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Impact of natural background radiation on chromosomes in female residents of high background radiation area of Kerala

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High levels of natural radiation areas are found in different parts of the world like Yangjiang in China, Guarapari in Brazil, Ramsar in Iran and Kerala in India. These areas are considered as appropriate places for the study regarding health risks as a result of continuous exposure to elevated level of radiation, if any, on the inhabitants there. Karunagappally, lying on the south west coast of Kerala in India is known for HBR due to natural deposit of monazite sand containing thorium (8-10%), uranium (0.3%) and its decay products. The present study analysed the chromosomes in peripheral lymphocytes from the female inhabitants of HBRAs of Karunagappally and compared their results with female inhabitants of adjacent normal background radiation areas (NBRAs). Peripheral blood samples from 110 female inhabitants of HBRA of Karunagappally and 100 samples from NBRAs were collected in heparinized vials and cultures were set up employing standard microculture techniques, slides were prepared, coded and stained with giemsa. Well spreaded metaphases were analysed for chromosome aberrations. Fluoroscence *in-situ* hybridization (FISH) using whole chromosome probe (WCP) 1, 2, 4 and X was performed in representative samples. The frequencies of chromosomal aberrations in HBRAs and NBRAs were 5.85±3.7 and 0.27± 0.58 per hundred cells respectively. All statistical analysis were done using SPSS version 21 to assess the Group statistics in experimentals for mean age, mean cumulative dose and chromosomal aberration frequency. FISH does not reveal any translocation among the chromosomes 1, 2, 4 and X. Background radiation had effect on the frequencies of chromosomal aberration in the inhabitants of HBRA and was found significant compared to inhabitants from NBRAs. The lack of any stable aberrations/translocations in chromosomes can be considered as one of the reasons for not having any serious ill effects or increased cancer incidence due to the radiation exposure in the area.

Keywords: Chromosomal aberrations, Dicentrics, FISH, HBRAs, Ring chromosome

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Natural radiation is a major component of radiation exposure for the general population. The level of background radiation varies from >1 mGy per year to 45mGy per year and the areas with a dose level of < 1.5 mGy per year are considered as normal level natural radiation area (NBRA) whereas areas having the background dose > 1.5mGy are considered as high background radiation areas (HBRA)[1]. Areas with unusually high level of background radiation are found in different parts of the world like Yangjiang in China, Guarapari in Brazil, Ramsar in Iran and Karunagappally in India. The high levels of radiation in these areas are due to hot springs with high radium content (Ramsar in Iran) or due to monazite bearing sand (Yangjiang in China, Guarapari in Brazil, and Karunagappally in India)[2]. Cytogenetic studies done in the HBRAs reveal a positive correlation with chromosomal aberrations in the lymphocytes of exposed persons[3]. Karunagappally, lying on the Southwest coastal belt of Kerala, India, 55 km long and 0.5 km wide, extends from Neendakara in Kollam district in south to Purakkadu in Alappuzha district in north. The area has monazite deposit containing thorium, illemnite, rutile, zircon and silmanite in the beach sands with 8-10% thorium [4,5]. The background radiation level in this area varies from <1.0 mGy to 45 mGy/y and up to 70 mGy /y reported in some areas[6].

Ionising radiation is a strong clastogen, causing chromosome breakages that result in cytogenetic aberrations in exposed cells[7]. Chromosomal aberration is a reliable biodosimetric tool and dicentric and ring chromosomes are indicators of radiation exposure[8]. Cytogenetic study using cord blood samples from newborns of the same area was conducted and the results showed that there was no significant variation in the frequency of aberrations in them compared to the newborns from NBRAs and also no significant effect on the induction of micronuclei frequency among infants[9]. The data of the frequency of chromosome aberrations among adult male individuals of the area was also available which does not show any significant difference from the NBRA individuals[10]. Hence the present study focuses the female inhabitants of the region with continuous exposure over a period of 30 years or more to have any cytogenetic effect induced by background radiation exposure. Therefore we determined the frequency of chromosomal aberrations

in the peripheral lymphocytes of the female inhabitants of Karunagappally.

Materials and Methods

Study area

A radiation cohort (HBRA) comprising of 4 panchayats (Chavara, Neendakara, Panmana and Alappadu) with high levels of background radiation in the Karunagappally Taluk was selected for the study. Female residents in the age group of 30-65 years who were born and brought up there, had been staying there for a minimum period of 30 years, were selected so that the cumulative dose would be maximal. The selected 4 Panchayats had detectable levels of background radiation exposure. Cumulative dose of each subject from birth to the time of blood sampling was estimated based on indoor and outdoor radiation levels (Table 1) of each household and age specific house occupancy factors. The external gamma radiation levels in each participants house was carried out by dosimetry using sodium iodide (NaI) detector based Scintillometers and plastic Scintillometers provided by the Bhabha Atomic Research Centre (BARC), Mumbai[11]. Both inside house as well as outside house measurements were done. The mean of three readings done at a height of 1 m inside and outside of each house was taken for measurement. The measured absorbed doses in air due to gamma rays in survey meter were converted to annual dose (mGy/year) using a conversion factor of 0.078 (= 0.873 × 24 h × 365 days × 10^{-5}). Dose contributed by the gamma rays in each individual was derived as sum of 0.5 (occupancy factor) × the annual indoor dose and 0.5 (occupancy factor) × the annual outdoor dose. The occupancy factor was based on gender and age specific occupancy factors estimated[12]. Each participant completed a standard questionnaire designed to obtain relevant details of current health status, health history and life style. Those subjects associated with any of the confounding factors such as tobacco chewing, smoking habits, alcohol consumption, medicinal usage, severe infections or viral disease were excluded from participation in the study. Finally, 110 females in the age range of 30-60 years old were selected for the cytogenetic study from HBRAs. Peripheral blood (PB) samples (5-10mL) from these subjects were collected under sterile conditions by venipuncture into heparinized tubes after getting their written informed consent and 100 samples from NBRAs were also included. The study was approved by Institutional Human Ethics Committee [IRB/10-2011]. After collection, all blood samples were transported to the cytogenetics laboratory of Regional Cancer Centre, Thiruvananthapuram, Kerala and processed as quickly as possible within 3-4 h following blood sampling.

Table 1 — Inside house & outside house radiation levels (mGy/yr)

Panchayaths	Inside house radiation	Outside house radiation
	Median	Median
Chavara	3.28	4.54
Neendakara	2.41	4.16
Panmana	2.23	3.12
Alappadu	2.83	4.14

PB culture and harvesting

The whole blood samples were cultured in RPMI 1640 containing 20% fetal bovine serum, phytohaemagglutinin (PHA), penicillin/streptomycin, and amphotericin B at 37°C, and 5% CO_2 in a humidified atmosphere. The sample was cultured for 48 h; including colcemid solution for arresting the dividing lymphocytes in metaphase stage. After lymphocyte culturing, cells were harvested by standard cytogenetic procedures[13]. Cultures were centrifuged at 1000 rpm for 10 min, the supernatant was carefully removed and resuspended in hypotonic solution 0.075 M KCl at 37^0C for 15-20 min. After centrifugation at 1000 rpm for 10 min, the lymphocytes were fixed with Carnoy's solution (methanol/glacial acetic acid, 3:1). Fixation and centrifugation were repeated washed several times with Carnoy's solution, until the supernatants were clear. A drop of cell suspension from each sample was dropped onto a wet, clean microscopic glass slide and left to air dry. Then the slides were stained with fresh 5 % Giemsa solution and were evaluated under a microscope. All slides were coded and scored blindly. 1000-2000 metaphases per subject (from both parallel cultures) were scored. The total number of dicentrics and rings per hundred cells per each subject was evaluated. Final judgment was made by the review of at least three scorers.

Fluorescence *in situ* hybridization (FISH)

Chromosome painting has been shown to be a valid and rapid method for quantifying structural chromosome rearrangements in human lymphocytes. This method is particularly accurate for detecting stable aberrations[14]. FISH analysis using whole chromosome painting (WCP) probes (ASI) of chromosome numbers 1, 2, 4 and X were used for identifying translocations according to manufacturer's instructions. One drop of the suspension, already prepared for cytogenetic

analysis was dispensed onto a slide and each slide was dried at 72°C in 2x SSC for 2 min for denaturation, then protease treatment in pepsin at 37°C for 12-15 min, subsequent treatment in neutral buffered formalin (NBF) for 5 min for fixation and further treatment in 70%, 85% and 100% ethanol for dehydration. Next, WCP of chromosome 1 and chromosome X (1-Red, X-Green) along with hybridization buffer, supplied along with the probes was applied in one slide and WCP of chromosome 2 and chromosome 4 (2-Red, 4-Green) in another slide preparation and the slide was covered with a glass coverslip and sealed. Nuclear DNA was denatured by incubating the slides on a hot plate at 72°C for 2 min, followed by incubation overnight at 37°C in a humidified chamber to allow for hybridization.

The glass coverslips were removed and the slides were washed in 0.4 × SSC at 72°C for 2 min. After draining, the slides were then washed in 2x SSC/0.05% Tween-20 at room temperature and then air dried at room temperature. Finally, nuclei were counterstained with DAPI and the slides were covered with a glass coverslip and sealed. Soon after completion of the chromosome preparations, FISH images were captured in the fluorescence microscope (Olympus BX53, Tokyo, Japan). DAPI was used for analysing metaphases. SpectraCube SD200 spectral imaging system (Applied Spectral Imaging, Migdal Ha'Emek, Israel) was used for capturing the hybridization signals.

Statistical analysis

All statistical analysis were performed using the software SPSS version 21 to assess the Group statistics in experimentals for mean age, mean cumulative dose and chromosomal aberration frequency (CA) mean ± SD. Logistic regression analysis was done in comparison with the control groups in respect of age group association with the chromosomal aberration frequency. The level of statistical significance was set at the $P<0.05$ level.

Results and Discussion

The cumulative dose for each individual was determined by adding up the annual dose in a time dependent manner. The Cumulative doses of each participant in the HBRA, calculated were in the range of 59.25–745.89 mGy/year and in the controls, it was 12-18.5 mGy. The cumulative radiation dose in the study area compared to the control area is significantly high ($P<0.001$). Annual dose of each participant was calculated using the formula

Annual dose (mGy)=[{indoor dose y^{-1}-0.227}×OF indoor+{outdoor dose y^{-1} (mean) of ward or panchayat-0.252}×OF outdoor]×CF

[OF: Occupancy factor; CF: Conversion factor for air kerma to organ-specific absorbed dose presented by the International Commission on Radiological Protection Report (1996). Colon dose (0.78) was used for risk analysis, as it was used in the cancer risk analysis of atomic bomb survivors[15]; 0.227: air kerma values for the cosmic ray component of the measured radiation level indoor; 0.252: air kerma values for the cosmic ray component of the measured radiation level outdoor.]

The female inhabitants of the HBRA were with a mean age and SD of 46.75 ± 7.89 years, and 100 females from NBRA were with an average age of 38.97 ± 6.83 years. There was significant difference in age distribution of HBRA and NBRA inhabitants. About 1000-2000 metaphases per subject were scored for determining the frequencies of dicentric chromosome (chromosomes that have two centromeres), chromatid breaks (Fig. 1A), ring chromosomes (Fig. 1B) etc. The total chromosomal aberration (CA) frequency per hundred metaphases in the NBRA groups was 0.27 ± 0.58, whereas in the high background radiation exposed group, it was 5.85 ± 3.72 ($P<0.001$). The number of total aberrations (dicentrics, ring chromosomes, single strand breaks) in the inhabitants exposed to high cumulative radiation dose was higher than those in the low dose exposed group. The inhabitants of the HBRAs were grouped as follows; Group I >45years (n=69) and Group II ≤45 years of age (n=41). There was a significant increase in chromosome aberration (CA) frequency among study subjects and NBRA inhabitants who were >45 years of age (FR= 1.61; 95% CI: 1.38-1.87; $P<0.01$), but among the HBRA inhabitants, the CA frequency was significantly reduced who were >45 years (Group I), compared to those inhabitants of ≤ 45 years (Group II) FR=0.73; 95% CI: 0.63-0.86; $P<0.01$). Poisson regression analysis demonstrated that high cumulative dose (>400 mGy) exposed inhabitants had a significantly increased CA frequency compared with the low dose group (≤ 400 mGy) (FR=1.50; 95% CI: 1.21- 1.85; $P<0.01$). The mean chromatid aberration (CTA) frequency was more in the group II (4.0 ± 4.4) than the group I (2.7 ± 1.7) with a significant value ($P=$ 0.024). Dicentric chromosomes and ring chromosomes were also observed in both groups and the mean frequency of dicentrics and ring chromosomes (CSAs) were also more in group II (2.97 ± 2.1) compared to group I (2.44 ± 1.6) ($P=$ 0.139).

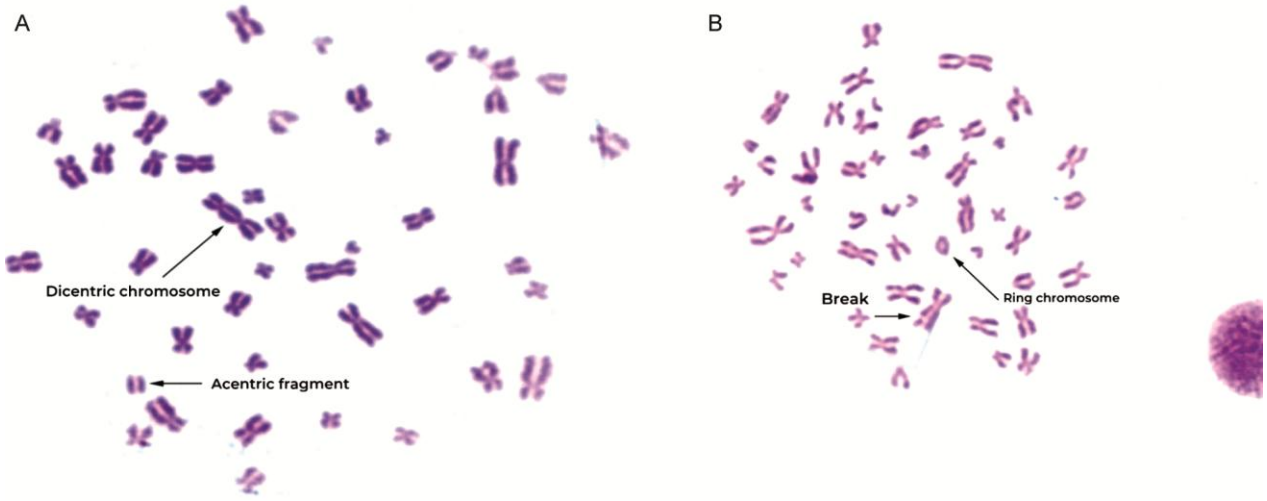

Fig. 1 — (A) Dicentric chromosome and acentric fragment (B) Ring chromosome and single strand break.

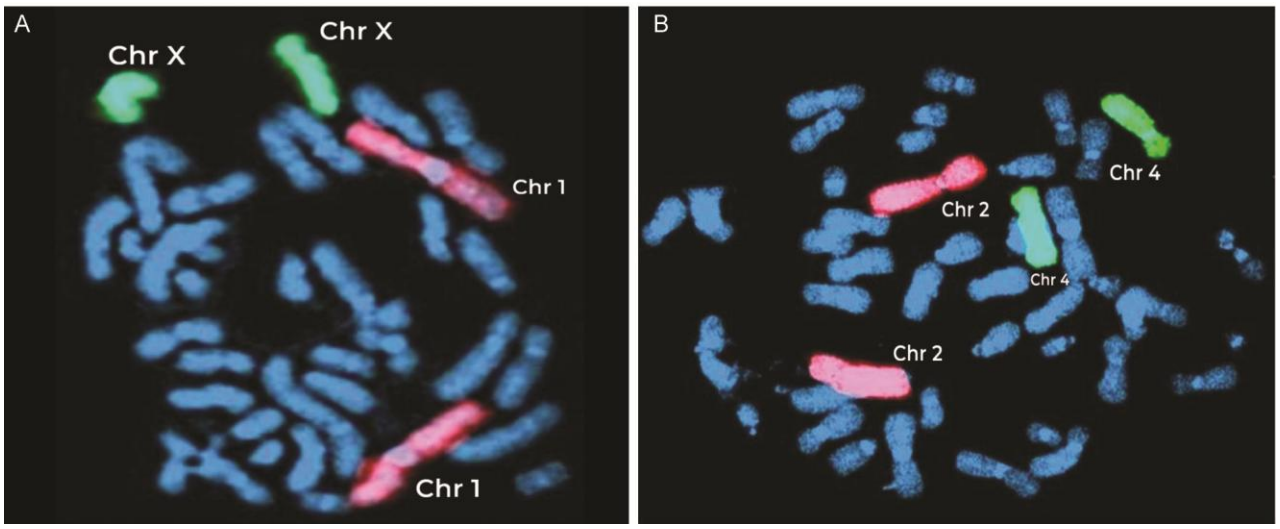

Fig. 2 — (A) Metaphase FISH image showing two normal chr 1 and two normal chr X (B) Metaphase FISH image showing two normal chr 2 and two normal chr 4.

The cytogenetic aberrations mostly SSBs were found in the large chromosomes, and to reveal any stable aberrations like translocations among the chromosomes 1, 2, 4 and X chromosomes, FISH analysis was done which does not show any translocation among the chromosomes (Fig. 2A & 2B).

Karuppasamy *et al.*[16] studied the frequencies of unstable (dicentrics & rings), stable (translocations & inversions), and other types of chromosome aberration in adult men from both high background radiation areas (HBRAs) and normal background radiation areas (NBRAs) of Kerala and did not find any statistically significant difference., Our study finds significant increase in the frequency of chromosomal aberrations (breaks, dicentrics and ring chromosomes) in the female inhabitants compared to those from normal areas. Even though the age distribution showed significant difference between them, the significant high cumulative radiation in the study area attributed the exposure risk of the inhabitants. There is lack of chromosomal translocations in the inhabitants of HBRAs , which was confirmed by FISH. The difference in findings from the same area may be due to the fact that the selection of the male participants, they won't spend much time inside home or nearby and also another fact is that they only selected 27 people from normal background radiation areas while they had 70 individuals from HBRAs, so the sampling done had some effect on the different findings. The major drawbacks of the previous cytogenetic (70 individuals) and the present study (110 individuals)

Table 2 — Chromosome aberration (CA) frequency in HBRA inhabitants and NBRA controls

Parameters	NBRA(Controls)		HBRA inhabitants		FR (95%CI)	*P* value
	n	mean CA±SD	n	mean CA±SD		
Age						
≤45yrs	82	0.32±0.63	41	7.0±5.1	1	*P*<0.01**
>45yrs	18	0.05±0.24	69	5.2±2.4	1.61 (1.38-1.87)	
Age(among exposed groups)	-	-				
≤45yrs	-	-	41	7.0±5.1	1	*P*<0.01**
>45yrs	-	-	69	5.2±2.4	0.73 (0.63-0.86)	
Cumulative radiation exposure	-	-				
≤195 mGy	-	-	73	5.2±2.6	1	*P*<0.01**
>195 mGy	-	-	37	7.2±5.1	1.41 (1.20-1.65)	
Total	100	-	110	-		

[195 mGy was the mean cumulative dose exposure in the inhabitants; Compared with controls in respective of age group, *P*<0.01**; Comparison within inhabitants in respective of age group, *P*<0.01**; Compared high versus low dose exposed groups, *P*<0.01**]

are the poor sampling method, the selection of a small group of residents for study from a very large group (a population of nearly 100,000) in the area[17].

The previous findings held in the area were also reviewed here. There is no evidence that cancer occurrence is consistently higher because of the levels of external gamma radiation exposure in the area. There is a recent report of the low frequency of double strand breaks in peripheral blood mononuclear cells of individuals from high level natural radiation areas of Karunagappally in Kerala[18]. Cytogenetic studies on newborns from both high and normal background radiation area revealed that the frequencies of chromosomal aberration and karyotype anomalies were very similar[19]. This may be due to the fact that the newborns had not much background radiation exposure in both areas. Studies also revealed no significant effect on the induction of micronuclei frequency among the newborns due to elevated level of naturally occurring radiation[20]. There are previous finding that there is an increase in the frequency of dicentric and ring chromosome aberrations from HBRAs of China[21]. Reports of chromosomal aberration studies from HBRAs of Ramsar showed a significant positive correlation in the cytogenetic results with the highest level of exposure[22]. In HBRAs of Brazil, studies observed an increase in the frequency of aberrations, which is attributed to the elevated level of radiation in the areas[23]. These studies indicated that chromosomal aberration is definitely a reliable biodosimetric tool.

Chromosomal aberrations such as dicentric chromosomes and ring chromosomes or translocated chromosomes in peripheral blood (PB) lymphocytes reflect the effective exposure to background radiation doses received by individuals[24]. Dicentric chromosomes and ring chromosomes are mitotically unstable and are gradually eliminated from the body when it undergoes further cell division but translocated chromosomes are stable and hence retained in cells[25]. The frequency of chromosome translocations is higher than the number of dicentrics and ring chromosomes in residents of HBRAs in China. Subsequently, this report demonstrated that smoking may have influenced the result of increased chromosomal translocation frequency[26,27]. However, in our study the participation of the female residents, which does not carry the habits of confounding factors like smoking etc. may have influence the absence of chromosomal translocation among the chromosomes 1, 2, 4 and X which are considered representatives of all chromosomes. Further, the presence of more translocation among other chromosomes can be revealed only by performing FISH for all the other chromosomes, but the method is expensive and very complex; moreover, it is not usually performed for translocation assay. Zhang *et al*., 2010[28] described the concept of adaptive response in the residents of HBRAs of China, similarly the residents of the HBRA here also had the adaptation which is revealed through the low frequency of aberrations like dicentrics, ring chromosomes and breaks compared to similar studies from other HBRAs. This could be due to low induction or better repair capacity of the inhabitants of HBRAs of Kerala coast. The study held in high natural radiation areas of west Sulawesi, Indonesia found a low frequency of cytogenetic biomarkers [micronuclei (MN), nucleoplasmic bridge (NPB), and nuclear bud (NBUD)] in the residents is suggestive of the fact that the exposure does not create a negative impact there[29]. Moreover the studies done in hospital radiation workers in Indonesia found that red blood cell and monocyte counts were

significantly higher in radiation-exposed workers whereas white blood cells, hematocrit, mean corpuscular volume, and lymphocytes values were significantly lower in radiation exposed workers compared to controls[30]. Similar studies can be conducted in HBRA so that the long term exposure on various parameters can be well understood.

There are reports of *in vivo* radioadaptive response in the peripheral lymphocytes of the elder individuals revealed by the induction of low frequency of micronuclei after challenging dose exposures in the HBRA of Karunagappally[31]. This is in consistent with our reports of the low frequency of chromosome aberrations (CA) in the elder age group (Group I, >45 years) compared to those below 45 years (Group II). This could be due to the better adaptation due to more continuous exposure to the challenging radiation exposure in the environment. Moreover a strong repair mechanism is supposed to be present in the inhabitants for the better repair of the aberrations due to radiation exposure. Studies demonstrate the active involvement of some base excision repair (BER) genes and upregulation of proteins such as APE1, FEN1 and LIGASE1 in primed cells in radio-adaptive response *in vivo* in healthy individuals exposed to a priming dose followed by a challenging dose of gamma irradiation[32]. Similarly there will be the active involvement of the various repair pathways like BER, NER etc in adaptive response shown by the residents of HBRAs against the challenging natural background radiation exposure[33].

Conclusion

In conclusion, our results reported that radiation exposed inhabitants had higher frequencies of CA frequency compared with those exposed to normal background radiation exposure. The chromosomal aberrations are intimately related with radiation exposure and as the aberrations found out were unstable, it can be suggested that they were repaired under body's defense mechanism and are being escaped from the chance of developing cancer. Hence the study supports the concept of adaptive response in the inhabitants of HBRAs of Kerala coast. The sample size is the major limitation of the study. In the future, large homogeneous study populations are needed to reach a firm conclusion and proper analysis of the impact of various relevant genes in DNA repair pathways that might involved in adaptive response of the inhabitants of HBRA and various other biological parameters is also recommended.

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Conflict of interest

The authors have no conflict of interest to declare.

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