COMPARISON OF THE CYTOGENETIC RADIOADAPTIVE RESPONSE INDUCED BY NATURAL BACKGROUND RADIATION WITH OCCUPATIONAL EXPOSURE IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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It is clearly shown that low doses of ionising radiation can induce resistance to subsequent higher exposures, a phenomenon known as , radioadaptive response. In this study we compared induction of cytogenetic radioadaptive response by High Natural Background Radiation (HNBR) in Ramsar, a city in the north part of Iran, has the highest level of natural background radiation in the world and X-Ray occupational exposure as conditioning doses in human peripheral blood lymphocytes. Thirty healthy control individuals, living in Ramsar but in ordinary background radiation areas, 15 healthy individuals from Talesh Mahalleh, a region with very high level of background radiation and 7 X-Ray radiographers working in Ramsar hospital located in an area with normal natural background ionising radiation were evaluated. Peripheral blood samples were obtained and exposed to challenge dose of 0 and 2 Gy. Lymphocytes were scored using analysis of metaphase, for the presence of chromosomal aberrations. An adaptive response was observed in HNBR and radiation workers groups in comparison with sham controls. Also, compared with occupationally exposed group a significant marked increase in adaptive response was observed in HNBR group. These findings indicate that, although both natural background radiation and occupational exposure could induce cytogenetic radioadaptive response but it is more significant with natural background ionising radiation.

Short Title: radioadaptive response induced by natural background and occupational exposure

Introduction

There is now little doubt that low doses of ionising radiation (conditioning dose) can induce resistance to subsequent higher (challenge) exposures. This phenomenon is termed radioadaptive response, first reported by Olivieri et al. in 1984 [1]. Later, many scientists supported this hypothesis [2,3] and described probable involved mechanisms [4,5,6,7]. Induction of cytogenetic radioadaptive response was shown by in vitro pre-exposing of human cells to radiation [2,3] as well as in vivo studies [8,9]. A cytogenetic adaptive response study in human lymphocytes of hospital workers occupationally exposed to X and gamma rays has shown that chronic exposure to radiation make hospital workers lymphocytes less sensitive to higher doses [10]. A similar study revealed that children who received low doses of radiation after Chernobyl accident , had significantly lower chromosomal damage compared to non-exposed controls [11]. In this study we compared induction of cytogenetic radioadaptive response in residents of high natural background radiation (HNBR) area in Ramsar and occupationally exposed radiation workers. People in some areas of Ramsar – a city in northern part of Iran- receive a large amount of dose from background radiation [12]. We considered X and local gamma rays as conditioning doses in radiation workers and Ramsar inhabitants respectively. We also intend to present more confirmation regarding to in vivo radioadaptive response in human lymphocytes following low and chronic doses of radiation in HNBR areas residents and X-ray medical radiographers.

Material & Methods

Venous blood samples were drawn from 52 healthy donors (30 control individuals, living in Ramsar in an area with ordinary background radiation (conditioning dose = 0) ,15 individuals from Talesh Mahalleh, a region with extraordinary high level of background radiation (max. conditioning dose = 260mGy/y) and 7 X-Ray radiographers working in Ramsar hospital located in an area with normal natural background radiation (max. conditioning dose = 20 mGy/year) into heparinized vacutainers. Every attempt was made to match samples from donors with controls in as many aspects as possible. No medicines were taken by the donors for at least 1 month before sampling . Each blood sample was divided into two parts; one as the pre-exposed control and the other for exposure to 2 Gy gamma radiation. Samples were irradiated with a ^{60}Co source (model 780 Teratron, Canada) at room temperature. Whole blood culture were prepared by adding 0.5 ml blood to 4.5 ml culture medium consisting of RPMI-1640 (Gibco BRL) supplemented with 0.2 mM L-glutamine, 15% fetal

calf serum (Gibco BRL), 100 IU/ml penicillin and 50 µg/ml streptomycin. PHA (Gibco BRL) at a concentration of 5 µg/ml was used to stimulate division of lymphocytes in culture. Blood cultures were incubated at 37 °C for 48 hours and 2 hours prior to harvesting, colcemid (Gibco BRL) was added to cultures at a final concentration of 0.1µg/ml to arrest the dividing lymphocytes in mitosis. The cells were collected by centrifugation and treated with a hypotonic solution containing 0.075 M KCl ,for 10 min to obtain good preservation of the cytoplasm. After centrifugation, lymphocytes were fixed in a 3:1 mixture of methanol:glacial acetic acid and then dropped onto cooled, clean slides and air dried. Slides were stained with 5% Giemsa (Merck). The culture technique is followed according to protocol recommended by IAEA* [13].One hundred mitoses were analysed for each sample and chromosomal aberrations (isogaps, breaks dicentrics and rings) were scored. Statistical analysis was done by student t-test and ANOVA with SPSS software.

Results and Discussion

The percentage of the total chromosomal aberrations (CA%) in control non-exposed individuals, radiation workers and HNBR residents before and after irradiation at 2Gy are shown in table 1. Results showed that CA% -before exposing to 2 Gy- was significantly ($p<0.05$) higher in radiation workers (9.37%) and HNBR residents (9.84%) when compared with non-exposed individuals (4.88%) (figure 1). The above result is in full agreement with the results of previous reports[14,15,16] indicating that exposure to radiation in medical radiation workers -even below dose limit, recommended by ICRP* [17]- could lead to detectable genetic effects. There are two different reports related to the cytogenetic assay findings of residents in Ramsar. One report shows a higher chromosomal aberrations compared with controls[18] but the other indicates no significant difference [19]. Our results also indicate that there is no significant difference between CA% in radiation workers and HNBR residents.It should be noted that the annual absorbed radiation dose received by residents of Ramsar is higher than the recommended level by ICRP for public or in some areas even higher than the annual limits for radiation workers[20]. So we suppose that this observation could be due to the fact that no overt dose was given to ramsar residents [19] and also might be due to the existence of significant inter-individual variations[21], that can be related to systematic inter-individual differences in radio-sensitivity[22].When blood samples were irradiated at 2 Gy, it was found that the chromosomal aberration for radiation workers (13.78%) and HNBR

* International Atomic Energy Agency

* International Commission on Radiation Protection

residents (11.03) were significantly lower than non-exposed controls ($p<0.01$). These results are in agreement with previous reports [23,24]. Results also showed that the reduction of CA% after 2 Gy exposure in HNBR residents is completely marked compared with occupationally exposed group in this respect (Figure 1). We feel that, this finding is related to different condition of exposure. Most families in Ramsar have lived in HNBR area for many generations and have received doses for their entire lives, but regarding to radiation workers, The exposure is job related and normally starts at the age of 18.We conclude that more radio-resistance observed in lymphocytes of HNBR residents might be due to longer exposure time for induction of initial DNA damage in these cells leading to sufficient induction of an active DNA repair mechanism[25].

Conclusion

These findings indicate that both natural background radiation and occupational exposure could induce cytogentic radioadaptive response and this process is more significant in high natural background radiation residents.

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	Challenge Dose = 0 Gy				Challenge Dose = 2 Gy			
Chromosomal Aberration (%)	Gap	Dicentric	Ring	Total (Mean ± SD)	Gap	Dicentric	Ring	Total (Mean ± SD)
Controls	3.23	1.65	0	4.88±1.21	9.20	5.39	2.11	16.70±1.45
X-Ray Radiographers	4.99	3.58	1.10	9.37±0.78	6.33	5.94	1.81	13.78±0.52
HNBR Residents	5.12	3.51	1.21	9.84±0.54	6.11	3.64	1.28	11.03±0.60

Table 1. Total chromosomal aberration of lymphocytes in non-exposed controls, X-Ray Radiographers and HNBR residents before and after exposure to 2 Gy gamma radiation as challenging dose.

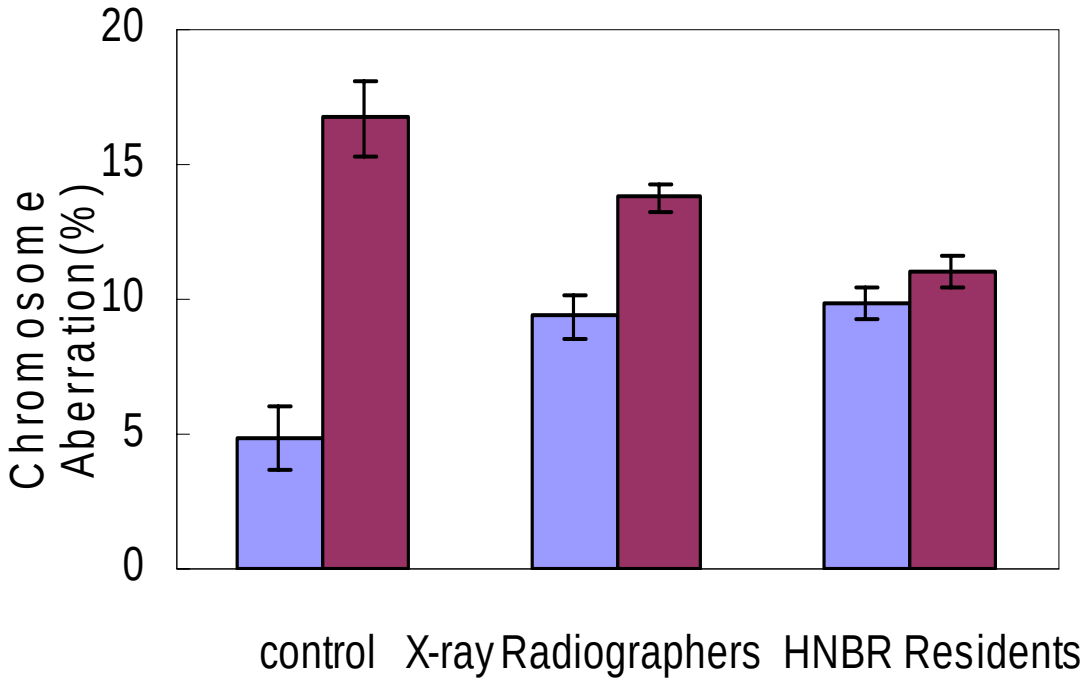

Fig. 1. The percentage of total chromosomal aberration of lymphocytes in non-exposed controls, Radiation workers and HNBR residents before (left columns) and after (right columns) exposure to 2 Gy gamma radiation. Error bars show the standard error of means.

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