Mutat Res Gen Tox En 828 (2018) 23–29

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Short communication

Frequency of chromosome aberrations among adult male individuals from high and normal level natural radiation areas of Kerala in the southwest coast of India

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 (BSG), Bhabha Atomic Research Centre (BARC), Kollam 691 001, Kerala, India
[b] LLRRS, RB & HSD, BSG, BARC, Trombay, Mumbai 400 085, India

ARTICLE INFO

Keywords:
Chromosome aberrations
Chronic low-dose radiation
Normal level natural radiation area (NLNRA)
High level natural radiation area (HLNRA)
Monazite bearing Kerala coast

ABSTRACT

Chromosome aberration analysis was carried out in peripheral blood lymphocytes of adult male individuals from normal level natural radiation areas (NLNRA, ≤1.5 mGy/year, N = 27) and high level natural radiation areas (HLNRA, > 1.5mGy/year, N = 70) of Kerala coast in southwest India. The mean age of individuals from NLNRA and HLNRA was 40.9 ± 9.4 and 43.7 ± 12.4 years, respectively, with an overall mean of 42.9 ± 11.6 (range: 18–80). Whole-blood cultures were set up and about 260 metaphases were scored per individual. The frequency of chromosome aberrations was calculated per 1000 cells. The overall basal frequency of unstable (dicentrics and rings), stable (translocations and inversions) and other (fragments and breaks) aberrations was 1.54 ± 0.25, 4.1 ± 0.40 and 6.66 ± 0.51, respectively. Individuals of NLNRA and HLNRA had statistically similar frequency of unstable (2.11 ± 0.64 v/s 1.39 ± 0.26; RR = 0.66; 95% CI: 0.33-1.33), stable (4.60 ± 0.94 v/s 3.97 ± 0.44; RR = 0.86; 95% CI: 0.55-1.36) and other (7.85 ± 1.23 v/s 6.36 ± 0.56; RR = 0.81; 95% CI: 0.57-1.15) chromosome aberrations. Frequencies of unstable, stable and other chromosome aberrations did not show any dose response after stratification of HLNRA samples into three dose groups (1.51–5.0 mGy/year, 5.01–10 mGy/year and > 10.0 mGy/year). Smokers showed an increase in other chromosome aberrations ($P < 0.001$), but smoking was not associated with unstable and stable aberrations. Alcohol consumption and tobacco chewing had no significant association with any type of chromosome aberrations. In conclusion, chronic low dose radiation prevailing in Kerala coast did not show any significant effect on the basal frequency of chromosome aberrations among the adult population.

1. Introduction

The human population living in the high level natural radiation areas (HLNRA) of Kerala coast is chronically exposed to elevated levels of natural background radiation. The coastal belt (55 km long and 0.5 km wide strip from Neendakara panchayat of Kollam district in the south to Purakkad panchayat of Alappuzha district in the north) has natural deposits of monazite sand containing 8–10% of ^{232}Th, which is highest in the world. The area is thickly populated and inhabited for generations [1–4]. Due to patchy distribution of monazite in the beach sand, there is a wide variation in the background radiation dose levels (< 1.0–45.0 mGy/year). The average radiation dose in the normal background areas of Kollam district is 1.2 mGy/y with a range of < 1.0–1.5 mGy/y. Hence, areas with a dose level of up to 1.5 mGy/year was considered to be normal level natural radiation areas (NLNRA) and those with a dose level of > 1.5 mGy/year as HLNRA [5]. As compared to the other HLNRAs of the world such as Guarapari in Brazil, Yangjiang in China, and Ramsar in Iran, Kerala coastal belt is unique for its dose range, population size and, interspersed NLNR and HLNR areas. Hence, it offers an opportunity to study the *in vivo* dose-response of biological and health indicators of chronic low-dose radiation exposure in humans.

* Corresponding author at: Low Level Radiation Research Laboratory, Low Level Radiation Research Section, Radiation Biology & Health Sciences Division, Bio-Science Group, Bhabha Atomic Research Centre, Beach Road, Kollam 691 001, Kerala.
** Corresponding author at: Low Level Radiation Research Section, Radiation Biology & Health Sciences Division, Bio-Science Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India.
E-mail addresses: karuppasamy.vetrivel@gmail.com (K. C.V.), biraj@barc.gov.in (B. Das).

https://doi.org/10.1016/j.mrgentox.2018.02.002
Received 2 June 2017; Received in revised form 15 January 2018; Accepted 7 February 2018
Available online 10 February 2018
1383-5718/

Biological studies pertaining to the effect of low dose and low dose-rate exposure below 100mSv have important implications in risk estimation and radiation protection science. However, the shape of the dose response curve is not clear yet at low dose and low dose-rate exposures, especially in humans. The linear no threshold (LNT) hypothesis is well debated as it extrapolates the biological effect at high acute dose exposures to the low dose region. Hence, studies using various biological endpoints in individuals from HLNRA assume paramount importance in understanding the effect of low dose radiation in humans.

Several studies have been conducted in this population in order to assess the biological and health effects of high level natural radiation exposures which includes skeletal and dental variations in wild rats [6], demographic survey to understand infertility and infant mortality rate in human population [7], incidence of congenital malformations and chromosome anomalies among consecutive newborns [8,9], studies on the basal frequency of micronuclei and telomere length attrition in newborns and adults [10–13], case-control study on cleft lip/palate and mental retardation [14], sex-ratio at birth[15], quantification of basal level DNA strand breaks [16] and, DNA double strand breaks (DSBs) [17] in PBMCs from healthy adults. This population also did not show any increase in any type of cancer [18–20]. So far, none of the studies have shown any adverse health effect due to high levels natural radiation exposure in the population. Interestingly, DNA damage and repair kinetics study using comet assay and, micronuclei frequency in elder individuals after challenge dose of gamma radiation, suggested that *in vivo* chronic low-dose radiation > 5.0 mGy/year may induce an adaptive response [21,22]. Recently, our laboratory has also shown lower level of DSB induction, efficient repair of DSBs and abundance of DNA damage response and repair genes in HLNRA groups [23,24].

Unstable (like dicentrics) and stable (like translocation) chromosome aberrations in human peripheral blood lymphocytes are often used to detect and measure the effect of exposure to ionizing radiation. Studies using conventional cytogenetic techniques and FISH suggested that induction of dicentric and translocation occurs at the ratio 1:1 [25], though some other studies using FISH suggested ratios other than 1:1 [26]. Unstable chromosome aberrations such as dicentric and ring chromosomes are sensitive and reliable indicators of radiation exposure even at low dose and low dose-rate. Dicentric chromosomes were studied in lymphocytes of chronically exposed population, Chernobyl clean-up workers, occupationally exposed hospital staff and nuclear power plant workers [27–30]. Unstable chromosome aberrations like dicentrics may be gradually eliminated during cell division. In contrast, cells with stable chromosome aberrations such as translocations and inversions can persist for a longer time and are indicators of radiation induced damage especially when the population is exposed over a long period of time or in case of retrospective bio-dosimetry [31–34].

In the present study, an attempt was made to estimate and compare the frequency of different type of chromosome aberrations including unstable, stable and other chromosome aberrations in adult male individuals from NLNRA and different background dose groups of HLNRA of the Kerala coast.

2. Materials and methods

2.1. Chemicals used in the study

RPMI 1640 medium, L-glutamine, bovine serum, Giemsa stain, phytohaemagglutinin (PHA), colcemid were procured from Sigma Aldrich Corporation, St Louis, MO, USA. Other chemicals used were benzyl penicillin (Alembic Pharmaceuticals, Mumbai, India), streptomycin (Abbott Health Care Private Ltd., Mumbai, India), potassium chloride, DPX (Qualigens, Mumbai, India), methanol and acetic acid (Merck, Mumbai, India).

2.2. Experimental design and sample collection

Peripheral blood samples were obtained by venipuncture from 97 adult male individuals (HLNRA, N = 70; NLNRA, N = 27) in sterile heparinized vaccutainer tubes. 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 detailed questionnaire was used to obtain information on age, alcohol consumption, smoking and tobacco chewing. Blood samples were brought to the laboratory under ice-cold conditions and the whole-blood cultures were set up using standard procedure [35]. 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 at 37 °C for 51 h. Colcemid (0.04 µg/ml) was added to whole blood cultures at 49 h and subsequently terminated at 51 h, followed by hypotonic treatment (KCl, 0.075 M) for 20 min and fixation with methanol: acetic acid (3:1). The cell suspension was dropped on to clean slides and air-dried. The slides were blind-coded, stained in 2% Giemsa and mounted with DPX.

2.3. Scoring

Well-spread metaphases were analysed under light microscope (Olympus BX-60) at 100X oil immersion. Chromosomes were classified into seven groups viz., 'A' to 'G' based on its size and centromere position [36]. Each chromosome was critically analyzed to identify numerical and structural variation such as unstable aberrations (dicentrics and ring chromosomes), stable aberrations (translocations: unbalanced exchange between two chromosomes, and inversions: unbalanced exchange between p and q arm of the chromosome) and other chromosome aberrations (fragments and breaks). All the identified chromosome aberrations were confirmed by three experienced Cytogeneticists. The frequency of chromosome aberrations was calculated per 1000 cells.

2.4. Mitotic index

The influence of low dose radiation on cell cycle was evaluated by estimating mitotic index in a subset of 63 samples (NLNRA, N = 21; HLNRA, N = 42). Mitotic index was estimated from 1000 cells per sample as number of cells in mitosis/total number of cells × 100.

2.5. Dosimetry

Gamma-radiation levels in each donor's house were measured using a halogen quenched Geiger Muller (GM) tube-based 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) and outside (near the entrance) 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 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. Indoor and outdoor occupancy factor of 0.5 for adult male subjects between 25 and 50 years of age [37], was adopted, since, about 76% of our study subjects were in the age range of 25–50 years. The estimated individual dose was used to classify the sample as from HLNRA, if the dose is more than1.5 mGy/year and from NLNRA, if it is ≤1.5 mGy/year.

2.6. Statistical analysis

Independent *t*-test was used to compare mean age of the individuals from NLNRA and HLNRA. Poisson distribution was assumed for number