FEATURE ARTICLE

Genetic studies on human population residing in High Level Natural Radiation Areas of Kerala coast

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Abstract

For the past few decades, the monazite bearing coastal belt of Kerala in South West India is under investigation, to study the biological and health effects of natural chronic low dose exposure of background radiation, on human population residing in that area. The level of background radiation varies from >1mGy per year to 45 mGy per year and the areas with a dose level of ≤1.5 mGy per year are considered as Normal Level Natural Radiation Areas (NLNRAs), whereas areas having the background dose >1.5mGy per year are High Level Natural Radiation Areas (HLNRAs). The non-uniform distribution of radiation level in this coastal belt provides a unique opportunity to study in vivo dose response of biological parameters. A number of studies have been conducted in this area, including cytogenetic investigation on newborns and adults for chromosomal aberrations, monitoring the newborns for congenital malformations and genetic disorders, Health Audit Survey to find out prevalence of late onset diseases and DNA mutation rate of the families using hyper-variable markers such as mini- and microsatellites. In the present paper, the findings obtained from two recently published reports dealing with spontaneous frequency of micronuclei among newborns and telomere length of adult population from high and normal level radiation areas have been highlighted. The spontaneous frequency of micronuclei (MN) was estimated in 271 newborns, whereas telomere length was determined from 310 adults. Our data did not reveal any significant difference in HLNRA as compared to NLNRA in both the parameters studied. No dose response was observed. In conclusion, the elevated background radiation prevailing in Kerala coast has no significant effect on the induction of micronuclei in newborns and telomere length in adults, which is in agreement with other studies conducted so far. Using advanced molecular biological techniques, further studies are in progress, to understand the cellular and molecular response of human cells to low dose radiation exposure.

Introduction

Studies on biological and health effects of the human population living in High Level Natural Radiation Areas (HLNRAs) provide an important source of information on the effects of chronic low dose rate exposures to ionizing radiation. There are many areas in the world, where the natural background radiation level is high (sometimes 10 -100 times the normal levels) either due to high levels of radioactivity in soils, rocks and hot springs or due to high levels of indoor radon and its decay products. For instance, the level of background radiation is high in the hot springs at Ramsar (Iran) due to high Radium content and because of Thorium content of monazite containing sand in Yangjiang (China), Guarapari (Brazil) and the coastal belt of Kerala

in South West India. These areas give unique opportunity to study the effect of natural chronic low level radiation directly on human population at all stages of life.

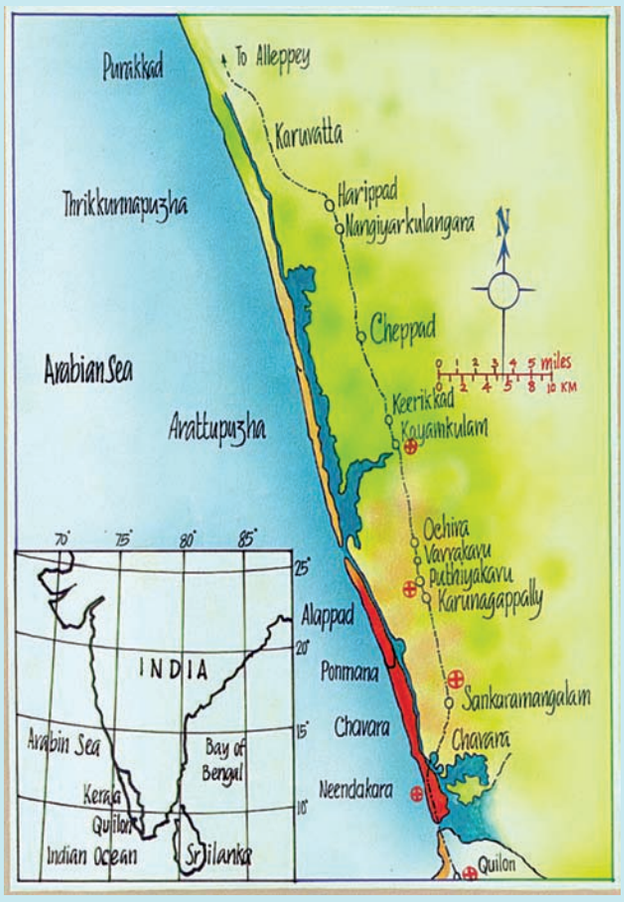

Fig. 1: Kerala coastal belt showing High Level Natural Radiation areas extending from Neendakara in south to Purrakad in north.

The monazite bearing coastal belt of Kerala is a strip of about 55kms, extending from Neendakara (Kollam district) in south to Purakkadu (Alapuzha district) in north (George et al. 1976). The radioactive component of this beach sand is monazite, which contains thorium (8-10%, highest in the World) and its radioactive daughter products. Due to non-uniform distribution of monazite in the beach sand, the level of background radiation in this area varies from <1.0 mGy to 45 mGy per year and in some areas it is as high as 70mGy per year. The per capita average dose received by the population residing in HLNRA is ~ 4mGy/year (Bharatwal and Vaze, 1958, Sunta, 1993). The areas with a dose range of ≤1.50 mGy/year are considered as Normal Level Natural Radiation Areas (NLNRA), whereas dose range of >1.50mGy/year is considered as HLNRA. This area is densely populated which is approximately 1000 years old. It is a difficult task to delineate the effect of natural background radiation, if any, on humans as there are many confounding factors such as age, gender, habits, diet, life style etc. in a population. But this area is unique as compared to other HLNRAs of the world, due to a great deal of variation in the level of background dose exposures, which allows the study of dose response.

Studies conducted in the past

A number of studies have been undertaken in the past including genetic studies in wild rats based on skeletal and dental variants (Grunberg, 1964 ; Grunberg et al. 1966) and a demographic study covering 70,000 individuals, to find out reproductive parameters, infant mortality etc. between high and normal level radiation areas (Gopal-Ayengar et al. 1971). Since 1975, the Bio-medical Group, BARC has its field laboratory at Kollam [presently Low Level Radiation Research Laboratory (LLRRL)] in order to carry out detailed genetic and epidemiological survey of the entire population. Studies conducted in this area include cytogenetic investigations on adults and newborns for chromosomal aberrations (Cheriyan et al. 1999, Thampi et al. 2002, 2005),

screening of the newborns for congenital malformations (Jaikrishan et al. 1999, Thampi et al. 2005), house-to-house Health Audit Survey (Thampi et al. 2005) and DNA mutation rate in the families using mini and microsatellite markers (unpublished data).

Initially, chromosome aberration analysis was carried out on adults using peripheral blood lymphocytes. Dicentrics (chromosomes with two centromeres) are considered as indicators of radiation-induced damage. The frequency of dicentrics in HLNRA adults was not significantly different as compared to NLNRA. From 1986 to 1996, cytogenetic investigation was carried out on newborns to establish the frequency of chromosomal aberrations (such as dicentrics, translocations, inversions, rings, chromosome breaks, gaps, etc.) as well as constitutional anomalies (both structural and numerical). Umbilical cord blood samples were collected from four local Government hospitals and lymphocyte cultures were set up at LLRRL, Kollam, Kerala. Cells with well spread metaphase were analysed for chromosomal aberrations. The frequency of chromosomal aberrations was reported per 10,000 cells. The frequencies of dicentrics were 1.87± 0.14 in HLNRA and 1.88± 0.34 in NLNRA, which were not significantly different from each other. Similarly, the frequency of stable aberrations (translocations, inversions) had a frequency of 3.46 ± 0.34 in HLNRA as compared to 3.26 ± 0.45 in NLNRA, which were not significantly different from each other. No significant difference was observed in the frequency of unstable aberrations (fragments, minutes and rings) in HLNRA (2.29 ± 0.17) and NLNRA (2.88± 0.42). Dose related increase in the frequency of any type of chromosomal aberrations was not observed in these samples (Cheriyan et al. 1999 and Thampi et al, 2005). Karyotype analysis of 23, 844 newborns (8004 from NLNRA and 15,840 from HLNRA) revealed the overall incidence of karyotype anomalies to be 4.86 ± 0.45 per 1000 newborns (HLNRA: 4.80 ± 0.55; NLNRA: 5.00 ± 0.79). The data is comparable with the published UNSCEAR reports (UNSCEAR, 1993).

Screening of newborns in hospital-based studies is one of the major programmes in this area, in order to establish the frequency of various congenital malformations including Downs Syndrome. So far, our data based on approximately 1,00,000 newborns did not show any significant increase in the frequency of any type of congenital malformations in HLNRA as compared to NLNRA (Jaikrishan et al. 1999, Thampi et al. 2002, 2005). The overall incidence of still births was 0.51% and that of malformations was 2.03%. Our statistical analysis of malformations and still births showed dependence on maternal age, gravida status, ethnicity, gender of the newborn and consanguinity, but does not suggest any correlation with radiation levels. The frequency of Downs Syndrome is also similar in both the areas with an overall frequency of 1 in 1471. The relatively lower incidence of still birth, malformations, Downs Syndrome could be due to the fact that our study group having ~85% mothers from the younger age group of 20-29 years.

The Health Audit Survey was initiated in collaboration with the Department of Health and Social Welfare, Government of Kerala to find out the prevalence of late onset diseases such as diabetes, hypertension, asthma etc. and malformations. The study also collects information on socio-demograpy, life style and reproductive history of married women. All these basic data is collected with the help of anganwadi workers, who have good rapport and interaction with the inhabitants. So far, Health Audit Survey carried out on six panchayats revealed that the pattern of both birth defects and late onset diseases was similar in both the areas (Thampi et al. 2005).

In addition to the above studies conducted in the field laboratory at Kollam, molecular genetic studies were initiated in 1995 at Low Level Radiation Studies Section (LLRSS), Radiation Biology and Health Sciences Division, Bio-Medical Group, Mumbai. For that purpose, human genomic DNA was isolated from blood samples of over 200 families (Father, mother and child trio) residing in that area. Over 40 hypervariable DNA markers such as minisatellites (autosomal) and microsatellites (includes autosomal and Ychromosomal) were analysed, to determine heritable mutation rate. No increase in the mutation rate at autosomal as well as Y-chromosomal markers was observed in HLNRA as compared to NLNRA. The data has not shown any dose response, suggesting that mutation rate at the mutational hot spots of human genome has not been affected due to natural chronic low dose exposure to radiation (unpublished data).

In the present article, the findings of two recently published reports in this study area have been discussed in detail. In one of the studies, we have determined and compared the spontaneous frequency of micronuclei among the newborns from HLNRA and NLNRA in order to assess the effect of elevated level of background radiation, if any, on the induction of micronuclei (Das and Karuppasamy, 2009). In the other study, telomere length was determined from HLNRA and NLNRA adults to find out the effect of natural background radiation, if any, on telomere length (Das et al. 2009).

I. Spontaneous frequency of micronuclei in newborns (published in IJRB, 2009):

The most frequently used biomarkers to study radiation-induced damage both *in vitro* and *in vivo* are chromosome aberration analysis in metaphase spreads or scoring micronuclei (MN) in binucleated (BN) cells. Micronucleus is a small nucleus present in the cell in addition to the main nucleus, indicating the presence of chromosomal damage. It may arise from a whole lagging chromosome (aneugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome after breakage (clastogenic event) which do not integrate in the daughter nuclei. Cytochalasin-Blocked Micronucleus (CBMN) assay is one of the most reliable and precise methods for assessing radiation-induced chromosome damage (Fenech & Morley 1985, 1986). It is also used as a biomarker to evaluate the utero effects to environmental exposures on umbilical cord blood samples (Milosevic-Djordjevic et al. 2007). In order to determine and compare the spontaneous frequency of MN among the newborns, cord blood samples were collected from 271 newborns (61 from NLNRA and 210 from HLNRA), born to mothers aged between 17 and 37 years (mean maternal age: 24.08 $\pm$ 4.23 years). Analysis of micronuclei was restricted to Cytochalasin Blocked binucleated (BN) cells only and the frequency of micronuclei was calculated per 1000 BN cells.

The frequency of BN cells with MN was observed to be 1.17 $\pm$ 0.04, in HLNRA newborns as compared to 1.23 $\pm$ 0.07 in NLNRA. These values are not statistically significant ($p>0.2$). In order to find out any dose-related changes in chromosomal/ DNA damage if any, we have categorized the samples into six different dose groups (NLNRA : d"1.5 mGy/year and five dose groups of HLNRA: 1.51 to 3.00 mGy/year, 3.01 to 6.0 mGy/year, 6.01 to 12.00 mGy/year, 12.01 to 18.00mGy/year and 18.00 to 28.12 mGy/ year. Odds ratios (OR) and confidence intervals (CI) have been calculated, to find out statistical significance, if any, in the mean MN frequency among the newborns from various dose groups with respect to control. It did not reveal any significant difference ($P>0.05$).

As shown in the Fig. 3, MN frequency did not show any increasing trend with respect to the dose groups studied. The baseline frequency of micronuclei in HLNRA newborns is not statistically different from NLNRA newborns, suggesting that, elevated level of naturally occurring radiation has

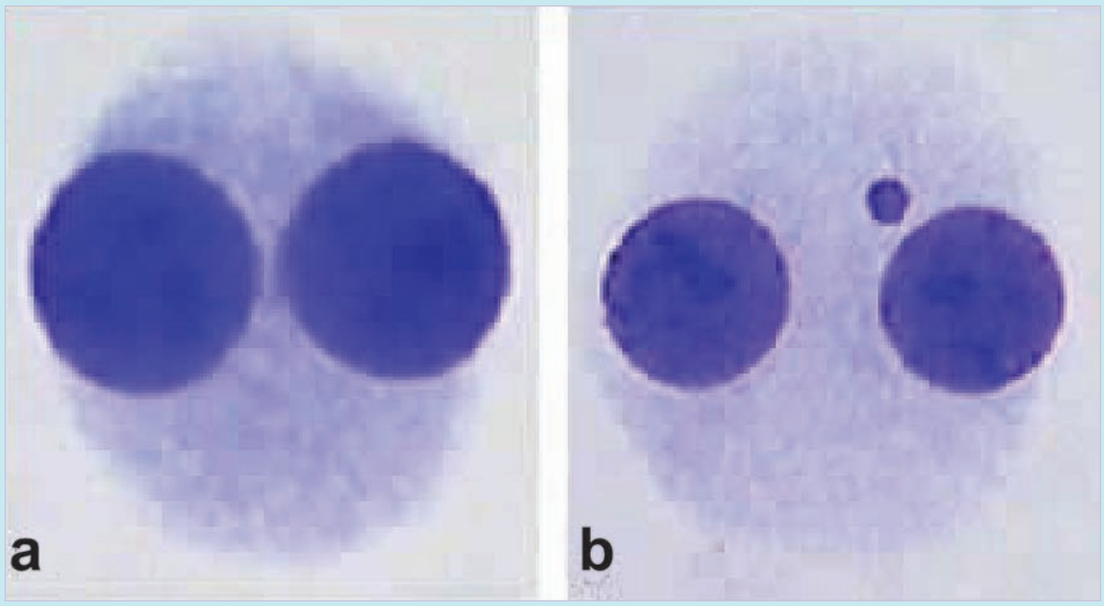

Fig. 2 : (a) Binucleated cell (b) Binucleated cell with a micronucleus

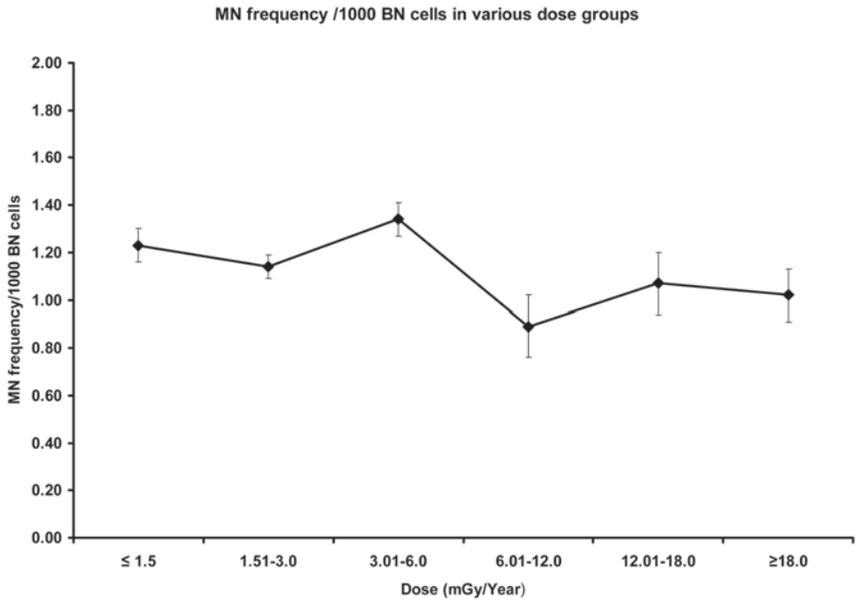

Fig. 3: The line graph represents the frequency of micronuclei/1000 BN cells in six different background dose groups [d"1.5mGy/year (N=61), 1.51-3.0 mGy/year (N=90), 3.01-6.0 mGy/year (N=69), 6.01-12.0 mGy/year (N=14), 12.01-18.0 mGy/year (N=16) and 18.01-28.1 mGY/year (N=21)]. N = The number of individuals studied in each dose group.Each point represents the mean frequency of micronuclei per 1000 BN cells for that particular dose group. For each point, the error bars indicate the standard error of the mean (SEM).

no significant effect on the induction of micronuclei frequency among the newborns. There is a maternal age-dependent increase, though not significant, in the frequency of MN among HLNRA and NLNRA newborns, except the age group >30 years (study samples consisted of ~85% mothers from the age group < 30 years). The MN frequency observed in females did not show any statistically significant difference as compared to males. The data was also in agreement with other reported studies.

In conclusion, due to elevated level of background radiation, there is no increase in the frequency of MN in HLNRA of Kerala coast as compared to the adjacent control area. Moreover, lower levels of micronuclei frequency observed among the newborns of HLNRA of Kerala coast could be indicative of adaptive response. The decreased frequency of MN at dose group of 6.01-12.00 mGy/year supports the phenomenon of hormesis, which needs to be explored further. To our knowledge, this is the first report which estimates the spontaneous frequency of MN among the newborns using cord blood samples from a natural high background radiation area.

II. Telomere length in adults (published in PLoS ONE, 2009):

The long, thread-like DNA molecules that carry our genes are packed into chromosomes and telomeres are the caps on their ends. There is a unique DNA sequence (TTAGGG)n in telomeres, which protects the chromosomes from degradation. Telomere length gets reduced with each round of cell division in normal human somatic tissues. It also shows inter-individual variation. Shortening of telomere length has been reported to be associated with aging, stress, diabetes, hypertension, obesity, dementia and many other age-related diseases.

So far, using cell lines, few *in vitro* experiments have shown telomere length variation in response to ionizing radiation. Till date, no *in vivo* data on

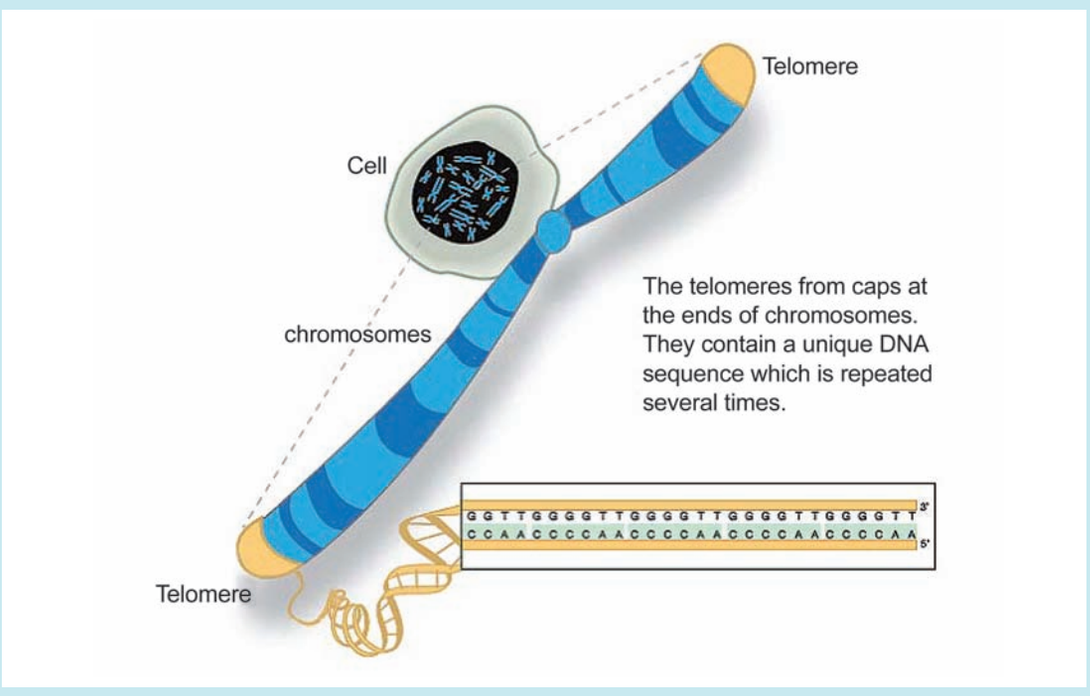

Fig. 4: Human chromosome showing telomere at the ends.

telomere length in human population exposed to elevated level of natural background radiation is available. We have determined telomere length in peripheral blood lymphocytes of 310 random, normal and healthy, age matched adult individuals (141 males and 169 females) of which 233 individuals were from HLNRA and 77 individuals were from NLNRA [mean maternal age in HLNRA; 26.24 ± 4.52 years and 25.69 ± 4.38 years], respectively. Genomic DNA was isolated from venous blood samples and telomere length was determined by using real time quantitative polymerase chain reaction. This method measures the factor by which the ratio of telomere repeat copy number to single gene copy number differs between a sample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers(S) which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) is represented as T/S ratio which is calculated to determine the telomere length.

The samples from HLNRA were stratified into four different dose groups on the basis of natural background dose level, which were as follows: ≤1.50mGy per year (NLNRA) and three HLNRA dose groups such as (1.51-3.00, 3.01-5.00 and >5.00 mGy per year). Telomere length was correlated with background dose groups. As shown in Fig.4, the telomere length observed in HLNRA adults was not significantly different from NLNRA. Our data did not show any dose dependent changes in the length of the telomere.

Inter-individual variation in the length of the telomere was observed among the samples. Longer telomere length is an indication of lesser

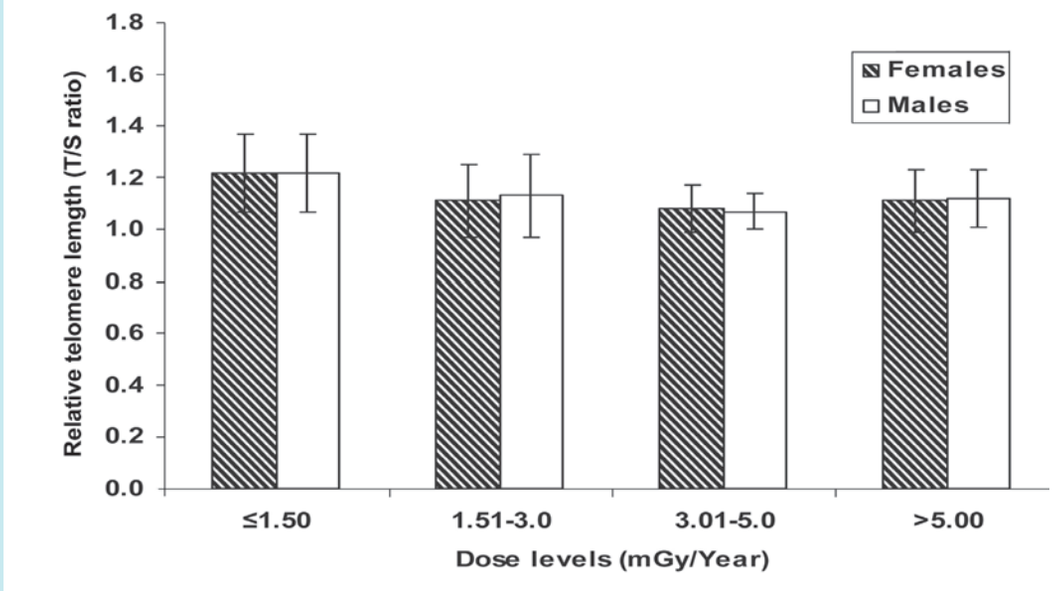

Fig. 5: Telomere length among male and female adults with respect to various background dose levels. [(d"1.50 mGy/year : 34 males + 43 females, 1.51-3.0 mGy/year: 67 males + 80 females, 3.01-5.0 mGy/year : 23 males + 27 females, >5.00 mGy/year : 17 males +19 females)].

DNA damage at the telomeric ends. Data analysis was done to compare the individuals from each group with shorter telomere length (defined as relative T/S ratio below the median) versus longer telomere length (defined as relative T/S ratio above the median). Logistic regression was used to compute the odds ratios (OR) and 95% confidence intervals (CI), for individuals with shorter and longer telomere lengths in all the three HLNRA dose groups (> 1.5mGy per year) with respect to the NLNRA (dose group ≤1.5 mGy per year), where odds ratio was taken as 1.00. The OR values found in various dose groups in HLNRA were not statistically different as compared to NLNRA, indicating that, elevated level of natural background radiation has no significant effect on telomere length in high background radiation areas of Kerala coast. There were no significant differences in telomere length between male and female adults in both the areas. Association of telomere length with aging has significant implications for human health. Data was correlated with age and indicated a negative correlation between the telomere length and the age in both NLNRA and HLNRA, with the limited sample size which is relatively from a younger age group.

In conclusion, the elevated level of natural background radiation has no significant effect on telomere length among the adult population residing in HLNRAs of Kerala coast. This could be an indication of better repair capacity of HLNRA individuals at telomeric ends. To our knowledge, this is the first *in vivo* study, addressing the dose response relationship between natural chronic background radiation and telomere length in human peripheral blood mononuclear cells. Telomere length attrition between males and females, association of telomere length and age, telomere length loss during different stages of life and contributions from genetic and environmental factors give rise to the large amount of variation of telomere length in a population. Efforts are in progress to collect data from older population and from each of the background dose groups, in order to provide a clearer picture of the association between telomere length, age and natural background radiation.

Future prospects

From this data, it is evident that it would be interesting and essential to understand the molecular and cellular mechanism occuring in human cells, in response to natural background radiation. It is worth pursuing the use of newer molecular biology techniques, in order to throw new light on this area of research. It might provide a deeper understanding of the phenomenon such as adaptive response, hormesis etc.. Perhaps, research in Kerala high background radiation area will contribute substantially towards Linear Non threshold (LNT) hypothesis, a well debated topic in radiation biology. At present, other ongoing studies in this area are Health Audit Survey covering 12 panchayats, DNA damage and repair studies using comet assay in adult population, adaptive response study using cytogenetic parameters like chromosomal aberration and micronuclei, global gene expression profile using microarray technology and structural or copy number variation study on selected malformations.

Contributors

Presently this work is being carried out under the supervision of Dr. M. Seshadri, Head, Radiation Biology and Health Sciences Division. The team members include researchers from LLRRL, Kollam, Kerala and LLRSS, RB&HSD, Bio-Medical Group, Trombay, Mumbai. [Mr. V. D. Cheriyan, Dr Birajalaxmi Das, Dr. G. Jaikrishan, Dr. Anu Ghosh, Mr. E.N. Ramachandran, Mr. C. V. Karuppasamy, Mrs. Shazia Ahmad, Mr. D.C. Soren, Dr. Sudheer K. R, Mr. P.K.M. Koya, Dr. Vivek Kumar P.R, Mr. Vinay Jain, Mr. V. J. Andrews, Mr. V. Anil Kumar and Mrs. Divyalakshmi Saini.

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Forthcoming Conference

International Conference on Physics of Emerging Functional Materials (PEFM-2010)

The above conference sponsored by BRNS, will be held at BARC, Mumbai, between Sep. 22-24, 2010. The conference focuses on the physics of emerging functional materials of 21st century, including organic semiconductors (both molecular and polymeric), materials at nanoscale, spintronic materials, novel superconducting and magnetic materials, soft matter etc. Contributed papers for poster presentation are invited on the following topics:

1. Organic semiconductors: Charge transport; photovoltaic, lighting and field effect transistors; flexible devices; molecular electronics 2. Soft matter: liquids, colloids, foams, polymers 3. Electronic and lattice phenomena in crystalline solids: semi- and superconductivity, magnetism, 4. Ferroelectricity, multiferroics, heavy fermions etc. 5. Non-crystalline solids: glass and ceramics, granular materials, quasicrystals 6. Biophysics 7. Experimental techniques: neutron, synchrotron, x-rays, positron, ions as probes 8. • Low dimensional structures: quantum dots, clusters, nanostructures, thin films. The template and guidelines for the preparation of manuscript can be downloaded from the AIP
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Paper submission :	July 15, 2010.
Acceptance notification :	August 1, 2010
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