GENESIS AND FUTURE OF BIOLOGICAL RESEARCH IN HIGH-LEVEL NATURAL RADIATION AREAS OF KERALA, INDIA

Vinay Jain[*1,3], M. Seshadri[2], P. K. M. Koya[1], P. R. Vivek Kumar[1] and Deepak Sharma[*1,3]
[1]Radiation Biology & Health Sciences Division
[2]Former Head, Radiation Biology & Health Sciences Division
Bhabha Atomic Research Centre
Mumbai - 400 085, India
[3]Homi Bhabha National Institute, Mumbai - 400 094, India.

[*]Email: vinayj@barc.gov.in; dsharma@barc.gov.in

Abstract

High level natural radiation areas (HLNRA) in India encompass a stretch of 55 km long and 0.5 km wide coastal belt along the Arabian sea, which extends from Kollam district to Alappuzha district of Kerala. The monazite containing beach sand contributes to 20-30 times higher background radiation in these areas compared to the global average of 2.4 mSv/year. In 1959, WHO's expert committee on radiation highlighted this area to investigate long-term biological effects of ionizing radiation. Early studies, during 1960-75 were focused on investigating genetic effects on black rats, radioactivity in food chain, demographic survey and aerial dosimetry. In 1975, "Monazite Survey Project" was started by Bio-science group, BARC to initiate studies involving epidemiology, cytogenetic analysis, cellular/molecular responses and cancer incidence in human populations. These investigations revealed no significant health risks and there was no increase in cancer incidence in the human population residing in HLNRA of Kerala. Current research focuses on radio-adaptive mechanisms,

genomic/epigenomic responses and addressing the low-dose radiation risk debate.

1. Introduction

Over millions of years, all forms of life on the earth have evolved in a radiation rich environment. We all are exposed to different types of radiations through natural as well as man-made sources. Although, naturally-occurring background radiation is the main source of exposure for living beings but the incidence of Hiroshima and Nagasaki atomic bombings in 1945, brought to the fore the urgent need to understand the long-term biological and health effects of ionizing radiation in human population. There are few areas around the world where the levels of natural background radiation from terrestrial and cosmic sources are significantly higher than the world average of 2.4mSv/year. These geographical places are termed as high-level natural radiation areas (HLNRA). Some of the predominant HLNRA's around the world are Guarapari (Brazil), Yangjiang (China), Ramsar (Iran) and Kerala (India). In early 1950's, the Karunagapally Taluk of Kollam district in Kerala was identified as one such area which had 2-20 times higher levels of background radiation due to thorium-232 containing monazite beach sand. The coastal zone from Sakthikulangara in Kollam district in the south to Purakkad in Alappuzha district (north) is a 55 km long and 0.5 km wide stretch skirted by Arabian sea and western ghats.

The large deposits of monazite sand in this coastal belt were discovered as early as 1909. The main sources of monazite are beach boulders and sedimentary rocks which include khondalites, chamockites, gneiss and granites present in the granulitic terrain of southern India mostly in parts of Tamilnadu and Kerala. They are transported to the sea by many lakes (e.g. Ashtamudi) and rivers (e.g. Kallada) flowing through this terrain and also some sort of panning action deposits the heavy metallic sand along the seacoast (UNSCEAR 2017 report).

The HLNRA of Kerala coast came to highlight through the WHO's expert committee report on "Effects of Radiation on Human Heredity" in the year 1959. The WHO committee mentioned that "Only in the light of more knowledge can decisions be taken to define more accurately the maximum amount of exposure which may be accepted by individuals and populations without risk of serious harm." At the same time, they noted that "Kerala area of India is one untapped source of information, which might be profitably investigated" (WHO technical report, 1959 and UNSCEAR 2017 report).

Dr. Homi Bhabha, the founder-architect of India's Atomic Energy program being the visionary leader envisaged the importance of biological research in nuclear establishment of our country and recruited Dr. A.R Gopal-Ayenger as first biologist to Head, Biology Division in Atomic Energy Establishment, Trombay. Dr. Gopal-Ayenger carried out pioneering work in establishing low dose radiation program and was a member of WHO expert committee on radiation which in its first report brought out a comprehensive plan with a section titled "The Kerala Project" for the study of long-term consequences to human population exposed to continuous doses of high-level natural radiation for several

generations. The committee further observed that "Such is the present status of knowledge of the somatic and genetic effects of chronic low-level exposures that any proper investigation of areas of high natural radiation is certain to contribute to the fund of biological knowledge and the ultimate specification of the genetic risks accruing from increasing exposure to ionizing radiations" (WHO technical report, 1959 and UNSCEAR 2017 report).

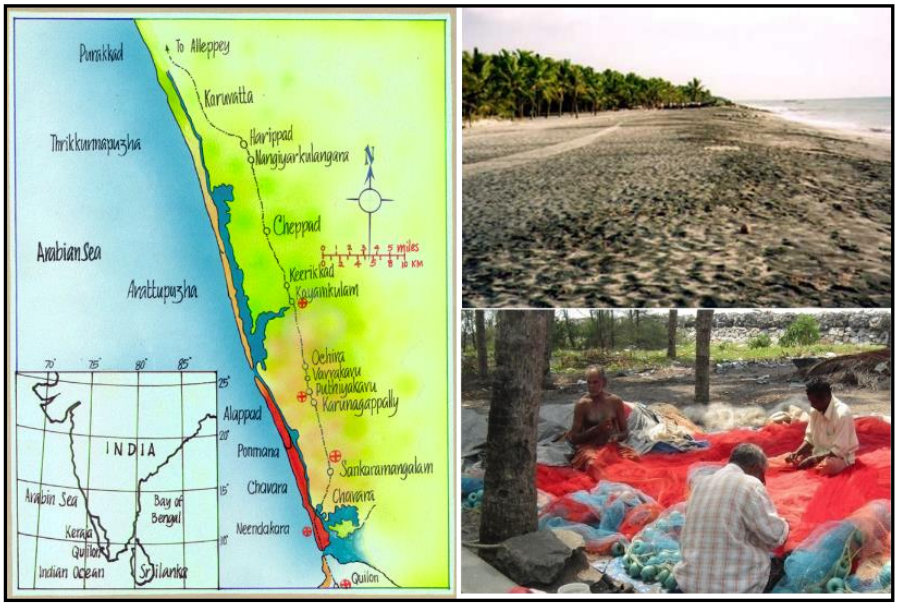

Fig. 1: Geographical representation of High-level natural radiation areas at Kerala coast in southwest India (left). The monazite containing beach sand and fishermen sitting on it (right).

2. Historical perspective:

2.1. Pioneering work on genetic effects of chronic radiation in HLNRA, Kerala:

The pioneering work to understand the genetic effects of chronic low-level radiation in Kerala was carried out on black rats, scientific name; "Rattus rattus L" by noted British geneticist Dr. Gruneberg in collaboration with Dr. Gopal-Ayenger and his team with the support of radiobiologist Dr. L.H. Gray. The work was started in the autumn of 1961 in a makeshift laboratory at Sree Narayana College at Kollam with the support of then principal Dr. M. Sreenivasan and Dr. S. Sivaprasad, a Professor of Zoology.

The black rats were chosen for this seminal study because they were the only mammals other than humans to exist in large numbers, Initially, it was thought to include one more mammal species-the bandicoots in the study, but due to insufficient numbers, it was later decided to exclude them. The study was carried out in a total of 896 black rats, out of which 438 rats were captured from house-holds of eight villages of Neendakara,

Puthenthura, Kovilthottam, Panmana, Cheriazhiekal, Alappad, Srayikkadu and Aziekal which were located near black sand beaches and 458 rats were caught from eight villages which were far from the coastal area and not exposed or migrated from high radiation areas. The radiation exposure dose on the coast was 7-8 times greater than the control value, although dosimetry was not very accurate during those days but the study was designed with great accuracy, precision and finesse.

Dr. Gruneberg and his team carried out detailed measurements of dental structure, minor and major changes in skeleton structure and reproductive pattern in these black rats. Analyzing the results with refined statistical procedures, Dr. Gruneberg and his team concluded and wrote in an epilogue that "There is no evidence for any consistent and systematic difference between the strip and control populations which might be reasonably attributed to radiation". He further stated "In view of the absence of any strong and consistent pattern of difference, the only possible conclusion seems to be that if there is any effect of radiation it is masked by the variation already existing within both areas from population to population." They summarized the absence of any genetic effects of exposure to high natural radioactivity in the black rat of Karunagapally with four possible scenarios. 1. Gamma rays at extremely low dose rate do not produce mutations. 2. Mutations were nullified by natural selection. 3. There might have been genetic change but those were masked by environmental change. 4. Accidents of sampling might have obscured a real effect (Gruneberg et al, 1966, A.P. Jayaraman 2005).

The studies on black rats of Karunagapally were further taken up by Mohapatra et al. at Utkal University, Bhubaneshwar, Orissa. During 1982-1986, they caught 345 black rats; 135 from Chavara,125 from Chatrapur and 85 from Bhubaneshwar and carried out cytogenetic studies on the bone marrows of these rats and discovered paired dot-like structures called double-minutes in the black rats captured from radioactive zone. They concluded that "the double minutes are gene-amplifying elements which cook up extra protein to counter the cell from harmful assaults and the appearance of double minutes in the progenitive tissue of rats is supposed to defend the animal from the background radiation shock."

In 1972, Dr. Gopal-Ayenger and his team conducted a demographic survey for various reproductive parameters such as fertility index, sex ratio and infant mortality in 70,000 human residents of these areas. No significant difference in these parameters were reported in residents of HLNRA in comparison to residents of adjacent normal level natural radiation areas (NLNRA).

2.2. Pioneering work on radioactivity in food chain in HLNRA, Kerala:

In another pioneering work during 1970's, Dr. Gopal-Ayengar and his colleagues carried out a dietary survey to collect data on the quantity of diverse foodstuff eaten by four hundred families of twenty-three south west coastal villages in Kerala and measured the profile of radioactive inputs in these food items. The study revealed the signature of the food style of Kerala coastal villages. They observed that cereals, roots and tubers and flesh foods make up most of the diet followed by milk and milk products. Dr. Gopal-

Ayenger and his team assiduously estimated daily intake of radioactivity by an individual through their dietary habits. They found that alpha and gamma activity was highest in fish and lowest in milk and coconut, respectively. Beta activity was highest in lady's finger and lowest in rice. Potassium 40 was maximum in plantains and minimum in rice. Tapioca is eaten in Kerala as energy source to meet the short supply of cereals during draught conditions. In studies to measure the uptake of natural radioactivity, it was observed that thorium content of tapioca was proportional to the thorium content of the soil and potassium was also found in high measure in tapioca (Mistry et al, 1970 and A.P. Jayaraman 2005). The seminal findings of these pioneering works prompted multiple questions and to answer these questions a new project "The Monazite Survey Project" was established in BARC.

2.3. Monazite Survey Project established in Kerala:

In 1975, Monazite Survey Project (MSP) was initiated by Bio-science/Bio-Medical group, BARC with an outstation lab set up at Medical College Health Unit, Neendakara, Kerala. Mr. K.P George was designated as first Officer-In-Charge of the laboratory. The project was started with a goal to study the biological and health effects of high background radiation in human population. The major works envisaged under the projects were dosimetry survey, Health audit and demographic survey of the population, Cytogenetic studies in newborn and adult population to detect karyotype anomalies and chromosomal aberrations in HLNR and NLNR areas, Cytogenetic studies among occupational workers from various DAE units, Cytogenetic studies on plants and rodents among others. A country wide survey of outdoor natural background radiation levels reported highest air-kerma in the monazite areas of Kerala. The studies showed the average per capita dose received by the population of this area was about four times the normal background radiation levels.

In 1976, Kochupillai et al. published a study in Nature, where they reported higher prevalence of Down's syndrome in the HLNRA population. However, the study was highly criticized for its shortcomings in design and interpretation and a strong rebuttal was written by Dr. Sundaram, then Director, Bioscience (Medical) Group and was published in the same journal.

Subsequently, Bhabha Atomic Research Centre, Mumbai signed a Memorandum of Understanding (MoU) with Department of Health & Family Welfare, Directorate of Health Services, Govt. of Kerala in 1986 to carry out collaborative studies in human population of normal and high-level natural radiation areas of Kerala coast. The major studies initiated under this MoU were cytogenetic analysis among newborns to estimate the frequency of chromosome aberration and karyotype anomalies, newborn survey to identify major congenital malformations and /or genetic disorders. The MoU is continued since then.

In 1988, the field laboratory of MSP was shifted from Neendakara to a rental space in Indian rare earth limited (IREL) campus, Beach Road, Kollam. The MSP project received major momentum and thrust under the leadership of Dr. Anil Kakodkar, then Director, BARC and Dr. P.S. Chauhan, then Head, Cell Biology Division, BARC &

Project Manager, MSP. Dr. Kakodkar inaugurated the new building of Monazite Survey Project (MSP) on September 4, 1999 at Kollam, Kerala, in the presence of Dr. A.M Samuel, then Director, Bio-Medical group. Dr. Kakodkar rechristened MSP as Low-Level Radiation Research Laboratory (LLRRL). At the same time, an independent section Low Level Radiation Research Section (LLRRS) was also created in Bio-Medical group, BARC at Trombay and Dr. M. Seshadri was made Section Head and Project Manager.

Fig. 2: Dr. Anil Kakodkar, then Director, BARC, inaugurating the new building of Monazite Survey Project (Low level Radiation Research Laboratory) at Kollam, Kerala

In his inaugural address, Dr. Kakodkar appreciated the importance of the studies being carried out at MSP and their significance to the people of the monazite belt. He also emphasized that the facilities at MSP (LLRRL) should be continuously updated so that people from other center's in the country will be encouraged to interact with BARC scientists to seek answers to the basic biological questions pertinent to the exposure of human population to continuous low-level radiation. He desired that LLRRL should become a unique centre for research in this area of life sciences and health sciences (BARC Newsletter 1999). Over the years, multiple collaborative studies with different universities and research institutes have been carried out at LLRRS Mumbai and LLRRL, Kollam. In 2010, for the first time in India, 7th International conference on high levels of natural radiation and radon areas was organized by Bio-Medical Group, BARC in association with International Committee on High Level Natural Radiation and Radon Areas (ICHLNRRA) and Indian Association for Radiation Protection (IARP) and Indian Aerosol Science and Technology Association (IASTA) at Mumbai, India.

2.4. Health effects of high background radiation in HLNRA, Kerala:

In 1990's, Regional Cancer Centre (RCC), Trivandrum in collaboration with BARC, initiated an epidemiology study to investigate health effects of HBR on Karunagapally population. A cohort of 385,103 residents of the Karunagapally taluk was selected for the epidemiological survey. During 1990-97, house to house surveys were carried out to record indoor and outdoor dose and document personal information on sociological and demographic factors including lifestyle, diet, tobacco chewing and alcohol consumption. In total, personal information on 359,619 subjects in 71,674 households which constituted 93% of the population was collected. (UNSCEAR 2017 report).

First cancer registry was established in Karunagapally in 1990. Dr. M. Krishnan Nair and his team from RCC Trivandrum established a sub-cohort of 173,067 individuals from six panchayats (Alappad, Chavara, Neendakara, Panmana, Oachira and Thevalakkara) and followed them up for more than 2 decades to investigate cancer incidence rate in these individuals. Recently, the results of above study were published and it was reported that "there is no elevated risk of cancer associated with high background radiation exposure in this cohort (Amma et al. 2021)". Moreover, the excess relative risk (ERR) of cancer excluding leukemia was -0.05/Gy (95% CI:-0.33, 0.29).

3. Current status of biological studies at LLRRS, BSG and Future direction:

For the last 3 decades, scientists at LLRRS, Mumbai and LLRRL, Kollam have carried out extensive investigations on biological and health effects of HBR in Kerala population. Multitude of studies involving epidemiology, cytogenetics, and molecular biology have been carried out in blood samples collected from HLNRA and NLNRA population. The prevalence of congenital malformation/birth defects has been analyzed in more than 2,10,000 newborns, chromosomal abnormalities have been analyzed in 27,295 newborns as well as in adult population. DNA damage studies using multiple biomarkers such as gamma-H2AX, telomere length, micronuclei, *HPRT* gene mutation, excess PCC fragments, and DNA strand breaks (comet assay) have been carried out. High-throughput transcriptomics and proteomics analysis have been done to understand the molecular mechanisms of radio-adaptive response in Karunagapally population. These studies did not reveal any adverse effect of chronic high background radiation in human population. Moreover, better and more efficient DNA repair mechanism were shown to be active in HLNRA population. All these studies have been published in reputed peer-reviewed journals. The importance of these studies is reflected in several reports of United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), where these publications have been included. The ongoing and future studies are being focused on deciphering the mechanism of radio-adaptive response and healthy ageing in HLNRA population. Large-scale multi-omics studies are being planned to investigate transgenerational changes at genomic and epigenomic level in multiple organism models to elucidate the effect of high-level natural radiation on living beings. These studies will provide in-depth insights into genetic changes in response to chronic low dose radiation

exposure and also might answer the linear no threshold (LNT) conundrum at low dose radiation.

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