articles have demonstrated radioadaptive response in plant cells (Cortes *et al.* 1990), insects (Fritz-Niggli and Schaeppi-Buechi 1991), Chinese hamster V79 cells (Ikushima 1987, 1989a, 1989b), cultured human lymphocytes (Wiencke *et al.* 1986, Shadley and Wolff 1987, Wolff *et al.* 1988, Shadley and Wiencke 1989, Sankaranaryanan *et al.* 1989), human embryonic and HeLa cells (Ishii and Watanabe 1996), cultured lymphocytes from occupationally exposed persons (Barquinero *et al.* 1995, Gourabi and Mozdarani 1998), cultured animal lymphocytes (Flores *et al.* 1996), and *in vivo* studies on laboratory animals (Wojcik and Tuschl 1990, Cai and Liu 1990, Farooqi and Kesavan 1993, Liu *et al.* 1992).

There are also reports indicating lack of radioadaptive response in cultured human lymphocytes (Bosi and Olivieri 1989, Olivieri and Bosi 1990, Hain *et al.* 1992). However, long-term follow up studies indicate that lack of radioadaptive response is not a temporary effect (Mortazavi *et al.* 1999) and in contrast with the early reports of Olivieri and Bosi (1990) does not depend on transient physiological factors (Mortazavi *et al.* 2000, Ikushima and Mortazavi 2000). It has been recently reported that adaptive response studies may have implications in manned deep space journeys. Mortazavi *et al.* proposed that individuals who failed to show an adaptive response in ground-based *in vitro* studies, would not be good candidates for space travel (Mortazavi *et al.* 2003).

Mortazavi *et al.* previously reported preliminary results of their cytogenetical, immunological and hematological studies on the residents of HLNRA of Ramsar (Mortazavi *et al.* 2002a, Ghiassi-Nejad *et al.* 2002, and

Mortazavi *et al.* 2002b) suggesting that exposure to high levels of natural background radiation can induce radioadaptive response in human cells. Lymphocytes of Ramsar residents when subjected to 1.5 Gy of gamma rays showed fewer induced chromosome aberrations compared to residents in a nearby normal background control area whose lymphocytes were subjected to the same radiation dose. In this paper we report the effect of cumulative dose on the radioadaptive response.

Materials and Methods

1. Cell Culture

In the 1st phase of the study, venous blood samples were taken from 15 and 30 healthy volunteers of both sexes who lived in HLNRA and NLNRA respectively. The maximum measured dose rate of gamma radiation was 155 $\mu\text{Sv h}^{-1}$. In the 2nd phase, seven healthy volunteers from HLNRA and five healthy volunteers from a nearby NLNRA with dose rates from 0.07 to 0.11 $\mu\text{Sv h}^{-1}$ served as controls. It should be noted that in the 2nd phase, due to our selection criteria for study participants (inhabitants who received the annual doses higher than 300 mSv), only limited number of volunteers from HLNRA were available for this study. All participants were non-smokers with no alcohol or drug consumption, history of medical irradiation or viral infections. Standard conditions for cell cultivation, irradiation and analysis of chromosome aberrations were used (Ikushima and Mortazavi 2000). Separate cultures were made from each blood sample, using 0.3 ml blood in 4.7 ml Ham's F10 medium (Gibco), supplemented with 20% fetal calf serum (FCS Gibco), 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, 1.0% L-glutamine and 1.0% phytohemagglutinin (PHA, Gibco) for mitogenic stimulation. The lymphocytes were incubated in the dark at 37°C for 48 hours after PHA stimulation

2. Irradiation

Following this incubation, cells were given a challenge dose of either 2 Gy or 1.5 Gy of Co-60 gamma radiation at a dose rate of 114 mGy/sec. Some of the culture flasks were sham irradiated to assess either the frequency of chromosomal aberrations induced by natural radiation alone in HLNRA residents and the spontaneous frequency of aberrations in NLNRA residents.

After the challenge dose all the culture flasks were incubated a further either 2 (1st phase) or 6 (2nd phase) hours. Colcemid was added 2 hours before the end of this incubation at a final concentration of 0.25 µg/ml to arrest the dividing lymphocytes at metaphase.

3. Slide Preparation

After the two or six hour incubation, the cells were exposed to 0.075 M KCl for 10 min at 37°C and fixed with methanol-acetic acid (3:1 v/v). The fixed cells were dropped onto wet slides, air dried and stained with Giemsa. For each data point, about 200 well-spread metaphases were blind scored for chromosomal aberrations. The number of chromosome aberrations was determined. Gaps (achromatic lesions smaller than the width of a chromatid) were included in the statistical analysis of the 1st phase but in order to enhance the reliability of the results, these lesions were excluded in the statistical analysis of the 2nd phase.

4. Data Analysis

Our basic measurement was the mean chromosomal aberration per cell (MCA). MCA_H indicates cells from a HLNRA and MCA_N indicates cells from a NLNRA. If a challenge dose of 2 or 1.5 Gy was given, this is indicated by “+CD”. If no challenge dose was given, it is indicated by “+0”. We expect a challenge dose to produce an increase in MCA from both the HLNRA and the NLNRA subjects. If the high background dose does not function as a priming dose, we define the “expected MCA” for subjects from high background areas, MCA_{HE} as :

$$MCA_{HE} = MCA_{H+0} + (MCA_{N+CD} - MCA_{N+0}) \quad 1$$

Standard error of the MCA_{HE} was calculated according the equation :

$$SE_{MCA_{HE}} = [(SE_{MCA_{H+0}})^2 + (SE_{MCA_{N+CD}})^2 + (SE_{MCA_{N+0}})^2]^{1/2} \quad 2$$

The coefficient of induced adaptive response (k) in each experiment was calculated as follows :

$$k = MCA_{H+CD} / MCA_{HE} \quad 3$$

Standard error of the k was calculated according the equation :

$$(SE_k / k)^2 = (SE_{MCA_{H+CD}} / MCA_{H+CD})^2 + (SE_{MCA_{HE}} / \text{Expected } MCA_{HE})^2 \quad 4$$

In this equation, SE_k is standard error of the k, $SE MCA_{H+CD}$ and $SE MCA_{HE}$ are standard errors of observed and expected MCA respectively. A k value of less than one indicates a radioadaptive response. If $k=1$, it means that there is no radioadaptive (a simple additivity) effect. Finally, if k is significantly greater than 1, it means that a synergistic effect¹ was induced. The statistical significance of increased or decreased frequencies of chromosome aberrations was evaluated using Student's t-test.

Results

Table 1 shows the number of study participants, age, sex, and potential maximum annual dose from exposure to external gamma rays in 15 and 30 individuals from HLNRA and NLNRA respectively who participated in our study. Table 1 also shows the mean frequencies of chromosomal aberrations per cell with and without the 2 Gy challenge dose and whether there was a radioadaptive response. Overall results show a statistically significant radioadaptive response with a k-value less than one (Table 2).

The age, sex, and mean cumulative effective radiation doses from exposure to external gamma rays in the 12 subjects are shown in Table 3. The average cumulative effective dose in the 7 residents of HLNRA was 2500 mSv (2.5 Sv) which is about 170 times greater than for the 5 controls who received only 15 mSv. Table 3 also shows the mean frequencies of chromosomal aberrations per cell with and without the 1.5 Gy challenge dose and whether there was a radioadaptive response. Five out of 7 residents of HLNRA, demonstrate a statistically significant radioadaptive response with k-values less than one. The k-values were less than one but the differences were not statistically significant in 2 individuals with the highest cumulative effective doses (Table 4).

Table 5 shows no significant difference between background levels of MCA in non-irradiated cells of residents of HLNRA and the controls. There were fewer chromosomal aberrations in the cells of the residents of HLNRA exposed to 1.5 Gy compared to the controls ($P<0.001$).

1. Synergism means the observed effect of exposure to a combination of substances is greater than the sum of the effects of each substance administered individually (e.g. $2+2 = 5$).

After plotting the k-value versus cumulative lifetime dose, we fit a second-order polynomial curve to the data, forcing a y-intercept value of zero. This seems appropriate because, at zero dose, we do not expect to see induced chromosomal damage. The resulting curve is not unlike those suggested by other researchers reporting on adaptive response (Ikushima 1989a) and suggests that these results are consistent with adaptive response induced by exposure to the elevated levels of natural background radiation found in Ramsar as we reported earlier (Ghiassi-Nejad et al., 2002). What is important about these findings is the apparent relationship between the degree of adaptive response (indicated by the k-value) and cumulative lifetime dose among all study participants. The radioadaptive response of the residents of HLNRA was more pronounced (lower k values) at higher cumulative doses except for 2 residents, whose cumulative doses were much higher than the others. That is, increased natural radiation decreased the *radiation* sensitivity of the cells. In view of the known detriment produced by exposure to high levels of radiation, this finding is expected and may reflect the transition from the adaptive response regime to the detrimental response regime in the radiation dose-response curve.

Discussion

Although the induction of adaptive response has been indicated in a wide variety of both *in vitro* and *in vivo* human studies, our study is the first extended experiment on the induction of adaptive response in humans by high levels of natural radiation. The overall results of the 1st phase of our study showed a significant adaptive response when the cultured lymphocytes of the 15 inhabitants of HLNRA were exposed to a 2 Gy Gamma challenge dose. These results confirm the previous results obtained in other *in vivo* human studies such as radiation worker studies (Barquinero *et al.* 1995, Gourabi and Mozdarani *et al.* 1998).

The results obtained in the 2nd phase indicate that residents of areas with extraordinary levels of natural radiation (annual doses up to 260 mGy) show a significant radioadaptive response. It was observed that the 5 persons who received cumulative doses of 360-950 mGy showed a significant radioadaptive response while the two individuals with the highest cumulative doses (6800 and 8400 mGy) failed to show a significant radioadaptive

response. That is, 70% showed an adaptive response from living in the HLNRA. No participant from the HLNRA had an increase in radiation damage ($k > 1$) compared to the controls.

We consider of great potential importance that high levels of natural radiation can serve as the priming or conditioning dose. Other human studies came from normal background areas and studies in which the adapting dose to the cells was given *in vitro*. These studies showed various responses as indicated in the following. There was a radioadaptive response in none of the three donors (Hain *et al.* 1992); in 2 of 6 donors (Pereira Luis and Pova 1992) and in 1 of 8 donors (Gajendiran 2001). In our study, the adaptive response observed in the residents who were exposed to doses up to 260 mGy y^{-1} , were significant.

In this study the k-values of the induced adaptive response ranged from 0.25 to 0.79 (Table 4). There was no significant difference between the $MCMA_{+0}$ of the residents of HLNRA and NLNRA (Table 5). This finding contradicts that of Fazeli *et al.* (1990), who reported that numbers of chromosomal aberrations in lymphocytes of HLNRA residents were significantly higher than in NLNRA residents.

The following factors explain the importance of the radioadaptive response observed in the residents of HLNRA of Ramsar:

1. Studies on the inhabitants of the contaminated areas after Chernobyl accident showed no adaptive response in the lymphocytes of the inhabitants chronically exposed to fallout and then challenged with a high dose radiation (Padovani *et al.* 1995). However, when the same samples were challenged with bleomycin rather than ionizing radiation, an adaptive response was observed (Tedeschi *et al.* 1995). This may be due to living in an area with elevated levels of natural radioactivity for many years vs. living in a contaminated area for a much shorter time. Assessing the radioadaptive response of inhabitants who moved to the HLNRA of Ramsar 5-20 years ago, is planned for the future.
2. Adaptive responses have been usually observed by exposing the cells *in vitro* to a low dose radiation in the range of 1-10 cGy (Redpath *et al.* 2001). These doses are considerably lower than the lifetime doses that induced adaptive response in the inhabitants of HLNRA of Ramsar.

3. Excluding a few studies, which have been performed on the induction of radioadaptive response in radiation workers (Barquinero *et al.* 1995, Gourabi and Mozdarani *et al.* 1998), *in vivo* adaptive response studies were typically performed on laboratory animals. Extrapolating animal data to humans may not be valid.
4. The relative frequency of individuals who show no radioadaptive response in some *in vitro* studies (cultured cells were exposed to both adapting and challenge doses in a laboratory) is considerably greater than the frequency observed in this study (Hain *et al.* 1992, Pereira Luis and Povo 1992, Vijayalaxmi *et al.* 1995, Gajendiran *et al.* 2001).
5. Gadhia (1998) suggested that aging could abolish the adaptive response. He found statistically significant radioadaptive response in all of blood donors aged 5-45 years and no radioadaptive response in all 12 donors aged 65 years. We observed significant radioadaptive responses in 2 individuals who were much older than 65 years (Table 3). Our findings suggest that in *in vivo* studies, aging does not seem to influence the induction of radioadaptive response.
6. Our study suggests that high levels of natural radiation may enhance radiation-resistance in non-cycling lymphocytes. As the majority of our lymphocytes are in the resting phase phase(G_0), any implication of radioadaptive response in radiation protection strongly depends on the possibility of induction of radioadaptive response in G_0 phase. There is still a great controversy over the possibility of induction of radioadaptive response in G_0 of the cell cycle. While some investigators claimed the existence of a significant radioadaptive response in G_0 phase of the cell cycle, others reported that this response is only observable in cycling lymphocytes (Shadley *et al.* 1987, Moquet *et al.* 1989, Wang *et al.* 1991). Our results on the high-level natural radiation induced radioadaptive response, clearly showed that the non-cycling lymphocytes of the inhabitants demonstrated a significant response.
7. We note that the seven data points assembled in this study appear to be a “U” shaped curve, similar in shape to those recently reported by Vilenchick and Knudson (2000) and other researchers (Ikushima 1989) in previous studies. Further work is needed to determine if this shape is maintained when more data are available.

8. Some of the discrepancies noted above may be due to the difference between administering the conditioning dose in vivo versus in vitro and over many years versus a short period of time. These discrepancies must be resolved through further studies in order to confirm the findings reported here and in our previous paper (Ghiassi-Nejad and Mortazavi 2001, Ghiassi-nejad et al., 2002). In particular, we feel that further studies in Ramsar are of sufficient importance that the international community, perhaps in conjunction with the RERF or the IAEA, should consider establishing a long-term research institute in Ramsar to help us gain a better understanding of the radiation biology and radioecology of this region.

Conclusion

Our studies suggest that chronic exposure to elevated levels of natural radiation could make the cultured lymphocytes more resistant to subsequent high doses of radiation. A relationship was found between the cumulative dose of each study participants and the magnitude of the induced radioadaptive response. Results of our experiments showed that high levels of natural radiation in inhabitants whose cumulative doses were up to 1 Gy significantly decreased radiation damage as measured by reduced chromosomal aberrations in irradiated lymphocytes. This can be considered a beneficial effect of high natural radiation. If the LNT hypothesis is true, public health is best served by relocating HLNRA's inhabitants. However several statistically significant epidemiological studies of populations exposed to occupational doses of radiation show decreases of cancer mortality and mortality from all causes (Matanoski 1991, Pollycove 1998, and Berrington *et al.* 2001). Populations in areas with high levels of natural radiation such as Kerala, India (Nair *et al.*, 1999; Jaikrishan *et al.*, 1999) or Yangjiang, China (Tao *et al.*, 2000) show no adverse health effects when compared to low-dose populations. Furthermore, several studies of large populations suggest beneficial health effects of higher than normal background doses of ionizing radiation, i.e., lower cancer mortality rates (Frigerio et al 1973; Cohen 1995, 1996; Wei 1997; Jagger 1998). Our findings on the biological effects of prolonged exposure to high levels of natural radiation in the inhabitants of HLNRA's of Ramsar, showed no apparent harmful health effects. We have been reported

previously that the health effects of prolonged exposure to high levels of natural radiation may contradict current ultra-conservative radiation protection regulations (Mortazavi et al. 2002c). Governments should adopt public health measures and policies that are cost-effective in risk reduction by considering the financial, social and psychological impact on their citizens (Karam et al., 2002, Mortazavi 2002). Based on our results we suggest that worldwide research on the residents of high level natural radiation areas help scientists better justify if LNT model of radiation risk is appropriate as the basis for public health measures.

Acknowledgement

This project was supported by National Radiation Protection Department (NRPD), Iranian Nuclear Regulatory Authority, Rafsanjan University of Medical Sciences, and Babol University of Medical Sciences.

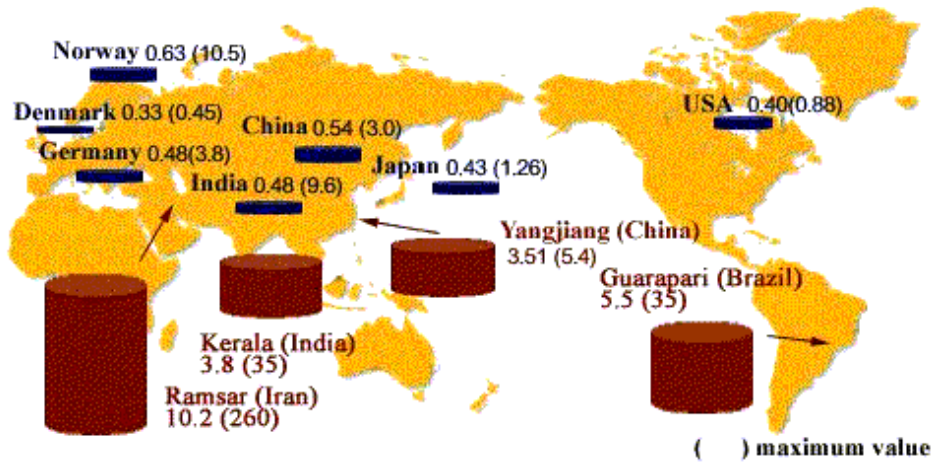


Figure 1. Average and Maximum annual background absorbed doses (mGy/yr) to the inhabitants of some countries and for areas with high levels of natural radiation (Used with permission of Radiation Research Foundation, Kyoto, Japan).

Table.1 Mean frequency of chromosome aberrations* in non-irradiated and 2 Gy irradiated cells of NLNRAs and HLNRAs residents.

Area	No. of Participants	Age (years)	Sex	Maximum Annual Dose (mGy)	MCA ^c in non-irradiated cells	MCA ^c in cells exposed to 2 Gy Gamma rays	Induction of radioadaptive response
HLNRAs ^a	15	30.40 ± 1.92	5F 10M	260	0.099 ± 0.003 ^d	0.111 ± 0.003	Positive (P<0.001)
NLNRAs ^b	30	33.77 ± 1.33	11F 19M	1.05	0.049 ± 0.003	0.167 ± 0.004	ND ^e

a Normal background radiation area

b High background radiation area

c Mean chromosome aberrations per cell

d Mean ± SE

e Not determined

* Gaps were included in the statistical analysis

Table. 2 Induction of radioadaptive response in the residents of HLNRA of Ramsar.

Cases	MCA ^a				k-Value
	Adapting Dose Alone	Challenge Dose Alone	Controls	Expected Observed	
15 HLNRA's Inhabitants ^b	0.099 ± 0.003 ^c	0.167 ± 0.004	0.049 ± 0.003	0.217 ± 0.006 0.111 ± 0.003	0.514 ± 0.055

a Mean chromosome aberrations per cell

b High background radiation area

c Mean ± SE

* Gaps were included in the statistical analysis

Table.3 Mean frequency of chromosome aberrations in non-irradiated and 1.5 Gy irradiated cells of NLNRA and HLNRA residents.

Case No.	Age (years)	Sex	Cumulative dose (mSv)	MCA ^c in non-irradiated cells	MCA ^c in cells exposed to 1.5 Gy Gamma rays	Induction of radioadaptive response
1 (NLNRA) ^a	69	F	11	0.02 ± 0.01	0.17 ± 0.03	ND ^d
2 (NLNRA)	29	M	18	0.01 ± 0.01	0.14 ± 0.03	ND
3 (NLNRA)	37	M	12	0.02 ± 0.01	0.24 ± 0.04	ND
4 (NLNRA)	44	F	17	0.01 ± 0.01	0.16 ± 0.03	ND
5 (NLNRA)	26	M	15	0.02 ± 0.01	0.23 ± 0.04	ND
1 (HLNRA) ^b	23	M	360	0.01 ± 0.01	0.12 ± 0.02	Positive (P<0.05)
2 (HLNRA)	47	F	390	0.01 ± 0.01	0.13 ± 0.03	Positive (P<0.05)
3 (HLNRA)	70	F	360	0 ± 0	0.09 ± 0.03	Positive (P<0.01)
4 (HLNRA)	57	F	6800	0.02 ± 0.01	0.14 ± 0.04	Negative
5 (HLNRA)	63	M	8400	0.02 ± 0.01	0.15 ± 0.05	Negative
6 (HLNRA)	75	F	950	0.03 ± 0.02	0.05 ± 0.02	Positive (P<0.001)
7 (HLNRA)	55	F	480	0.01 ± 0.01	0.06 ± 0.02	Positive (P<0.01)

a Normal background radiation area

b High background radiation area

c Mean chromosome aberrations per cell

d Not determined

* Gaps were excluded in the statistical analysis

Table.4 Induction of radioadaptive response in the residents of HLNRA of Ramsar.

Case No.	MCA ^b					k-Value
	Adapting Dose	Challenge Dose	Controls	Expected	Observed	
1 (HLNRA) ^a	0.01 ± 0.01	0.19 ± 0.02	0.02 ± 0.004	0.18 ± 0.02	0.12 ± 0.02	0.67 ± 0.13
2 (HLNRA)	0.01 ± 0.01	0.19 ± 0.02	0.02 ± 0.004	0.18 ± 0.02	0.13 ± 0.03	0.72 ± 0.20
3 (HLNRA)	0 ± 0	0.19 ± 0.02	0.02 ± 0.004	0.17 ± 0.02	0.09 ± 0.03	0.53 ± 0.20
4 (HLNRA)	0.02 ± 0.01	0.19 ± 0.02	0.02 ± 0.004	0.19 ± 0.02	0.14 ± 0.03	0.74 ± 0.22
5 (HLNRA)	0.02 ± 0.01	0.19 ± 0.02	0.02 ± 0.004	0.19 ± 0.02	0.15 ± 0.03	0.79 ± 0.28
6 (HLNRA)	0.03 ± 0.02	0.19 ± 0.02	0.02 ± 0.004	0.20 ± 0.03	0.05 ± 0.02	0.25 ± 0.12
7 (HLNRA)	0.01 ± 0.01	0.19 ± 0.02	0.02 ± 0.004	0.18 ± 0.02	0.06 ± 0.02	0.33 ± 0.13

a High background radiation area

b Mean chromosome aberrations per cell

** Gaps were excluded in the statistical analysis*

Table.5 Frequency of chromosomal aberrations in non-irradiated and irradiated cells of the residents of high background radiation areas and the residents of the control area.

Study group	Sample size	Cumulative dose (mSv)	MCA ^a in non-irradiated cells	MCA ^a in cells exposed to 1.5 Gy
HLNRA ^b	7	2534	0.014 ± 0.004	0.106 ± 0.015
NLNRA ^c	5	14.6	0.016 ± 0.002	0.188 ± 0.020
P-value ^d			Not significant	< 0.001

a Mean chromosome aberrations per cell

b High background radiation area

c Normal background radiation area

d Student's *t*-test.

* Gaps were excluded in the statistical analysis

References

Barquinero J.F., Barrios L., Caballin M.R., Miro R., Ribas M., Subias A. and Egozcue J. Occupational exposure to radiation induces an adaptive response in human lymphocytes, *Int. J. Radiat. Biol.* 1995; **67**, 187-91.

Berrington, A, Darby, SC, Weiss, HA, Doll, R. 100 years of observation on British radiologists: mortality from cancer and other causes 1897-1997 *Br J Radiol.* 2001; **74**, 507-519.

Boothman DA, Bouvard I, Hughes EN. Identification and characterization of X-ray-induced proteins in human cells. *Cancer Res.* 1989; **49**, 2871-2878.

Bosi A., and Olivieri G. Variability of the adaptive response to ionizing radiation in humans. *Mutat. Res.* 1989; **211**, 13-17.

Cai L.and.Liu S.Z. Induction of cytogenetic adaptive response of somatic and germ cells *in vivo* and *in vitro* by low dose X-irradiation, *Int. J. Radiat. Biol.* 1990; **58**, 187-194.

Cohen B.L. Test of the linear no-threshold theory of radiation carcinogenesis for inhaled radon decay products. *Health Phys.* 1995; **68**, 157-174.

Cohen, B.L. Problems in the radon versus lung cancer test of the linear no-threshold theory and a procedure for resolving them. *Health Phys.* 1996; **72**, 623-628,

Cumulative Dose and Induced Radioadaptive Response

Cortes F, Dominguez I, Mateos S, Pinero J, Mateos JC. Evidence for an adaptive response to radiation damage in plant cells conditioned with X-rays or incorporated tritium. *Int J Radiat Biol.* 1990; **57**, 537-41,

Farooqi, Z., Kesavan, , PC. Low-dose radiation-induced adaptive response in bone marrow cells of mice, *Mutat. Res.* 1993; **302**, 83-9.

Fazeli T.Z. Cytogenetic studies of inhabitants of a high background radiation area of Ramsar. *Proceeding of International Conference on High Levels of Natural Radiation (ICHLNR)*, Ramsar, Iran, 1990; 459-464.

Flores M.J., Pinero J., Ortiz T., Pastor N., Mateos J.C. and Cortes F. Both bovine and rabbit lymphocytes conditioned with hydrogen peroxide show an adaptive response to radiation damage, *Mutat. Res.* 1996; **372**, 9-15.

Frigerio, N.A., Eckerman, K.F. and Stowe, R.S. Carcinogenic Hazard from Low-Level, Low-Rate Radiation, Part I, Rep. 1973; ANL/ES-26. Argonne Nat. Lab.

Fritz-Niggli H, Schaepfi-Buechi C. Adaptive response to dominant lethality of mature (class A) and immature (class B) oocytes of *D. melanogaster* to low doses of ionizing radiation: effects in repair-proficient (yw) and repair-deficient strains (mei 41D5 and mus 302D1). *Int J Radiat Biol.* 1991; **59**, 175-84.

Gadhia P.K., Possible age-dependent adaptive response to a low dose of X-rays in human lymphocytes, *Mutagenesis.* 1998; **13**, 151-152.

Gajendiran N, Tanaka K, Kumaravel TS, Kamada N. Neutron-induced adaptive response studied in go human lymphocytes using the comet assay. *J Radiat Res (Tokyo).* 2001; **42**, 91-101.

Ghiassi-nejad, M; Mortazavi, SMJ; Cameron, JR; Niroomand-rad, A; Karam, PA; Very High Background Radiation Areas of Ramsar, Iran: Preliminary Biological Studies. *Health Physics.* 2002; **82**, 87-93.

Gourabi H., and Mozdarani H. A cytokinesis-blocked micronucleus study of the radioadaptive response of lymphocytes of individuals occupationally exposed to chronic doses of radiation, *Mutagenesis.* 1998; **13**, 475-80.

Hain J., Jaussi R., and Burkart W. Lack of adaptive response to low doses of ionizing radiation in human lymphocytes from five different donors. *Mutat. Res.*, **283**, 137-44, 1992.

Hayata I, Tabuchi H, Furukawa A, Okabe N, Yamamoto M, Sato K. Robot system for preparing lymphocyte chromosome. *J Radiat Res (Tokyo)*; 33 Suppl: 231-41, 1992.

Ikushima T. Chromosomal responses to ionizing radiation reminiscent of an adaptive response in cultured Chinese hamster cells, *Mutation Research*, Vol. 180, No 2, 215-221, 1987.

Mart. SMJ., Shab. M.A., Ghiassi N.M., Mozd. H. (2005) Asian J. Exp. Sci., 19(1), 19-39

Ikushima T. Radioadaptive response: characterization of a cytogenetic repair induced by low-level ionizing radiation in cultured Chinese hamster cells, *Mutation Research* , Vol. 227, No 4, 241-246, 1989a.

Ikushima, T. Radioadaptive response: a novel chromosomal response in Chinese hamster cells in vitro, In: *Chromosomal Aberrations, Basic and applied aspects*, G. Obe and AT. Natarajan eds. Springer-Verlag, Berlin, pp. 151-161, 1989b.

Ikushima, T., and Mortazavi, S. M. J. Radioadaptive response: its variability in cultured human lymphocytes, In: *Biological Effects of Low Dose Radiation*. Yamada T, Mothersil C, Michael BD, and Potten CS, eds. Elsevier, Amsterdam, 1st edition, pp. 81-86, 2000.

Ishii K. and Watanabe M. Participation of gap-junctional cell communication on the adaptive response in human cells induced by low dose of X-rays, *Int. J. Radiat. Biol.* , **69**, 291-9, 1996.

Jaikrishan G, Andrews VJ, Thampi MV, Koya PK, Rajan VK, Chauhan PS. Genetic monitoring of the human population from high-level natural radiation areas of Kerala on the southwest coast of India. I. Prevalence of congenital malformations in newborns. *Radiat Res*, 152 (6 Suppl), S1 49-53, 1999.

Jagger, J. Natural background radiation and cancer death in Rocky Mountain states and Gulf Coast states. *Health Phys.* **75**, 428-430, 1998.

Jaworowski Z. Beneficial effects of radiation and regulatory policy. *Australas Phys Eng Sci Med*, **20(3)**, 125-38, 1997.

Karam PA, Leslie SA. Calculations of background beta-gamma radiation dose through geologic time. *Health Phys*, **77(6)**, 662-7, 1999.

Kellerer AM. Risk estimates for radiation-induced cancer—the epidemiological evidence. *Radiat Environ Biophys*, **39(1)**, 17-24, 2000.

Kratz FL. Radioresistance in natural populations of *Drosophila nebulosa* from a Brazilian area of high background radiation. *Mutat Res*, **27(3)**, 347-55, 1975.

Liu S.Z., Cai L., Sun S.Q. Induction of a cytogenetic adaptive response by exposure of rabbits to very low dose-rate gamma-radiation, *Int. J. Radiat. Biol.*, **62**, 187-90, 1992.

Matanoski G: Health effects of low-level radiation in shipyard workers final report. 471 pages Baltimore, MD, DOE DE-AC02-79 EV10095, (1991)

Mettler FA, Sinclair WK, Anspaugh L, Edington C, Harley JH, Ricks RC, Selby PB, Webster EW, Wyckoff HO. The 1986 and 1988 UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) reports: findings and implications. *Health Phys*, **58(3)**, 241-50, 1990.

Cumulative Dose and Induced Radioadaptive Response

Mortazavi, S. M. J., Mozdarani H, Ikuhima T, and Sharafi AA. Radiation Hormesis and Adaptive Responses Induced by Low Doses of Ionizing Radiation. *Journal of Kerman University of Medical Sciences*, Vol. 6, No. 1, 50-60, 1999.

Mortazavi, S. M. J., Ikuhima, T., Mozdarani, H., Sharafi, A. A. and Y. Ishi. Is low-level pre-irradiation of human lymphocytes an absolutely beneficial phenomenon. A report on the extra-ordinary synergism. *Kowsar Medical Journal*, Vol 5, No 4, 235-240, 2000.

Mortazavi SMJ, Ghiassi-nejad M, Niroomand-rad A, Karam PA, Cameron, JR. How should governments address high levels of natural radiation and radon? Lessons from the Chernobyl nuclear accident, *Risk: Health, Safety and Environment*, 13/1.2, 31-45, 2002a.

Mortazavi, SMJ, Karam P.A. High Levels of Natural Radiation in Ramsar, Iran: Should Regulatory Authorities Protect the Inhabitants? *Iranian Journal of Science (Germany)*, **2** (2), 1-9, 2002b.

Mortazavi, SMJ., Ghiassi-nejad M., and Ikushima, T., Do the findings on the health effects of prolonged exposure to very high levels of natural radiation contradict current ultra-conservative radiation protection regulations, In: *Radiation and Homeostasis*. Sugahara T, Nikaido O, and Niwa O. Eds, pp. 19-21, Elsevier, Amsterdam, 2002c

Mortazavi, SMJ., Risk Assessment: Extraordinary Levels of Natural Radioactivity in the Environment and the Problems Associated with Induced Radioresistance, In: *Proceedings of the International Conference on Radioactivity in the Environment*. Borretzen P, Jolle T, and Strand P. Eds, pp. 110-113, 2002.

Mortazavi SMJ, Cameron JR, and Niroomand-rad A. Adaptive response studies may help choose astronauts for long-term space travel, *Advances in Space Research*, **31** (6), 1543-1552, 2003.

Moquet JE, Prosser JS, Edwards AA, Lloyd DC. Sister-chromatid exchanges induced by mitomycin C after acute or chronic exposure of human lymphocytes to a low dose of X-rays. *Mutat Res*, **227**(4), 207-13, 1989.

Nair MK, Nambi KS, Amma NS, Gangadharan P, Jayalekshmi P, Jayadevan S, Cherian V, Reghuram KN. Population study in the high natural background radiation area in Kerala, India. *Radiat Res*; 152 (6 Suppl), S1 45-8, 1999.

Olivieri G, Bodycote J. and Wolff S., Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, *Science*, **223**, 594-597, 1984.

Olivieri G., and Bosi A. Possible causes of variability of the adaptive response in human lymphocytes. In *Chromosomal Aberrations Basic and Applied Aspects* (G. Obe and E. D. induced DNA damage in adapted cells, *Mutat. Res.*, 358, 193-8, 1990.

Mart. SMJ., Shab. M.A., Ghiassi N.M., Mozd. H. (2005) Asian J. Exp. Sci., 19(1), 19-39

Padovani L., Appoloni M., Anzidei P. and Tedeschi B. Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? Challenge with ionizing radiation, *Mutat. Res.*, 332, 33-8, 1995.

Pereira Luis JH and Pova VL. Individual variability of adaptive response of human lymphocytes primed with low dose gamma rays. In: *Low Dose Irradiation and Biological Defence Mechanisms*. Pp. 315-317, Elsevier Science Publication, Tokyo, 1992.

Pollycove M. Nonlinearity of radiation health effects. *Environ Health Perspect*, 106 Suppl **1(9806)**, 363-8 1998.

Redpath JL, Liang D, Taylor TH, Christie C, Elmore E. The shape of the dose-response curve for radiation-induced neoplastic transformation in vitro: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. *Radiat Res*; **156(6)**, 700-7, 2001.

Samson L. and Carins J., A new pathway for DNA repair in *Escherichia coli*, *Nature*, **267**, 281-282, 1977.

Samson L. and Schwartz J.L., Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines, *Nature*, 287, 861-863, 1980.

Sankaranarayanan K., Von Duyn A., Loos M., and Natarjan, A.T. Adaptive response of human lymphocytes to low level radiation from radioisotopes or X-rays. *Mutat. Res.*, **211**, 7-12, 1989.

Shadley J.D., Wolff S. Very low doses of X-rays can cause human lymphocytes to become less susceptible to ionizing radiation, **2**, 95-96, 1987.

Shadley J.D. and Wiencke J.K. Induction of the adaptive response by X-rays is dependent on radiation intensity, *Int. J. Radiat. Biol.*, **56**, 107-118, 1989.

Sobue T, Lee VS, Ye W, Tanooka H, Mifune M, Suyama A, Koga T, Morishima H, Kondo S. Residential radon exposure and lung cancer risk in Misasa, Japan: a case-control study. *J Radiat Res (Tokyo)*, **41(2)**, 81-92, 2000.

Sohrabi M. Recent radiological studies of high background radiation areas of Ramsar. *Proceeding of International Conference on High Levels of Natural Radiation (ICHLNR)*, Ramsar, Iran, 3-7, 1990.

Sohrabi M. World high level natural radiation and/or radon prone areas with special regards to dwellings. In: *Proceeding of the 4th International Conference on High Levels of Natural Radiation (ICHLNR)*, Beijing, China, 1996 (Wei L, Suahara T and Tao Z Ed), pp. 3-7, 1997.

Cumulative Dose and Induced Radioadaptive Response

Tao Z, Zha Y, Akiba S, Sun Q, Zou J, Li J, Liu Y, Kato H, Sugahara T, Wei L. 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, 2000.

Tedeschi B, Caporossi D, Vernole P, Padovani L, Appolloni M, Anzidei P, Mauro F. Do human lymphocytes exposed to the fallout of the Chernobyl accident exhibit an adaptive response? 2. Challenge with bleomycin. *Mutat Res* 23; **356(2)**, 299-300, 1996.

UNSCEAR. Sources and effects of ionizing radiation, Report to the general assembly, New York. United Nations Scientific Committee on the Effects of Atomic Radiation, Sources and Effects of Ionizing Radiation, 2000.

Vijayalaxmi, Leal B.Z., Deahl T.S., Meltz M.L. Variability in adaptive response to low dose radiation in human blood lymphocytes: consistent results from chromosome aberrations and micronuclei. *Mutat. Res.*, **348**, 45-50, 1995.

Wang ZQ, Saigusa S, Sasaki MS. Adaptive response to chromosome damage in cultured human lymphocytes primed with low doses of X-rays. *Mutat Res*, **246(1)**, 179-86, 1991.

Wei, L. High background radiation area - an important source of exploring the health effects of low dose ionizing radiation. In: Wei, L.; Sugahara, T.; Tao, Z., ed. *High Levels of Natural Radiation: Radiation Dose and Health Effects*. Beijing, China; Amsterdam, The Netherlands: Elsevier: 1-66, 1997.

Wiencke J.K., Afzal V., Olivieri G. and Wolff S. Evidence that the [3H] thymidine induced adaptive response of human lymphocytes to subsequent doses of X-rays involves the induction of chromosomal repair mechanism, *Mutagenesis*, **1**, 375-380, 1986.

Wojcik A. and Tuschl H. Indications of an adaptive response in C57BL mice pre-exposed *in vivo* to low doses of ionizing radiation, *Mutat. Res.*, **243**, 67-73, 1990.

Wolff S., Afzal V., Wiencke J.K. and Olivieri G., Human lymphocytes exposed to low doses of ionizing radiation become refractory to high doses of radiation as well as to chemical mutagens that induce doublestrand breaks in DNA., *Int. J. Radiat. Biol.*, 53, 39-48, 1988.

Zou J, Sun Q, Akiba S, Yuan Y, Zha Y, Tao Z, Wei L, Sugahara T. A case-control study of nasopharyngeal carcinoma in the high background radiation areas of Yangjiang, China. *J Radiat Res (Tokyo)*, 41 Suppl : 53-62, 2000.