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CYTOGENETIC STUDIES ON NEWBORNS FROM HIGH LEVEL NATURAL BACKGROUND RADIATION AREAS OF KERALA COAST, SOUTH INDIA

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ABSTRACT

The human population residing in the monazite bearing high level natural background radiation (HLNBR) areas of Kerala, along the South-West coast of India provides unique opportunities of assessing directly in man, the health effects of chronic low-level radiation exposure. The per capita dose received by this population is nearly four times the normal background radiation level. While this is the average dose, the radiation levels prevailing in these HLNBR areas are in the range of 1 to over 35 mGy per year. Chromosomal aberration studies in the lymphocytes of newborns and adults from these areas have been in progress for two decades. So far, 4156 newborn babies from HLNBR and 7321 from normal background radiation (NBR) areas have been screened for the incidence of chromosomal aberrations (dicentrics and rings). The mean frequency of dicentrics and rings did not show any significant difference between the newborns in the control and the HLNBRA population. Assessment of the frequency of micronuclei in cytochalasin-B blocked binucleated lymphocytes of 49 newborns from control areas and 131 newborns from radioactive areas also showed similar values. While an age-dependent increase in chromosome aberration frequency was observed in the adult samples from control and the study areas, the regression analysis of the data indicated a marginally higher slope for the samples from HLNBRA. Karyotype anomalies recorded so far among the newborns have not revealed any significant difference in the incidence of numerical (including Down syndrome) and structural alterations between the control and the exposed populations. A noteworthy observation, herein reported for the first time from any HLNBR area is that there is no discernible increase in the incidence of micronuclei and chromosomal aberrations in the peripheral lymphocytes of newborn babies hailing from HLNBR areas, where their ancestral generations have lived for several hundreds of years.

INTRODUCTION

There is growing scientific interest, as well as controversy, over the relationship between health effects in humans chronically exposed to low doses of ionizing radiation at extremely low dose rates over prolonged period of time. The usual approach for assessment of low level radiation hazard is to extrapolate linearly to zero dose from well documented effects observed with high doses at high dose-rates. However, the validity of such extrapolation has remained unestablished in the absence of any statistically reliable evidence of detrimental effects of radiation at low doses [1]. On the contrary, the accumulating evidence of radioadaptive responses, the mechanisms of which are being currently elucidated, shows that effects elicited by low and high doses of radiation are not only diverse, but can be entirely counteracting [2,3,4]. Therefore, direct human studies pertaining to low level radiation exposures assume profound significance and these are also more appropriate for consideration of risk assessment to occupational workers. The inhabitants of the high level natural background radiation (HLNBR) areas, a coastal strip situated along the west coast of Kerala State in South India, serve as an ideal population to evaluate the possible biological and health effects of low level chronic radiation in a large human population. The radioactivity of these monazite bearing HLNBR areas (about 55 km long and 0.5 km. wide) is primarily due to thorium (also traces of uranium) content ranging from 8-10.5%, the highest reported in the world. ^{232}Th and its decay products contribute to the

elevated background radiation levels. The radiation belt is frequently interrupted by stretches which do not contain monazite deposits and thus have normal background radiation (NBR) levels. Historical records of this coastal strip indicate that it has been inhabited for over several centuries. Eversince, the importance and necessity of assessing the biological and health impact of HLNBRA in the Kerala coast was emphasised in a WHO meeting in 1959 [5], several investigations pertaining to dosimetry, cytological and radiochemical studies on different plant species and demographic and health studies in human populations have been undertaken [6-10]. Gruneberg et al. in a comprehensive study found no evidence of any genetic effects in the black rats, (Rattus rattus) trapped from the monazite belt [11-12]. Dosimetric studies show the average background radiation level prevailing in this area to be around 4 times the normal, with a wide range from 1 to over 35 mGy per year.

A field laboratory located at site in Kollam, adjoining the HLNBR areas has been pursuing a comprehensive programme for assessment of biological and health effects of radiation on human population for several years. Studies on the incidence of chromosomal aberrations in lymphocytes of newborns and adults (including young, adult and aged group of men and women) from NBR and HLNBR areas , which form part of the continuing programme of the Monazite Survey Project, are summarised in this communication.

MATERIALS AND METHODS

Metaphase preparations were made from short term *in vitro* PHA stimulated whole blood cultures employing standard microculture techniques. The slides were coded and stained in lacto-aceto-orcein or Giemsa. About 80-100 well spread metaphases were scored for dicentrics, rings, acentric fragments, minutes, translocations, inversions as well as chromatid aberrations like gaps and breaks. The incidence of dicentrics and rings are presented here, while detailed analysis of the total cytogenetics data are underway for critical evaluation. All major chromosomal aberrations were cross checked by each of the five cytogeneticists engaged in this project. Micronuclei (MN) were scored in cytochalasin-B blocked binucleated lymphocytes using cord blood samples with standard procedures as reported elsewhere [4]. From the coded slides stained in Giemsa, about 5000 binucleate lymphocytes have been analysed for each sample.

RESULTS AND DISCUSSION

Studies in Newborns : The data on the frequency of dicentrics and rings from a total of 11477 newborns, with 7321 samples (628960 cells) analysed from NBR and 4156 samples (360089 cells) from HLNBR areas, available so far, have been presented in Table I. The frequency of dicentrics and rings per cell was 1.84×10^{-4} in NBR and 1.92×10^{-4} in HLNBR samples. Thus, the incidence of chromosome aberrations in the newborns do not indicate any significant difference between the samples from normal and HLNBR areas. Considering the fact that a substantially large number of newborns have been analysed, it is highly unlikely that further increase in sample size will basically alter the observed trend. However, categorisation of the newborns of the HLNBR areas showed a very marginal upward trend with increase in the radiation levels. Both from HLNBR and NBR areas about 77% pregnancies occurred in women below 25 years of age and 97% below 30 years. In fact, less than 0.5% deliveries occurred among women above 35 years of age.

TABLE I. DICENTRICS AND RINGS AMONG THE NEWBORNS

Area	No. of Samples	Cell Analysed	Dicentrics + Rings	
			No.	Frequency*
NBRA	7321	628960	116	1.84 ± 0.17
HLNBRA	4156	360089	69	1.92 ± 0.23

* Dicentrics and rings per cell ± S.E x 10^{-4}

Data on the incidence of micronuclei (MN) in cytochalasin-B blocked binucleated lymphocytes of newborns presented in Table II, also do not indicate any significant difference between the samples from NBR and HLNBR areas, the frequency of micronucleated cells being 1.14 x 10^{-3} and 1.12 x 10^{-3} per binucleate cell, respectively. A slightly higher frequency of MN was observed in the female babies as compared to the males, thereby suggesting, as reported by others that sex can be one of the variable affecting the spontaneous frequency of micronuclei. Although, reports on the frequency of MN in adults have been published in literature, there is a paucity of such data among the newborns. To our knowledge no report on chromosome aberrations or micronuclei in the lymphocytes of newborns has been published from any HLNBR area, so far.

TABLE II. MEAN FREQUENCY OF MICRONUCLEATED LYMPHOCYTES AMONG NEWBORNS.

Area	No. of Samples	Cells Analysed	No. of Micro-nucleated Cells	Frequency*
NBRA	49	239734	266 (311)	1.14 ± 0.76
HLNBRA	131	602001	657 (740)	1.12 ± 0.76

Figures within parenthesis denote total number of micronuclei. * Micronucleated cell per binucleate lymphocyte ± S.D x 10^{-3}.

Studies in adults : The limited data available so far from 582 adult subjects include 279 (47727 cells scored) from NBR and 303 samples (53092 cells) from HLNBR areas. The frequency of dicentrics and rings observed among the samples of different age groups of adults have been presented in Table III. The incidence of dicentrics and rings in the lowest age group (≤ 20) is slightly, but not significantly, higher in the HLNBR as compared to the NBR population. The chromosome aberration frequency increased from 7.07 ± 2.89 to 26.13 ± 6.16 in the NBR population and from 10.44 ± 2.46 to 27.78 ± 16.0 per cell x 10^{-4} in the exposed population across the different age groups. In the NBR samples, a marked increase in aberration frequency was observed in the age group of ≥ 60 yrs., while in case of HLNBR it was higher from 40 years onwards. The highest age group in HLNBR suffers from the limitation of small sample size at present. A linear regression analysis of the data (the frequency of dicentrics and rings plotted against increasing age) showed a reasonable correlation with slightly higher slope for the HLNBR samples (Y = 0.3414 x + 2.8022, correlation coefficient = 0.845) as compared to NBRA (Y = 0.2977 x + 0.8112, correlation coefficient, = 0.7552). The initial tendency for increase in dicentrics and rings in the ≤ 20 yrs. age group was not seen in the 21-40 yrs of age group. The age dependent increase in chromosome aberrations is in confirmity with the reports in literature.

TABLE III. DICENTRICS AND RINGS IN THE LYMPHOCYTES OF ADULT SUBJECTS OF DIFFERENT AGE GROUPS.

	N B R A			H L N B R A		
Age Group	No. of Samples	Frequency*	Mean Age ± S.D	No. of Samples	Frequency*	Mean Age ± S.D
<=20	47 (8484)	7.07 ± 2.89	15.3 ± 5.2	98 (17244)	10.44 ±2.46	13.9 ± 4.5
21-40	150 (25062)	9.98 ± 1.99	27.7 ± 4.8	150 (25561)	9.78 ± 1.96	28.2 ± 5.9
41-60	41 (7293)	9.60 ± 3.63	50.5 ± 5.5	48 (9207)	16.29 ± 4.21	48.4 ± 5.5
> 60	41 (6888)	26.13±6.16	72.9 ± 9.1	7 (1080)	27.78 ± 16.0	65.0 ± 3.7

Figures in parenthesis denote number of cells analysed. * Dicentrics and rings per cell ± S.E x 10^{-4}.

During the metaphase analysis for chromosomal aberrations in the newborns, both numerical and structural karyotype abnormalities (KA) were recorded. A total of 65 variants (31 numerical and 34 structural) have been scored among 11,477 newborns. The overall population frequency of KA is 0.56%, with 0.59% in NBRA and 0.53% in HLNBRA. Analysis of karyotypic variants is being pursued by G-banding to identify the inter and intra chromosomal rearrangements. The 8 cases of Down Syndrome, so far confirmed are all primary trisomies with an overall frequency of one DS in about 1640 births. However, the number of individual karyotypic anomalies is still too small, and a sufficiently larger data base will be required to make any reliable estimates. In this context, it is important to mention that the state of Kerala with highest literacy in India, has lowest infant mortality and smallest family size. The total number of births from the HLNBR areas are also less due to relatively smaller population size in the limited HLNBR areas than the NBR areas. The studies are designed to continue for the next several years to obtain a satisfactory number of live borns from HLNBR areas for rigorous comparisons.

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