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Short communication

Peripheral blood lymphocyte micronucleus frequencies in men from areas of Kerala, India, with high vs normal levels of natural background ionizing radiation

C.V. Karuppasamy[a,*], E.N. Ramachandran[a], V. Anil Kumar[a], P.R. Vivek Kumar[a], P.K.M. Koya[a], G. Jaikrishan[a], Birajalaxmi Das[a,b,**]

[a] Low Level Radiation Research Laboratory, Low Level Radiation Research Section (LLRRS), Radiation Biology & Health Sciences Division (RB&HSD), Bio-Science Group, Bhabha Atomic Research Centre (BARC), Beach Road, Kollam 691 001, Kerala, India
[b] LLRRS, RB&HSD, BARC, Trombay, Mumbai 400 085, India

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ABSTRACT

We have measured the frequencies of micronuclei (MN) in adult male individuals living in areas of the Kerala coast, southwest India, with either high (HLNRA, >1.5 mGy/year) or normal levels of natural ionizing radiation (NLNRA, ≤1.5 mGy/year). Blood samples were obtained from 141 individuals, 94 from HLNRA and 47 from NLNRA, aged 18–72, and were subjected to the cytokinesis-block micronucleus (CBMN) assay. An average of 1835 binucleated (BN) cells per individual were scored. The overall frequency of MN (mean ± SD) was 11.7 ± 6.7 per 1000 BN cells. The frequencies of MN in the HLNRA (11.7 ± 6.6) and NLNRA (11.6 ± 6.7) were not statistically significantly different (P = 0.59). However, a statistically significant (P < 0.001) age-dependent increase in MN frequency was observed among individuals from both HLNRA and NLNRA. No natural background radiation dose-dependent increase in MN frequency was seen. MN frequency was not influenced by tobacco smoking or chewing but it was increased among individuals consuming alcohol. Chronic low-dose radiation in the Kerala coast did not have a significant effect on MN frequency among adult men.

1. Introduction

Humans are constantly exposed to natural background radiation. The level of exposure varies from place to place due to altitude and radioactive mineral deposits. In some areas, levels of natural background radiation exposure are much higher (10–100×) compared to normal levels, due to the presence of radioactivity in soils, rocks, or hot springs. The most prominent high level natural radiation areas (HLNRA) include Guarapari, Brazil; Yangjiang, China; Kerala, India; and Ramsar, Iran. Populations residing in these areas are exposed to low dose/low dose-rate radiation for generations, throughout their lives. The effect of low dose and low dose-rate exposure below 100 mSv has important implications in radiation protection science. The linear-no-threshold (LNT) dose-response relationship extrapolates to low-dose exposures from high acute-dose exposures. Studies of persons from HLNRA provide an opportunity to understand better the biological effects of low-dose exposures.

The coastal belt of Kerala state in southwest India, extending from Neendakara panchayat of Kollam district in the south to Purakkad panchayat of Alappuzha district in the north (about 55 km long and 0.5 km wide) is unique among the world's HLNR areas. The radiation levels in this region range from <1.0 mGy/year to 45.0 mGy/year due to the natural deposits of monazite (1% of beach sand) containing ^{232}Th (8–10%), ^{238}U (0.3%), and their corresponding decay products. The area is densely populated and has been so for more than 1000 years [1–4].

The importance of studies on populations exposed to elevated level of background radiation was emphasized by WHO as early as 1959 [5]. Some of the investigations carried out include skeletal and dental variation in wild rats [6], cytological studies in plants [7], demographic characterization of human population [8],

Abbreviations: GM, Geiger-Muller; HLNRA, high level natural radiation area; NLNRA, normal level natural radiation area; BN, binucleated; CBMN, cytokinesis-block micronucleus assay.

* Corresponding author.
** Corresponding author at: Low Level Radiation Research Section, RB &HSD, Bio-Science Group, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai 400 085, India.
E-mail addresses: karuppasamy.vetrivel@gmail.com (C.V. Karuppasamy), biraj@barc.gov.in, birajalaxmi@yahoo.co.in (B. Das).

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chromosomal aberration analysis in adults [9], cancer incidence [10–12], cytogenetic investigations on newborns for chromosomal aberrations, karyotype anomalies [4,13,14], frequency of micronuclei [15,16], genetic monitoring of newborns for congenital malformations [17], determination of telomere length in newborns and adults [18,19], and a case-control study of mental retardation and cleft lip/palate [20]. None of the above studies reported any significant differences between the HLNRA and NLNRA populations. An investigation using the alkaline comet assay showed that the effect of age on spontaneous DNA damage in peripheral blood lymphocytes of the individuals was influenced by their area of residence (HLNRA/NLNRA) [21].

The cytokinesis-block micronucleus (CBMN) assay is useful in population monitoring. It is faster and simpler than scoring chromosomal damage in metaphase spreads and it provides measures of both chromosome breakage and loss [22–24]. This assay is also reliable for assessing radiation-induced chromosome damage [25–28]. In this study, we have assessed the effect of chronic low-dose and low-dose-rate radiation by measuring the frequencies of micronuclei (MN) in men from the HLNRA and NLNRA of the Kerala coast.

2. Materials and methods

2.1. Materials

RPMI 1640 medium, L-glutamine, 10% fetal calf serum (FCS), Giemsa stain, phytohaemagglutinin (PHA), cytochalasin B and dimethyl sulfoxide were obtained from Sigma-Aldrich Co., St. Louis, MO, USA. Other chemicals used were benzyl penicillin (Alembic Pharmaceuticals, Mumbai, India), streptomycin (Abbott Health Care Private Ltd., Mumbai, India), potassium chloride and DPX (Qualigens, Mumbai, India), methanol and acetic acid (Merck, Mumbai, India).

2.2. Experimental design

Peripheral blood samples were obtained in sterile heparinized vaccutainer tubes, by venipuncture, from 141 adult males (94 from HLNRA and 47 from NLNRA). Written informed consent was obtained from each individual, and the study was carried out with the approval of the medical ethics committee, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. A questionnaire was used to obtain information on age, consumption of alcohol, tobacco smoking, and chewing. Blood samples were brought to the laboratory on ice and the whole blood culture was set up using standard micro-culture techniques as described by the International Atomic Energy Agency (IAEA) [29].

Briefly, heparinized blood (0.5 ml) was added to RPMI 1640 (4.5 ml) supplemented with benzyl penicillin (100 U/ml), streptomycin (100 μg/ml), 1% L-glutamine (200 mM), 10% FCS, and PHA (10 μg/ml), followed by incubation for 44 h at 37 °C. Cultures were treated with cytochalasin-B (6 μg/ml) in dimethyl sulfoxide, incubated for 72 h, and further processed with mild hypotonic treatment using potassium chloride (0.075 M) for 5 min, followed by fixing the cells in 3:1 methanol:acetic acid. The cell suspension was dropped carefully onto pre-cleaned, ice-chilled glass slides and allowed to dry at room temperature. Prepared slides were coded, stained with 2% Giemsa for 20 min at pH 6.8 and mounted using DPX. Slides were scored under the bright-field microscope (Olympus BX-60) at 400× magnification for binucleated (BN) cells with well preserved cytoplasm. MN was confirmed at 1000× magnification. Enumeration of MN was done as described by Fenech et al. [30].

2.3. Dosimetry

The gamma radiation level at the residential house of each of the 141 donors was measured using a halogen-quenched Geiger-Muller (GM) tube survey meter consisting of a GM tube and a microprocessor-based digital display (Type ER-709, Nucleonix Systems, India). Measurements were done at a height of 1 m inside (the main room having maximum occupancy, inrad) and outside (near the entrance, outrad) of each house. The mean of three readings was taken for each measurement. The radiation exposure in air (μR/h) due to γ-rays was converted to an annual dose (mGy/year), using a conversion factor of 0.0765 (= $0.873 \times 24\,h \times 365$ days $\times 10^{-5}$). The individual dose was derived as sum of 0.5 × the annual indoor dose and 0.5 × the annual outdoor dose. The coefficient of 0.5 is the occupancy factor for both indoor and outdoor for male subjects between 25 and 50 years of age, as estimated by Nair et al. [31].

2.4. Statistical analysis

The frequency of MN was calculated per 1000 BN cells. ANOVA was used to compare the mean age across different dose groups and chi-square test was used to assess the difference in the distribution of MN across dose and age groups. Comparison of mean MN between NLNRA and HLNRA and across different dose groups was carried out after controlling for the effect of age using analysis of covariance (ANCOVA), as the distribution of age of the study subjects was not similar across different dose groups. Relationship of the frequency of MN with age, background radiation dose, smoking, drinking, and chewing was explored using multiple regression analysis. Statistical analysis was carried out using STATISTICA version 9.1, Stat Soft Inc. [32].

3. Results

The mean age (mean ± SD) of HLNRA and NLNRA individuals was 42.6 ± 10.6 and 38.9 ± 9.1 years, respectively, with an overall mean of 41.3 ± 10.2 years (range, 18–72). The independent *t*-test suggested that the mean age of individuals from HLNRA was higher compared to NLNRA, t = 2.07, P = 0.04. The annual dose in mGy/year received by individuals from HLNRA in our study ranged from 1.53–34.92 with an average of 8.0 ± 6.77 (Mean ± SD), and in NLNRA, it ranged from 0.88–1.50 with an average of 1.35 ± 0.12.

A total of 258,845 BN cells were scored from 141 samples with an average of about 1835 BN cells per sample. The distributions and frequencies of MN in peripheral blood lymphocytes of individuals from HLNRA and NLNRA are shown in Table 1. The overall frequency of MN was 11.69 ± 6.6 per 1000 BN cells. A total of 169,145 BN cells were scored in 94 individuals from HLNRA, where a total of 1844 MN was observed in 1637 BN cells, with a frequency of 11.73 ± 6.59 per 1000 BN cells. Similarly, 89,700 BN cells were scored in 47 individuals from NLNRA and a total of 998 MN was observed in 886 BN cells, with a frequency of 11.62 ± 6.69 per 1000 BN cells. ANCOVA indicated that the frequency of MN (adjusted for age) was similar in individuals from HLNRA and NLNRA, $F_{(1,138)} = 0.29$, P = 0.59. The samples from HLNRA (N = 94) were further stratified into four groups, based on background dose levels (Table 1). ANCOVA suggested that the MN frequencies (adjusted for age) per 1000 BN cells across different dose groups were similar, $F_{(4,135)} = 0.16$, P = 0.96.

Linear regression analysis (Fig. 1) did not suggest any relationship between frequency of MN and background dose levels. However, an age-dependent increase in the frequency of MN was observed (Table 2). Analysis of variance suggested that the difference in frequencies were statistically significant among the four age groups, $F_{(3,137)} = 5.44$, P = 0.001. Post-hoc analysis revealed the