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Spontaneous frequency of micronuclei among the newborns from high level natural radiation areas of Kerala in the southwest coast of India

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Abstract

Purpose: The present study was an attempt to determine the spontaneous frequency of micronuclei (MN) in newborns from High Level Natural Radiation Areas (HLNRA) and the adjoining Normal Level Radiation Areas (NLNRA) of the monazite-bearing Kerala Coast in Southern West India using Cytochalasin Blocked Micronuclei (CBMN) assay.

Materials and methods: Human umbilical cord blood samples were collected from a total number of 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). Lymphocyte cultures were set up following microculture techniques and cultures were terminated at 72 hours. Cytochalasin B at a concentration of 4.0 μg/ml was added to the lymphocyte cultures at 44 h. Enumeration of micronuclei was restricted to Cytochalasin Blocked binucleated (BN) cells only.

Results: The frequency of MN among the newborns from NLNRA (1.40 $\pm$ 0.12) per 1000 BN cells was not statistically significant as compared to HLNRA newborns (1.33 $\pm$ 0.04) per 1000 BN cells. Our data did not show any radiation dose response. Odds ratios (OR) and confidence intervals (CI) have been calculated to see statistical significance in the mean MN frequency among the newborns from various dose groups with respect to control and it did not reveal any significant difference ($p > 0.05$). A marginal increase in the frequency of micronuclei was observed among the female newborns as compared to males with increasing mothers' age groups except for the mothers aged > 30 years, though not statistically significant.

Conclusion: The baseline frequency of micronuclei in HLNRA newborns is not statistically different from NLNRA newborns suggesting that elevated level of naturally occurring radiation has no significant effect on the induction of micronuclei frequency among the newborns.

Keywords: *Cord blood, micronuclei, Cytochalasin blocked micronuclei (CBMN) method, High Level Natural Radiation Area (HLNRA), Normal Level Natural Radiation Area (NLNRA)*

Introduction

The biological and health effects of chronic natural low level ionizing radiation exposure on human population has been a thrust area of research in radiation biology. There are some 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. The High Level Natural Radiation Areas (HLNRA) of the world are of great interest, because they provide unique opportunity to study the health effects of chronic low level radiation exposure directly on humans. In south west India, the thickly populated monazite sand-bearing areas of Kerala coast provide such an ideal situation to investigate the health effects of low level, low dose rate radiation on human population exposed for many generations. In this area, deposits of radioactive minerals occur in littoral formations along the coastal line and the most concentrated deposits of monazite are located along a coastal strip of about 55 km, extending from Neendakara (Kollam district) in south to Purakkadu (Alapuzha district) in north (George et al. 1976). The radioactive component of

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this beach sand is monazite, which contains thorium and its radioactive daughter products. It has been estimated that the per capita average dose received by the population residing in HLNRA is $\sim$4 mGy/year (Bharatwal and Vaze 1958, Sunta 1993). However, the background level in this area ranged from $\leq$1.0 mGy to over 45 mGy per year (Thampi et al. 2005). The areas with a dose range of $\leq$1.50 mGy/year are considered as normal level natural radiation areas (NLNRA), whereas a dose range of $>$1.50 mGy/year is considered as HLNRA. Several investigations have been carried out to study the biological and health effects of low level chronic radiation on human population residing in HLNRA of Kerala coast (Cheriyan et al. 1999, Jaikrishan et al. 1999, Foster et al. 2002, Thampi et al. 2005).

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. The Cytochalasin-Blocked Micronucleus (CBMN) assay provides a comparatively less tedious and simple method of scoring chromosomal damage. A number of studies have been conducted in which MN has been scored as an alternative approach for quantifying chromosome damage, because it offers a measure of both chromosome breakage and chromosome loss (Schmid 1975, Countryman and Heddle 1976). More especially, in cultured human lymphocytes, it is one of the most reliable and precise method for assessing radiation induced chromosome damage (Fenech and Morley 1985, Fenech 1993, 2000, Muller et al. 1996, Fenech et al. 2003, Gutierrez-Enriquez and Hall 2003).

Although CBMN assay has been used extensively as a biomarker to evaluate the effect of genotoxic agents in adult population and children, data on peripheral cord blood lymphocytes are comparatively limited. However, recently, many groups have contributed substantial data to establish the relationship between MN frequency and environmental pollution using cord blood samples (Bockay et al. 2005, Neri et al. 2006, Milosevic-Djordjevic et al. 2005, 2007). Micronuclei frequency on cord blood samples has been used as a biomarker to evaluate the utero effect to substantial changes in the environment (Milosevic-Djordjevic et al. 2005). Considering the importance of cord blood and newborn studies, these details are highly relevant to human health. As compared to chemicals/drugs, very few studies have been reported studying the effect of ionizing radiation and human cord blood samples using CBMN assay. In the present study, we have made an attempt to estimate and compare the spontaneous frequency of MN among the newborns from HLNRA and the adjoining control areas/NLNRA of Kerala coast.

Materials and methods

Subjects

For the present investigation, umbilical cord blood samples were obtained from four hospital units located in HLNRA and the adjoining NLNRA. Cord blood samples were collected from 271 random, healthy and normal newborns (138 males and 133 females) born to mothers aged between 17 and 37 years with a mean maternal age of 24.08 $\pm$ 4.23. The samples from HLNRA included 109 males and 101 females (mean maternal age: 24.18 $\pm$ 4.26), whereas 29 males and 32 females (mean maternal age: 23.75 $\pm$ 4.10) were from NLNRA. All the samples were collected with informed consent with institutional ethical/human experimentation approvals and under Memorandum of Understanding (MOU) between Kerala state Health Department and Bhabha Atomic Research Center, Mumbai. Data pertaining to pregnancy history, life-style, occupation, duration of stay in HLNRA, mother's diet during pregnancy, habits etc., are recorded in proformae designed under the World Health Organization (WHO) guidelines. The average radiation levels for the family are incorporated for each newborn and the data entry is verified and validated for internal consistency. As per the data sheets, all the newborns were born to mothers who were non-smokers and they did not take any drugs during pregnancy. The diet of the parents of the collected newborns was similar in both HLNRA and NLNRA, because of the similarities in the socio-economic status.

The study area is a belt about 55 km long and 0.5 km wide and the radiation dose ranges from 1.0–45 mGy/year. The average radiation dose in NLNRA of Kollam district is 1.2 mGy per year with a range of $<$1.0–1.5 mGy per year. Hence area with an exposure above 1.5 mGy per year is considered as High Level Natural Radiation area (HLNRA). The classification of high level and Normal level natural radiation is based on the level prevailing at the residence of the mother. Greiger Muller Counters were used for measuring the terrestrial gamma radiation and average area dose was calculated from 0.5 km grid.

Lymphocyte culture

Umbilical cord blood samples were collected from the pre-cleaned cord in sterile heparinized vials and stored under refrigeration. Whole blood cultures were set up the following day using standard microculture techniques as described in International Atomic Energy Agency (IAEA), 1986. The cultures were incubated for 72 h in the presence of phytohaemagglutanin (PHA) (Sigma-Aldrich Corporation, St Louis, MI, USA), at 37°C.

CBMN method

Cytochalasin B (Cyt-B) (Sigma-Aldrich Corporation, St Louis, MI, USA), was prepared as a stock solution in sterile Dimethyl Sulphoxide (DMSO) (Sigma-Aldrich Corporation, St Louis, MI, USA) at a concentration of 2 mg/ml., stored in aliquots at -20°C until use. The aliquots of Cyt-B was diluted in phosphate buffered saline (PBS, pH 6.8) and was added to the cultures at 44 h after the commencement of the culture at a final concentration of 4 μg/ml (Fenech and Morley 1985). The cultures were harvested at 72 h and slide preparations were made. Cultures were processed following mild hypotonic treatment (KCl, 0.075M) (Qualigens, Mumbai, India) for 2 min, and then fixed twice in methanol and glacial acetic acid (3:1). Cell suspensions in fresh fixative were dropped carefully from a pasteur pipette onto clean ice chilled slides and allowed to dry slowly at room temperature. Coded slides were then stained with 2% Giemsa (Sigma-Aldrich Corporation, St Louis, MI, USA) for 20 min at P^H 6.8, mounted on DPX (Qualigens, Mumbai, Maharashtra, India) and used for analysis.

Scoring of MN

Slides were scored at 1000 × magnification. Binucleated (BN) cells with well preserved cytoplasm were only taken into consideration. On average 4155 BN cells (range: 4000–5600 cells) were analyzed from these samples. Binucleated cells and micronuclei were identified according to the criteria of Fenech (2000, 2007) and Fenech et al. (2003). For each lymphocyte culture four slides were prepared and coded before staining. The coded slides were supplied to the scorers who were expert cytogeneticists. Each MN scored was verified by the scorers before recording in the work sheet. After the completion of the scoring of all the samples, the information regarding each sample was revealed to the scorer so as to compile the data with respect to dose, the mother's age and gender of the newborn.

Statistical analysis

All the calculations were done using the statistical software Statosoft. The relationship between MN frequency and radiation dose, MN frequency and maternal age were determined using least squares linear regression analysis. Odds ratio (OR) and their confidence intervals (CI) were calculated in order to see statistical significance in the mean MN frequency among the newborns from various dose groups as compared to control.

An odds ratio is the ratio of probabilities of getting an observation given two different scenarios, i.e., BN cells with MN and BN cells without MN. Odds Ratio and CI are calculated as follows: For that purpose, if the table is set as:

	BN cells with MN	BN cells without MN
Exposed or HLNRA	a	b
Unexposed or NLNRA	c	d

Then OR = (a/b)/(c/d). [Odds for cells with MN among exposed (HLNRA) divided by Odd for cells without MN among unexposed (NLNRA)]. The calculation for 95% CI is as follows:

The Assumption in the present study is that, the sample size is large and the events are rare, then the log(OR) follows normal distribution. Standard error of $\log(\mathrm{OR}) = \sqrt{1/a + 1/b + 1/c + 1/d}$. Therefore 95% CI for $\log(\mathrm{OR}) = \log(\mathrm{OR}) \pm 1.96 \times \sqrt{1/a + 1/b + 1/c + 1/d}$. The 95% CI are then obtained by exponentiation. 95% CI for $\mathrm{OR} = e^{\log(\mathrm{OR}) \pm 1.96 \times \sqrt{1/a+1/b+1/c+1/d}}$.

Since in the present investigation, we have more than one dose exposure categories in HLNRA, the unexposed (NLNRA) group is considered as reference category (OR = 1) and the other OR is calculated, relative to this (The OR of each group is with respect to $\leq$1.5 mGy/year). The procedure is same wherein the 'unexposed' or NLNRA category in Table I will remain the same and the 'exposed' or HLNRA group will change according to the different dose groups.

Results

In HLNRA the frequency of BN cells with MN was observed to be 1.17 ± 0.04 per 1000 BN cells, whereas it is 1.23 ± 0.07 per 1000 BN cells among the newborns from NLNRA (Table I). These values are not statistically significant ($p > 0.2$). The overall frequency of MN is determined to be 1.35 ± 0.04 per 1000 BN cells (range: 0–6.5 micronuclei per 1000 BN cells) in all the newborns studied. Inter-individual variability was clearly observed among these samples (Table II). All the samples were categorized into six different dose groups (NLNRA $\leq$1.5 mGy/year and five dose groups of HLNRA: 1.51–3.00 mGy, 3.01–6.0 mGy, 6.01–12.00 mGy, 12.01–18.00 mGy and 18.00–28.12 mGy per year) in order to find out the relationship between radiation dose and MN frequency (Table I). Odds ratio (OR) and 95% confidence intervals (CI) were calculated with respect to $\leq$1.5 mGy/year taking the mean frequency of MN in various dose groups. The OR values found in various dose groups in HLNRA were not statistically different as compared to

Table I. MN frequency per 1000 BN cells at different background radiation dose levels, Odds ratio (OR) and 95% confidence intervals (CI).

Area	Background radiation dose range in mGy/year (mean)	No. of newborns analyzed	No. of BN cells analyzed	Frequency of MN per 1000 BN cells ± SEM (obs. no. of MN)	Frequency of micronucleated cells/1000 BN cells ± SEM	Odds Ratio (OR)	95% CI	
							Lower	Upper
NLNRA	≤1.50 (1.21)	61	269420	1.40 ± 0.07 (376)	1.23 ± 0.07 (329)	1		
HLNRA	1.51–3.00 (2.20)	90	378529	1.33 ± 0.06 (504)	1.14 ± 0.05 (433)	0.93366	0.80612	1.08576
	3.01–6.00 (4.11)	69	274085	1.51 ± 0.07 (413)	1.34 ± 0.07 (368)	1.09746	0.94052	1.26888
	6.01–12.00 (7.86)	14	52650	0.91 ± 0.13 (48)	0.89 ± 0.13 (47)	0.72891	0.59245	1.03404
	12.01–18.00 (14.37)	16	60710	1.14 ± 0.14 (69)	1.07 ± 0.13 (65)	0.87633	0.59970	1.09378
	18.01–28.14 (23.90)	21	90746	1.20 ± 0.11 (109)	1.02 ± 0.11 (93)	0.83538	0.678438	1.085115
	Total 1.51–28.14 (6.30)	210	856720	1.33 ± 0.04 (1143)	1.17 ± 0.04 (1006)			
NLNRA + HLNRA	Grand total (5.16)	271	1126140	1.35 ± 0.04 (1519)	1.19 ± 0.04 (1335)			

NLNRA = Normal Level Natural Radiation Area; HLNRA = High Level Natural Radiation Area; BN = Binucleated cell; MN = Micronuclei; SEM = Standard Error of the Mean; OR = Odds Ratio; CI = Confidence Intervals. The Odds Ratio (OR) of each group is with respect to ≤1.5 mGy/year which is considered as reference category (OR = 1) and other OR is calculated relative to this.

NLNRA. To find out, if there were any correlation between the spontaneous MN frequency and different background radiation dose levels, the scatter plot of the frequency of MN per 1000 BN cells in newborns was also analyzed. Twenty two samples were not included in the scatter plot as the MN frequency observed was zero in those samples (Figure 1). The regression analysis between MN frequency and different background radiation dose levels showed a negative correlation ($Y = -0.0098X + 1.6143$, $R^2 = 0.0026$), though not highly significant.

As shown in the Figure 2, MN frequency did not show any increasing trend with respect to six different dose groups. A marginal increase in the frequency of MN at dose group 3.01–6.00 mGy/year was observed as compared to control (≤1.50 mGy/year) and 1.51–3.00 mGy/year. Subsequently a clear-cut decline in the frequency of MN was observed in the dose group of 6.01–12.00 mGy/year and thereafter a marginal increase in the MN frequency in the later two dose groups was observed.

There is a maternal age dependant increase, though not significant, in the frequency of MN among HLNRA and NLNRA newborns except the age group >30 years. However, sample size analysed from this age group is comparatively small (Figure 3). Overall, the frequency of MN in female newborns was 1.39 ± 0.05 per 1000 BN cells whereas among males it was 1.31 ± 0.05 per 1000 BN cells (Table II). We have analyzed the frequency of MN in male and female newborns in various maternal age groups (Figure 4). An increasing trend in the frequency of newborns in females was observed as compared to males in both the areas, though not statistically significant ($p > 0.05$). However, there is decline in MN frequency in the age group ≥30 years.

Discussion

Several studies have been undertaken using CBMN assay as a means of assessing human exposure to radiation in lymphocytes (Fenech and Morley 1986, 1990, Kormos and Koteles 1988, Prosser et al. 1988, Littlefield et al. 1989, Erexion et al. 1991, Ban et al. 1993). There is a wide variation in the frequency of MN in adults ranging from 2–36 per 1000 BN cell (Fenech and Morley 1985, Ramalho et al. 1988, Huber et al. 1989, 1992, Eastmond and Tucker 1989). As compared to adults, studies on newborns using cord blood lymphocytes and children are limited (Aghamohammadi et al. 1984, Krishneja and Sharma 1991, Stankovic et al. 2004, Bockay et al. 2005, Levario-Carrillo et al. 2005, Neri et al. 2005, 2006, Milosevic-Djordjevic et al. 2007). There are some combined studies on newborns and children (Barala et al. 1998, Fellay-Reynier et al.

Table II. Distribution of MN frequency among male and female newborns in HLNRA and NLNRA.

Area	Sex	Mean background radiation dose (mGy/year)	No. of newborns analyzed	No. of BN cells analyzed	Frequency of MN per 1000 BN cells ± SEM	Frequency of Micronucleated cells per 1000 BN cells ± SEM
NLNRA	M	1.22	29	127900	1.42 ± 0.11 (182)	1.18 ± 0.10 (151)
	F	1.21	32	141520	1.37 ± 0.10 (194)	1.26 ± 0.10 (178)
HLNRA	M	6.18	109	442650	1.28 ± 0.05 (567)	1.11 ± 0.05 (491)
	F	6.44	101	414070	1.39 ± 0.06 (576)	1.24 ± 0.05 (515)
NLNRA+ HLNRA	M	5.13	138	570550	1.31 ± 0.05 (749)	1.13 ± 0.04 (642)
	F	5.18	133	555590	1.39 ± 0.05 (770)	1.25 ± 0.05 (693)
	Total M+F	5.16	271	1126140	1.35 ± 0.04 (1519)	1.19 ± 0.04 (1335)

NLNRA = Normal Level Natural Radiation Area; HLNRA = High Level Natural Radiation Area, BN = Binucleated cell; MN = Micronuclei, SEM = Standard Error of the Mean, M = Male, F = Female. Figures in parenthesis denote the observed numbers of micronuclei.

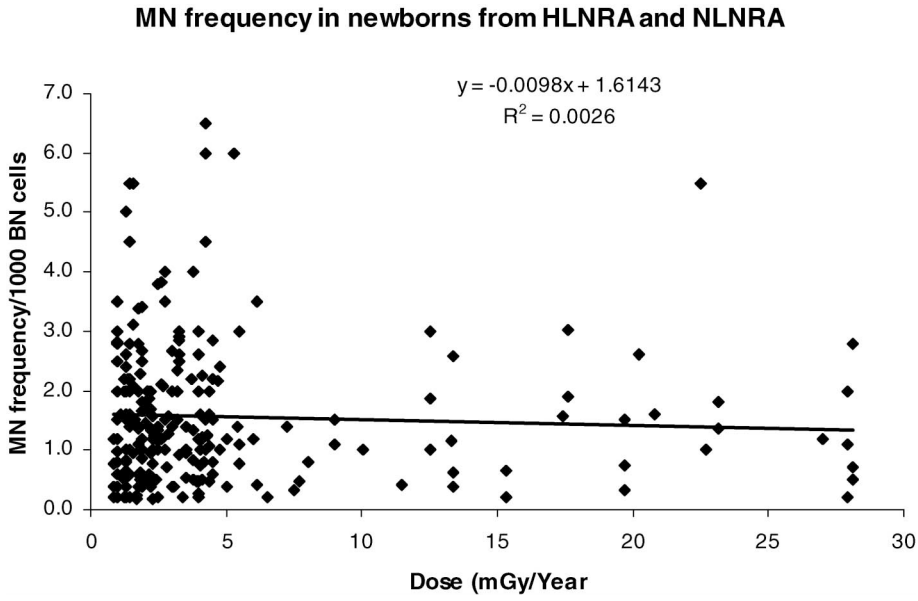

Figure 1. Distribution of MN frequency/1000 BN cells with respect to background radiation dose (mGy/year). Each point represents the frequency of micronuclei in each individual. The samples with zero MN have been omitted for clarity. The line drawn is the trendline.

2000, Shi et al. 2000, Maluf and Erdtmann 2001). There is also a wide variation of MN frequency in newborns. Prior to the development of CBMN assay (Fenech and Morley 1985), Aghamohammadi et al. (1984) analyzed 28 cord blood samples using 5-bromodeoxyuridine (BUdR) incorporation method and reported the frequency of MN to be ranged between 0.5 and 9.5 per 1000 cells with a modal value of 2.5. Krishneja and Sharma (1991) analyzed six cord blood samples using CBMN assay and reported the frequency of MN ranged from 0–1.5 per 1000 BN cell in 48 h culture. The MN frequency was found to be 3.74 ± 1.60 per 1000 BN cells in Serbia among 15 cord blood samples (Stankovic et al. 2004). Levario-Carrillo et al. (2005) have reported that the base line frequency of MN in newborns ranged from 1.00 ± 0.9 per 1000 BN cells in urban areas and 2.00 ± 1.5 per 1000 BN cells in agricultural area. Similarly, Milosevic-Djordjevic et al. (2005) have reported MN frequency in newborns to be 5.53 ± 3.02 (range: 1–13). A meta-analysis of 13 selected studies (age range 0–19 years), which included three studies on newborns have estimated the frequency of MN to be 1.70–9.92 per 1000 BN cells (Neri et al. in 2005). Our present study reports an overall MN frequency among 271 newborns to be 1.35 ± 0.04 per 1000 BN cells (range 0–6.5 per 1000 BN cells) as shown in Table II.

Numerous studies have demonstrated a relationship between the increase of micronuclei frequency in cord blood lymphocytes of newborns due to the exposure of environmental genotoxic agents/pollutants (Bockay et al. 2005, Neri et al. 2005, 2006, Milosevic-Djordjevic et al. 2005, 2007). The study using micronuclei in cord blood lymphocytes as

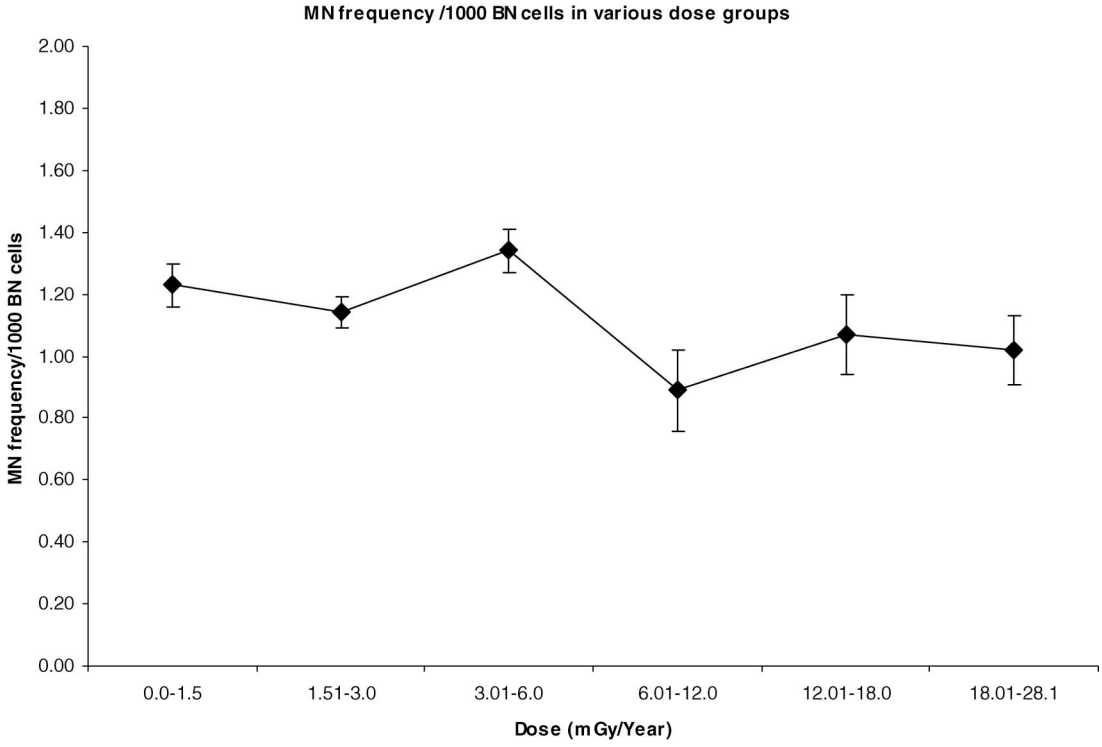

Figure 2. The line graph represents the frequency of micronuclei/1000 BN cells in six different background dose groups (0.0–1.5, 1.51–3.0, 3.01–6.0, 6.01–12.0, 12.01–18.0 and 18.01–28.1 mGy/year). Each point represents the mean frequency of micronuclei per 1000 BN cells for that particular dose group. For each point error bars indicate the standard error of the mean (SEM). N is the number of individuals studied in each dose group which is given in Table I.

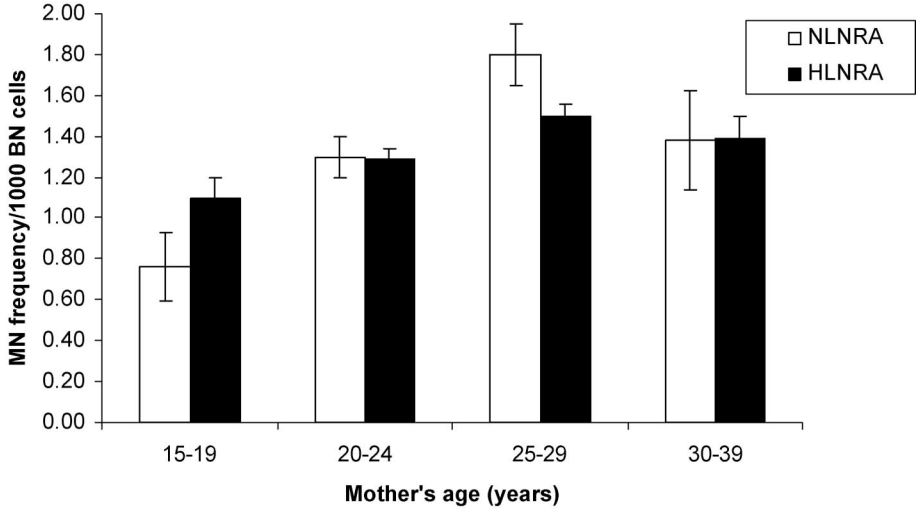

Figure 3. Histogram showing the frequency of MN/1000 BN cells in newborns from HLNRA and NLNRA with respect to four different mothers' age groups (15–19, 20–24, 25–29 and 30–39 years). The clear bar represents the mean frequency of MN/1000 BN cells in NLNRA and the solid bar represents the mean frequency of micronuclei per 1000 BN cells in HLNRA. The error bars indicate the standard error of the mean (SEM).

biomarker of transplacental exposure to environmental pollutants revealed that the frequency of MN in cord blood is increased from 5.53 ± 3.02 per 1000 BN cells to 9.36 ± 5.60 per 1000 BN cells (Milosevic-Djordjevic et al. 2005) and subsequently decreased to 4.73 ± 3.38 per 1000 BN cells after few years.

Many confounding factors such as smoking and gender have found to have increased the frequency of MN in fetal cord blood samples (Di Giorgio et al. 1994, Zalacain et al. 2006). On the contrary, there are also reports, which did not show any correlation between MN frequency and smoking (Bonassi et al.

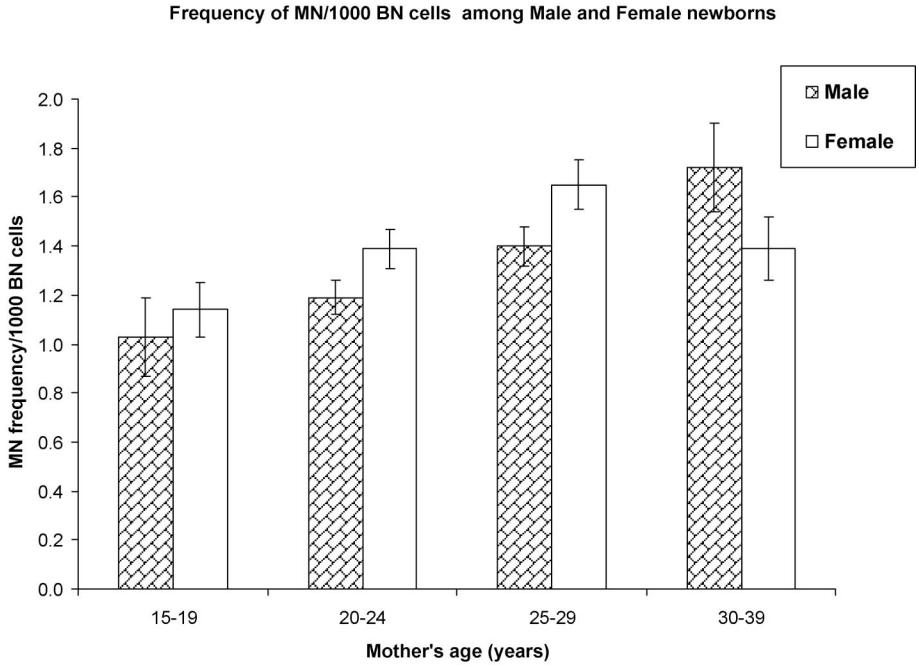

Figure 4. Histogram showing the frequency of MN/1000 BN cells in male and female newborns with respect to four different mothers' age groups (15–19, 20–24, 25–29 and 30–39 years). The shaded bars represent the mean frequency of MN/1000 BN cells in male newborns and the clear bars represent the frequency of MN per 1000 BN cells in female newborns. The error bars indicate the standard error of the mean (SEM).

2003). The effect of smoking on newborns was not highlighted in our study as the mothers of the studied newborns were non-smokers. Although the effect of gender on micronuclei frequency has been reported very often in adult populations, no such clear-cut relationship between gender and micronuclei frequency have been established in children and newborns (Aghamohammadi et al. 1984, Neri et al. 2005, Zalacain et al. 2006). Our results are also in agreement with the above observations as shown in Table II. In our study, the overall frequency of MN among male newborns did not differ significantly from females in both HLNRA and control. The MN frequency observed in females did not show any statistically significant difference as compared to males with respect to different maternal age groups, except the older age group ($\leq$30 years). However, a comparatively smaller sample size analyzed from this group could not be ruled out. The small sample size in this maternal age group is because in this study area (Kerala) a few deliveries are found at this particular age group. Similar findings are also reported by Zalacain et al. (2006), where no significant increase in MN frequency of newborns born from mothers older than 35 years was observed. We have observed inter-individual variations among the newborns studied from HLNRA and NLNRA, which is similar to the report by Neri et al. (2005).

Utero exposure to environmental pollutants can result in chromosomal aberrations, increased micronuclei frequency, carcinogenic DNA adducts and increased risk of childhood cancer. Many studies have reported genetic damage and exposure to pollutants, which has increased the frequency of micronuclei in newborns. Thus it is very important to study the effect of radiation if any, in newborns in HLNRA. The spontaneous frequency of MN among the adults has been reported from HLNRA of Ramsar, Iran (Mohammadi et al. 2006). So far, there are no reports available on the effect of radiation and the frequency of micronuclei in cord blood samples/newborns. To our knowledge, this is the first attempt to estimate the spontaneous frequency of MN among the newborns using cord blood samples from a natural high background radiation area.

It is also interesting that we did not observe significant difference in the baseline frequency of micronuclei in both the areas as shown in Table I. No dose response in the mean frequency of MN was observed. As compared to control ($\geq$1.50 mGy/year), there is a sharp decrease of MN frequency in the dose group 6.01–12.0 mGy/year and thereafter a marginal increase in subsequent dose groups (12.01–18.0 and 18.01–28.0 mGy/year) as shown in Figure 2. The decreased MN frequency at dose group of 6.01–12.00 mGy/year could be an indication of the phenomenon of hormesis. However, we require sufficient data to draw firm conclusions on this aspect.

Our results obtained from this study are also in agreement with the chromosomal aberration data on newborns from this area (Cheriyan et al. 1999, Thampi et al. 2005), although the mitochondrial

mutation rate study of Foster et al. (2002) reported increased germline point mutations between mothers and their offspring in HLNRA ($p < 0.01$) as compared to adjoining control areas. However, their study design had certain limitations. Considering the high mutation rate of mitochondrial DNA (mtDNA), the confounding factors such as chewing (which is more prevalent in the study area) and most importantly, radiation dosimetry was not appropriately addressed in their study. Mohammadi et al. (2006) on adults did not reveal any significant difference between MN frequency and radiation dose in Ramsar, Iran. Our data on newborns is in agreement with their data. However, there are also some studies on Ramsar, Iran, which have reported that residents of high background radiation areas of Ramsar have higher incidences of stable and unstable chromosomal aberrations as compared to the control group (Ghiassi-Nejad et al. 2004, Hayata et al. 2004).

From the data obtained in the present study, we conclude here that the newborns from high level natural radiation area have no significantly increased MN frequency as compared to the newborns from adjacent control area. The lower levels of micronuclei frequency in newborns of HLNRA of Kerala coast could be an indicative of adaptive response. Detailed research on this aspect would be worth pursuing in this area to understand the basic mechanism of DNA repair and adaptive response.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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