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Effect of chronic low dose natural radiation in human peripheral blood mononuclear cells: Evaluation of DNA damage and repair using the alkaline comet assay

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ABSTRACT

This study investigates whether peripheral blood mononuclear cells (PBMCs) from inhabitants of Kerala in southwest India, exposed to chronic low dose natural radiation *in vivo* (>1 mSv year^{-1}), respond with a radioadaptive response to a challenging dose of gamma radiation. Toward this goal, PBMCs isolated from 77 subjects from high-level natural radiation areas (HLNRA) and 37 subjects from a nearby normal level natural radiation area (NLNRA) were challenged with 2 Gy and 4 Gy gamma radiation. Subjects from HLNRA were classified based on the mean annual effective dose received, into low dose group (LDG) and high dose group (HDG) with mean annual effective doses of 2.69 mSv (N = 43, range 1.07 mSv year^{-1} to 5.55 mSv year^{-1}) and 9.62 mSv (N = 34, range 6.07 mSv year^{-1} to17.41 mSv year^{-1}), respectively. DNA strand breaks and repair kinetics (at 7 min, 15 min and 30 min after 4 Gy) were evaluated using the alkaline single cell gel electrophoresis (comet) assay. Initial levels of DNA strand breaks observed after either a 2 Gy or a 4 Gy challenging dose were significantly lower in subjects of the HDG from HLNRA compared to subjects of NLNRA (2 Gy, P = 0.01; 4 Gy, P = 0.02) and LDG (2 Gy P = 0.01; 4 Gy, P = 0.05). Subjects of HDG from HLNRA showed enhanced rejoining of DNA strand breaks (HDG/NLNRA, P = 0.06) during the early stage of repair (within 7 min). However at later times a similar rate of rejoining of strand breaks was observed across the groups (HDG, LDG and NLNRA). Preliminary results from our study suggest *in vivo* chronic low-level natural radiation provides an initial exposure that allows an adaptation to a subsequent higher radiation exposure, perhaps through improving DNA repair *via* an unknown mechanism. Therefore, further investigations would be necessary in this population to understand the biological and health effects of chronic low-level natural radiation exposures.

1. Introduction

Radioadaptive response is a phenomenon in which the cells pre-exposed to low dose of ionizing radiation or genotoxic chemicals exhibit an enhanced cellular resistance to the effect of a subsequent higher challenging dose of ionizing radiation. This phenomenon was first reported in human lymphocytes by Oliveri et al. [1], where cells pretreated with tritiated thymidine caused a decrease in chromosome aberration frequency on subsequent exposure to high dose X-rays. Later on, several laboratories have analyzed the radioadaptive response in human lymphocytes and other cellular systems by measuring indicators of cellular damage such as cell lethality, chromosome aberrations, micronuclei, mutation induction and DNA repair [2–6]. In general, pretreatment of cells with an acute *in vitro* low dose radiation in the range of 0.01–0.5 Gy, referred to as a priming dose [7], showed adaptation. However, adaptive effects were not observed in some of the experiments [8–10]. Indeed adaptive response is known to be influenced by experimental conditions and inter- and intra-individual variations [11,12].

Abbreviations: HLNRA, high level natural radiation areas; NLNRA, normal level natural radiation area.

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Additionally, several *in vivo* studies investigated the potential of a chronic low dose radiation in inducing an adaptive response. In humans these studies were mainly carried out in peripheral blood lymphocytes obtained from occupational radiation workers and from adults and children of areas contaminated due to Chernobyl accident [13–17]. Results from the subjects occupationally exposed to ionizing radiation indicate that chronic low dose radiation might induce adaptation in humans [13–15]. Either no adaptive response or a weak response was more frequent in Chernobyl studies [16,17].

As compared to the individuals of the above *in vivo* studies, population in high level natural radiation areas (HLNRA) is exposed to chronic low dose radiation for generations and during all stages of their life from conception to death. This provides an opportunity to investigate the potential of chronic low dose natural radiation in inducing an *in vivo* radioadaptation in humans [18,19]. Results of such studies may have important implications in radiation protection practices and human health.

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 HLNR areas of the world. The radiation levels in this region range from $\leq$1.0 mGy to 45.0 mGy year^{-1} and are due to the natural deposits of monazite (1% of beach sand) containing thorium 232 (8–10%), uranium (0.3%) and their corresponding decay products [20,21]. The area has a high density of population, wide range of doses and a stable non-migratory community.

Several studies have been conducted in this population to understand the biological and health effects of chronic low dose natural radiation exposures. Genetic monitoring to determine the incidence of major congenital anomalies, chromosomal aberrations and spontaneous frequency of micronuclei among newborns did not detect any significant difference between the two populations from HLNRA and normal level natural radiation area (NLNRA) [22–24]. Studies carried out among the adult population on telomere length, case-control study of mental retardation and cleft lip/palate and incidence of cancer also did not suggest any difference between HLNRA and NLNRA [25–27]. Our previous study 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). Spontaneous DNA damage increased with age in individuals from NLNRA, while a significant negative correlation was found in subjects from HLNRA [28]. An increased frequency of mitochondrial germ line point mutation was reported in a study with saliva samples from residents of the high radiation area compared to a nearby control area [29].

However, to the best of our knowledge, no investigations were carried out to test the potential of chronic low dose natural radiation on the induction of an *in vivo* radioadaptive response in this population. Thus a pilot study was conducted in 114 subjects (77 subjects from HLNRA and 37 subjects from NLNRA of Kerala) by estimating initial levels of DNA strand breaks after 2 Gy and 4 Gy gamma irradiation and repair of DNA strand breaks (after 4 Gy irradiation) in peripheral blood mononuclear cells.

Single cell gel electrophoresis (comet) assay provides a suitable tool for quantifying initial levels of DNA damage and its repair after a challenging dose of radiation, at the level of individual cells in the stationary phase [30–32]. We have used alkaline comet assay that quantifies DNA damage in terms of DNA strand breaks and alkali labile sites. Being sensitive to detect DNA damage after low dose radiation exposure [33], the alkaline comet assay seems well suited for studying the phenomenon of radioadaptive response in subjects exposed to chronic low dose of ionizing radiation [19,34]. In our study, we have also analyzed the influence of age and smoking habit of subjects on the phenomenon of radioadaptive response.

2. Materials and methods

2.1. Study population and blood sample collection

The study was carried out in 114 healthy adult male subjects with an average age of 37.54 $\pm$ 8.98 (range: 22–55 years). A group of 77 subjects was selected from Chavara and Neendakara panchayats, which are HLNR areas of Karunagapally taluk, Kollam district, Kerala. The average annual effective dose estimated for the 77 subjects from the HLNRA was 5.75 mSv. Consequently, subjects receiving $\leq$5.75 mSv year^{-1} were classified into low dose group (LDG) with a mean effective dose of 2.69 mSv year^{-1} and the subjects receiving >5.75 mSv year^{-1} were classified into high dose group (HDG) with a mean dose of 9.62 mSv year^{-1}. A control group of 37 subjects was selected from the adjacent NLNR areas ($\leq$1 mSv year^{-1}) of the Kollam district, Kerala. Characteristics of the subjects from various groups are shown in Table 1.

About 5 ml of whole blood was collected from each subject by venipuncture using heparin coated vaccutainer tubes (BD Vaccutainer systems). Samples were collected after obtaining written informed consent of all the volunteers participating in this study. The procedure was approved by the medical ethic committee, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai. Immediately after collection, the blood samples were transported to the laboratory in refrigerated conditions and were processed within 2 h.

2.2. 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, inrad) and outside (near the entrance, outrad) of each house. The mean of three readings was taken for each measurement. The survey meter readings measured absorbed doses in air (μR h^{-1}) due to γ rays and were converted to annual dose (mGy year^{-1}) using a conversion factor of 0.0767 (=0.8763 $\times$ 24 h $\times$ 365 days $\times$ 10^{-5}). The dose contributed by the γ rays was derived as sum of 0.5 (OF_{indoor}, occupancy factor) $\times$ the annual indoor dose and 0.5($OF_{outdoor}$) $\times$ the annual outdoor dose. The sex- and age-specific occupancy factors were estimated in a previous study conducted by Nair et al. [35] in 7711 individuals from this population (3783 males and 3928 females). They have shown an occupancy factor of 0.5 for both indoor and outdoor for male subjects between 25 and 50 years of age. Since we studied only male subjects and about 90% of our subjects are within the age range of 25–50 years, we have used occupancy factor of 0.5 for estimating individual dose. The annual effective dose (mSv year^{-1}) was obtained using a conversion coefficient of 0.7 Sv Gy^{-1}, as suggested by UNSCEAR for adults [36].

2.3. Peripheral blood mononuclear cells isolation and irradiation

Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood using Histopaque 1077 (Sigma–Aldrich Corporation, St. Louis, MO, USA). Isolated cells were washed twice with ice-cold phosphate-buffered saline (pH 7.5), and resuspended in cold RPMI-1640 medium (Sigma–Aldrich Corp.) supplemented with 20% fetal bovine serum (PAA Laboratories GmbH, Austria), counted in a hemocytometer and stored overnight in a refrigerator at 4 °C. PBMCs suspended in RPMI (10^6 cells/ml) from each sample had about 6–8 $\times$ 10^6 cells, which were split into three aliquots and marked as I, II and III, respectively. Aliquot I and II had 1–1.5 $\times$ 10^6 cells and aliquot III had 4–6 $\times$ 10^6 cells. Aliquot I was kept as control