Study of stillbirth and major congenital anomaly among newborns in the high-level natural radiation areas of Kerala, India

G. Jaikrishan · K. R. Sudheer · V. J. Andrews ·
P. K. M. Koya · M. Madhusoodhanan ·
C. K. Jagadeesan · M. Seshadri

Received: 24 February 2012 / Accepted: 25 July 2012 / Published online: 9 August 2012
© Springer-Verlag 2012

Abstract Monitoring newborns for adverse outcomes like stillbirth and major congenital anomalies (MCA) is being carried out in government hospitals since 1995 in and around high-level natural radiation areas, a narrow strip of land on the southwest coast of Kerala, India. Natural deposits of monazite sand containing thorium and its daughter products account for elevated levels of natural radiation. Among 141,540 newborns [140,558 deliveries: 139,589 singleton, 957 twins (6.81 ‰), 11 triplets (0.078 ‰), and one quadruplet] screened, 615 (4.35 ‰) were stillbirth and MCA were seen in 1,370 (9.68 ‰) newborns. Clubfoot (404, 2.85 ‰) was the most frequent MCA followed by hypospadias (152, 2.10 ‰ among male newborns), congenital heart disease (168, 1.19 ‰), cleft lip/palate (149, 1.05 ‰), Down syndrome (104, 0.73 ‰), and neural tube defects (72, 0.51 ‰). Newborns with MCA among stillbirths were about 20-fold higher at 190.24 ‰ (117/615) compared to 8.89 ‰ (1,253/140,925) among live births ($P<.001$). Logistic regression was carried

out to compare stillbirth, overall, and specific MCA among newborns from areas with dose levels of ≤ 1.5 , 1.51–3.0, 3.01–6.0 and >6 mGy/year after controlling for maternal age at birth, gravida, consanguinity, ethnicity, and gender of the baby. Clubfoot showed higher prevalence of 3.26 ‰ at dose level of 1.51–3.0 mGy/year compared to 2.33 ‰ at ≤ 1.5 mGy/year (OR=1.39; 95 % CI, 1.12–1.72), without indication of any clear dose–response. Prevalences of stillbirth, overall MCA, and other specific MCA were similar across different dose levels and were relatively lower than that reported elsewhere in India, probably due to better literacy, health awareness, and practices in the study population.

Keywords High-level natural radiation · Stillbirth · Major congenital anomaly · Clubfoot · Hypospadias · Cleft lip/palate · Down syndrome

Introduction

The estimation of risk in humans exposed to low-dose radiation is based on extrapolation from well-documented effects (UNSCEAR 1993) observed at high doses, largely delivered at high dose rates in laboratory models or during accidental exposures. Direct studies on humans exposed to low dose and dose rates is of great significance (WHO 1959) for assessment of risk to occupational exposure and radiation protection practices. There are several high background radiation areas in the world (UNSCEAR 2000) and among them is an important one, which is located in the Kerala state on the southwest coast of India. It is a stretch of coastal land extending from Neendakara Panchayat of Kollam district in the south to

G. Jaikrishan (✉) · K. R. Sudheer · V. J. Andrews ·
P. K. M. Koya · M. Seshadri
Low Level Radiation Research Laboratory, Radiation Biology and
Health Sciences Division, Bhabha Atomic Research Center,
Beach Road,
Kollam, Kerala 691 001, India
e-mail: anjali96@yahoo.com

M. Madhusoodhanan
Department of Paediatrics, Victoria Hospital,
Kollam 691 001, India

C. K. Jagadeesan
Directorate of Health Services, Government of Kerala,
Thiruvananthapuram 695 011, India

Purakkad in Alappuzha district in the north. About 55 km in length and 0.5 to 1.5 km width, the background radiation dose in the region ranges from <1 to 45 mGy/year and is due to the natural deposit of monazite sand containing radioactive thorium (8–10 %), uranium (0.30 %), and their decay products (Bharatwal and Vaze 1959; Sunta 1993). With a wide range of doses, high density of population, and stable non-migratory community, the area provides unique opportunity to investigate health effects of low-level chronic radiation exposure directly on human population. Normal and high-level natural radiation areas are interspersed due to non-uniform distribution of monazite sand and the former provide an inbuilt control population. Radiation may induce germline mutations that may cause or abet congenital anomalies. Therefore, newborns from high and normal-level radiation areas are being monitored for the past three decades to study the association of elevated levels of natural radiation with congenital anomalies detectable at birth (Jaikrishan et al. 1999; Cheriyan et al. 1999; Thampi et al. 2002, 2005). Further analysis is attempted to assess adverse pregnancy outcomes of stillbirth and overall as well as specific major congenital anomalies (MCA) like clubfoot, heart disease, Down syndrome, cleft lip/palate, neural tube defects, and hypospadias after controlling for the effects of confounding/competing factors like maternal age at birth, gravida status, ethnicity, consanguinity, and gender of the newborn.

Materials and methods

This study was carried out in selected government hospitals under the Directorate of Health and Family Welfare, Government of Kerala (Victoria Hospital, Kollam, Taluk Headquarters Hospital, Karunagapally, Government Hospital, Kayamkulam and Community Health Centre, Chavara) under a memorandum of understanding between Department of Atomic Energy, Government of India and Ministry of Health and Family Welfare, Government of Kerala. These hospitals cater to the needs of people from both high-level natural radiation area (HLNRA) and from normal-level natural radiation area (NLNRA). All women admitted for delivery with a gestational age of more than 28 weeks were included in the study and the newborn infant (alive or dead) was the primary study subject. Intrauterine death (fetal death before the onset of labor), stillbirth (death during the process of delivery), and death immediately after live birth without any physical stress were all considered as stillbirth in this study. Congenital anomaly was identified primarily by clinical examination of the newborns by pediatricians of the hospitals and was further verified by trained medical doctors of Low-Level Radiation Research Laboratory (LLRRL), Kollam who made regular visits to the participating hospitals. Congenital anomalies with serious structural, functional, or cosmetic disability requiring

surgical or medical management (Singh 1985) like congenital heart disease, hypospadias (a urogenital anomaly in the male in which the external urethral opening is either on the ventral aspect of the penis or in the perineum), clubfoot (an anomaly of the foot at the subtalar joint and having three components—plantar flexion, inversion, and adduction), cleft lip/palate (a craniofacial anomaly caused by non-fusion defects during the development of the face), Down syndrome (most common chromosomal anomaly seen among newborns with characteristic facies, muscle hypotonia, and intellectual impairment varying from mild to moderate), neural tube defects (improper development of the neural tube which is the progenitor of the brain and spinal cord, e.g., anencephaly, meningocele, meningomyelocele, encephalocele, and spina bifida), etc. were considered as MCA. Specific MCA in each newborn (other than those considered as part of a particular syndrome) was counted separately and an exhaustive list of MCA observed in the study is given as [Appendix](#). Data related to the socioeconomic profile, pregnancy history, life style, occupation, place of stay of the parents, etc. were collected from the family by trained staff nurses of the hospital and were recorded in a pre-coded proforma. Relevant details of the current pregnancy outcome were entered by the obstetrician/pediatrician. The completeness and consistency of information collected in the pre-coded proforma were ascertained during the stay of the mother and the newborn in the hospital by paramedical personnel of LLRRL. The entries were further verified during inclusion in the computer database using programs with range checks and were validated for internal consistency.

Dosimetry

The dosimetric profiling of the study area was carried out by dividing the area into small grids of 100 m². Ambient radiation dose level of each grid was estimated by taking the mean of several survey meter (halogen-quenched Geiger Muller tube-based survey meter consisting of the tube and microprocessor-based digital display) readings taken across the length and breadth of the grid at 1 m height from the ground. The dose levels so derived were assigned to all newborns with parental residence in the grid. The average radiation dose in control areas of Kollam district is 1.2 mGy/year with a range of <1.0 to 1.5 mGy/year. Hence, areas with a radiation exposure above 1.5 mGy/year were considered as HLNRA and those below 1.5 mGy/year, as NLNRA (Jaikrishan et al. 1999). This approximates to an equivalent dose of 1 mSv/year, which is in conformity with the external dose component of the world average dose of 2.4 mSv/year (UNSCEAR 2000).

Statistical methods

Comparison of the proportions of newborns with stillbirth/MCA across various subgroups was carried out using chi-

square test with continuity correction in two-by-two tables. MCA with at least 60 newborns were chosen for separate analyses so that doubling of prevalence in HLNRA relative to NLNRA could be detected with 80 % power and 5 % level of significance, assuming the ratio of the number of newborns from HLNRA to that in NLNRA to be 60:40 (Breslow and Day 1987). Logistic regression was employed for comparison of adverse birth outcomes like stillbirth, overall, and some most frequent MCA across dose groups after controlling for the effects of possible confounding/competing factors like maternal age at birth, gravida status, ethnicity, consanguinity, and gender of the newborn. Dose levels in milligray per year were categorized as ≤ 1.5 , 1.51–3.0, 3.01–6.0 and >6 with ≤ 1.5 as the reference category. Maternal age at birth in years was also categorized as 15–19, 20–21, 22–23 (reference), 24–25, 25–27, 28–29 and ≥ 30 . Categorization of the variables was done considering lower sample size at higher values of both dose levels and maternal age at birth. Logistic regression was also employed for modeling the relationship between the outcomes and maternal age at birth using STATISTICA software (StatSoft Inc. 2010). Statistical significance of the odds ratio (OR) obtained from logistic regression was judged by 95 % confidence interval (CI). OR is statistically significant at 5 % level if the 95 % CI does not include 1 (Khan and Sempos 1989), the value of OR under the null hypothesis of no difference between the HLNRA and NLNRA.

The study involved no intervention on human subjects and the study protocol was approved by the Institutional Ethical Committee. Blood samples from newborns with suspected chromosomal anomaly were collected after obtaining informed consents from the parents.

Results

During the study period from August 1995 to June 2011, a total of 141,540 newborns from 140,558 deliveries—139,589 singleton, 957 twins (6.81 %), 111 triplets (0.078 %) and one quadruplet—were monitored. MCA were detected in 1,370 newborns with a prevalence of 9.68 ‰. Frequency of stillbirth observed in the study was 4.35 ‰ (615 newborns) and MCA among stillbirths were about 20-fold higher at 190.24 ‰ (117 newborns) compared to 8.89 ‰ (1,253 newborns) among 140,925 live births ($P < .001$). The most frequent specific MCA was clubfoot (2.85 ‰) followed by heart disease (1.19 ‰), cleft lip/palate (1.05 ‰), Down syndrome (0.73 ‰) and neural tube defect (0.51 ‰). Hypospadias was another frequent MCA observed among 152 male newborns with a frequency of 2.1 ‰. The frequencies of other specific MCA observed in the study in the decreasing order of magnitude were microtia (33, 0.23 ‰), imperforate anus

(27, 0.19 ‰), hydrocephalus (26, 0.18 ‰), anencephaly (23, 0.16 ‰), micro penis (22, 0.16 ‰), dislocation of hip (21, 0.15 ‰), intersex (21, 0.15 ‰), Pierre Robin syndrome (20, 0.14 ‰), microcephalus (19, 0.13 ‰), spina bifida (19, 0.13 ‰), meningomyelocele (18, 0.13 ‰), achondroplasia (16, 0.11 ‰), diaphragmatic hernia (14, 0.10 ‰), meningocele (11, 0.08 ‰), polycystic kidney (9, 0.06 ‰), hydronephrosis (9, 0.06 ‰), fetal ascitis (6, 0.04 ‰), phocomelia (6, 0.04 ‰), albinism (5, 0.04 ‰), and digital aplasia (5, 0.04 ‰).

The variation in the frequency of clubfoot across the radiation dose groups of ≤ 1.5 , 1.51 to 3.0, 3.01 to 6.0 and >6 mGy/year was statistically significant ($P = .020$) without indication of any clear dose–response. Highest frequency of 3.26 ‰ was observed at the dose level of 1.51–3.0 and lowest frequency of 2.33 ‰ at ≤ 1.5 mGy/year. Similarly, there was statistically significant variation in the frequency of overall MCA at different dose levels ($P = .046$) with 7.16 ‰ at 3.01–6.0 mGy/year and 11.28 ‰ at >6 mGy/year. The differences in the prevalence of stillbirth, heart disease, Down syndrome, cleft lip/palate, neural tube defects, or hypospadias at different dose levels at parental residence were not statistically significant (Table 1). Clubfoot was more common among newborns of HLNRA compared to NLNRA (3.19 ‰ vs. 2.33 ‰; $P = .004$) but all other adverse birth outcomes including overall MCA were similar between HLNRA and NLNRA.

About 85 % of the deliveries were in the maternal age group of 20–29 years. As shown in Table 1, stillbirth (6.72 ‰) and overall MCA (13.81 ‰) were highest among mothers in the age group of 30 years or more. Maternal age at birth had a clear influence on stillbirth, Down syndrome, and overall MCA other than Down syndrome. The relationship was modeled using logistic regression with both linear and quadratic terms of maternal age at birth in the model (Fig. 1). The quadratic term was statistically significant for stillbirth and Down syndrome.

More than 90 % of mothers were either of primi or second gravida and both stillbirth and MCA were significantly higher for the gravida status of four or more. Clubfoot, congenital heart disease, cleft lip/palate, neural tube defect, and hypospadias were not associated with gravida status. The prevalence of Down syndrome was not similar among the four gravida groups and the highest frequency (1.42 ‰) was observed at gravida status of three (Table 1).

Frequency of stillbirth was not similar among newborns of different ethnic group ($P = .029$), the lowest being among Arayas (3.48 ‰) and highest among other Hindu ethnic group (5.55 ‰). Though the frequency of overall MCA ranged from 9.08 ‰ among Nairs to 12.19 ‰ among Arayas, the variation was well within the limits of sampling fluctuation. Similarly, the variation observed in the frequencies of Clubfoot, heart diseases, Down syndrome, cleft lip/

Table 1 Adverse pregnancy outcome (per 1,000) among newborns according to radiation levels at the area of parental residence, maternal, and newborn characteristics

Characteristic	N	%	SB (615)	Major congenital anomaly						
				Overall (1,370)	CF (404)	HD (168)	DS (104)	CLP (149)	NTD (72)	HS (152)
Radiation dose (mGy/year)										
≤1.5	55,250	39.0	4.05	9.48*	2.33*	1.38	0.71	1.00	0.51	2.48
1.51–3.0	73,074	51.6	4.71	10.02	3.26	1.08	0.77	1.08	0.55	1.90
3.01–6.0	8,518	6.0	3.40	7.16	2.58	0.70	0.70	0.70	0.23	1.37
> 6.0	4,698	3.3	3.83	11.28	3.19	1.49	0.64	1.92	0.43	2.04
Maternal age at birth in years										
15–19	8,833	6.2	4.98***	7.59***	2.83	1.36	0.34***	0.91	0.11	1.53
20–21	21,805	15.4	3.99	9.36	3.26	0.73	0.37	0.87	0.46	2.24
22–23	31,745	22.4	3.28	9.45	2.80	1.20	0.50	0.98	0.69	2.58
24–25	30,469	21.5	4.43	8.76	2.59	0.98	0.39	1.15	0.33	2.00
26–27	21,588	15.3	4.49	9.82	2.78	1.39	0.56	1.20	0.60	1.63
28–29	13,707	9.7	4.23	9.85	2.92	1.46	0.95	1.09	0.73	1.43
≥ 30	13,393	9.5	6.72	13.81	2.99	1.64	2.99	1.12	0.45	2.79
Gravida status										
1	64,596	45.6	4.03***	9.26**	3.19	1.04	0.45***	0.88	0.46	1.96
2	64,845	45.8	3.96	9.84	2.54	1.31	0.91	1.25	0.54	2.09
3	10,568	7.5	6.91	10.12	2.46	1.14	1.42	0.85	0.57	2.77
≥ 4	1,531	1.1	16.33	17.64	4.57	2.61	0.65	1.31	0.65	3.72
Ethnicity/religion										
Araya	2,297	1.6	3.48*	12.19	4.35	1.74	0.44	1.74	0.00*	0.83*
Ezhava	33,912	24.0	4.01	9.32	2.62	0.88	0.86	0.91	0.29	2.35
Nair	20,486	14.5	3.71	9.08	2.83	1.46	0.73	0.98	0.54	1.83
Viswakarma	8,260	5.8	3.75	9.20	2.42	0.85	1.09	0.73	0.24	1.67
Other Hindu	29,715	21.0	5.55	9.62	3.10	1.01	0.57	1.21	0.67	1.58
Christian	17,533	12.4	4.11	9.70	2.45	1.65	0.74	1.48	0.91	1.34
Muslim	29,337	20.7	4.33	10.50	3.14	1.30	0.68	0.89	0.44	3.18
Consanguineous marriage										
No	138,509	97.9	4.25***	9.54***	2.79**	1.17	0.73	1.05	0.48**	2.10
Yes	3,031	2.1	8.91	16.17	5.61	1.98	0.99	0.99	1.98	1.91
Number of birth ^a										
Singleton	139,589	98.6	4.00***	9.62	2.85	1.17	0.75	1.07	0.49	2.06
Twins	1,914	1.4	27.69	14.11	3.13	2.09	0.00	0.00	1.57	5.52
Gender of the newborn ^b										
Male	72,373	51.1	4.63	11.11***	3.43***	1.15	0.59	1.06	0.47	2.10
Female	69,146	48.9	3.99	7.88	2.24	1.23	0.88	1.04	0.55	—
Total	141,540	100.0	4.35	9.68	2.85	1.19	0.73	1.05	0.51	2.10

Figures in parentheses are the number of newborns with the adverse birth outcome

N number of newborns, SB stillbirth, include born dead and born alive but died immediately without any physical stress, CF clubfoot, HD heart disease, DS Down syndrome, CLP cleft lip/palate, NTD neural tube defects, HS hypospadias (frequency among male newborns)

^a Thirty-three newborns of 11 triplets and four newborns of a quadruplet delivery were not included

^b Twenty-one newborns with intersex were not included

*** $P < .001$; ** $P < .01$; * $P < .05$, testing the null hypothesis that the prevalence of the adverse birth outcome is equal across the subgroups using chi-square test

palate, and hypospadias across the ethnic groups was not large enough to be statistically significant (Table 1). However,

the variation in neural tube defects among the different ethnic groups was marginally significant ($P = 0.045$) with no neural

Fig. 1 Relationship of maternal age at birth with stillbirth, Down syndrome, and major congenital anomalies other than Down syndrome. Logistic regression was used to model the relationship with age as continuous variable. Coefficient of the quadratic term was statistically significant for both stillbirth and Down syndrome but not in major congenital anomalies other than Down syndrome

tube defects being recorded among Arayas and 0.91 % neural tube defects among Christians.

The analysis depicted in Table 1 also revealed that consanguinity was associated with higher frequency of stillbirth (8.91 % vs. 4.25 %; $P<.001$), overall MCA (16.17 % vs. 9.54 %; $P<.001$), clubfoot (5.61 % vs. 2.79 %; $P=.007$), and neural tube defects (1.98 % vs. 0.48 %; $P=.0013$). Overall frequency of MCA was higher among male newborns (11.11 %) compared to that among females (7.88 %, $P<.001$). Clubfoot was also higher among males than females (3.43 % vs. 2.24 %; $P<.001$). Newborn twins were more prone to stillbirth than singletons (27.69 % vs. 4 %, $P<.001$) but the difference in the prevalence of overall MCA between twins and singletons was not statistically significant (14.11 % vs. 9.62 %, $P=.061$).

None of the adverse pregnancy outcomes considered here was associated with paternal habits of smoking, chewing, or alcohol consumption. When MCA were grouped according to the anatomical system(s) involved in the anomaly, the number of newborns identified with MCA of different systems was: musculoskeletal, 479 (3.38 %); genitourinary, 226 (1.60 %); gastrointestinal, 227 (1.60 %); cardiovascular, 168 (1.19 %); central nervous, 114 (0.81 %); ear, 39 (0.28 %); eye, 24 (0.17 %); respiratory, 18 (0.13 %); and other systems, 20 (0.14 %). Except for the musculoskeletal system MCA, no system-wise anomalies showed any statistically significant variation across different dose levels prevailing at the area of parental residence (analysis not shown).

Multiple logistic regression analyses were carried out to assess the association of stillbirth, overall MCA, clubfoot, heart disease, cleft lip/palate, Down syndrome, neural tube defects, and hypospadias with radiation levels, after adjusting for the possible effects of maternal age at birth, gravida, gender, consanguinity, and ethnicity (Table 2). Frequency of stillbirth was not associated with radiation dose levels, the ORs (95 % CI) being 1.16 (0.98–1.37), 0.84 (0.57–1.24), and 0.87 (0.53–1.41) in the dose groups of 1.5–3, 3–6 and >6 mGy/year, respectively, compared to the reference category of ≤ 1.5 mGy/year; nor was there any dose-related trend in the prevalence of stillbirth ($P>.20$). Similarly, no MCA considered here except clubfoot was associated with background radiation dose levels. The highest prevalence of clubfoot was observed at the dose level of 1.51–3.0 mGy/year (3.26 %) followed by 3.19 % at >6.0 mGy/year, 2.58 % at 3.01–6.0 mGy/year and 2.33 % at ≤ 1.5 mGy/year, the reference category without indication of any dose–response. The frequency of clubfoot observed at 1.51–3.0 mGy/year alone was higher compared to the reference category (OR=1.39, 95 % CI, 1.12–1.72) and it was similar at other dose levels.

Discussion

There is global interest in the biological and health effects of chronic low-dose radiation exposure. The current study,

Table 2 Logistic regression analyses of stillbirth, overall major congenital anomaly, clubfoot, congenital heart disease, Down syndrome, cleft lip/palate, neural tube defects, and hypospadias among newborns on the prevailing dose levels at parental residence

Adverse pregnancy outcome at birth	Dose levels at parental residence in mGy per year			<i>P</i> for trend
	1.51–3.0 (<i>n</i> =73,061) OR ^a ; 95 % CI	3.01–6.0 (<i>n</i> =8,518) OR ^a ; 95 % CI	>6.0 (<i>n</i> =4,698) OR ^a ; 95 % CI	
Stillbirth ^b	1.16; 0.98–1.37	0.84; 0.57–1.24	0.87; 0.53–1.41	>.20
Overall MCA ^c	1.06; 0.94–1.18	0.78; 0.59–1.01	1.25; 0.94–1.67	>.20
Clubfoot	1.39 ^d ; 1.12–1.72	1.12; 0.71–1.76	1.36; 0.79–2.34	.042
Heart disease	0.78; 0.57–1.07	0.53; 0.23–1.22	1.13; 0.52–2.49	>.20
Down syndrome	1.09; 0.72–1.64	1.05; 0.44–2.48	1.14; 0.35–3.76	>.20
Cleft lip/palate	1.08; 0.77–1.53	0.72; 0.31–1.67	1.70; 0.83–3.49	>.20
Neural tube defect	1.07; 0.66–1.73	0.48; 0.11–2.03	0.69; 0.16–2.92	>.20
Hypospadias	0.78; 0.56–1.08	0.54; 0.23–1.25	1.02; 0.40–2.56	0.165

^a ≤1.5 mGy/year (*n*=55,242) was the reference category. OR adjusted for maternal age at birth (15–19, 20–21, 22–23, 24–25, 25–27, 28–29 and ≥30), gravida (1, 2, 3, and ≥4), ethnicity (Ezhava, Nair, Viswakarma, and other Hindu ethnic group including Araya, Christian, and Muslim), consanguinity (yes/no) and gender (male/female) of the newborn

^b Stillbirth includes born dead and born alive but died immediately without any physical stress

^c Major congenital anomaly

^d Statistically significant at 5 % level. Twenty-one cases of intersex not included in the analyses. Analysis of hypospadias carried out only among male newborns

carried out in government hospitals in the coastal area offering secondary level health care, covered about 142,000 newborns and provides interesting information in terms of adverse pregnancy outcomes like stillbirth and MCA. Those attending government hospitals mostly belonged to the lower socioeconomic groups from both HLNRA and NLNRA. The observed prevalences of stillbirth and MCA were relatively lower in the study population probably due to the features like lower maternal age at birth (more than 90 % of the deliveries occur before the age of 30 years), small family size, higher literacy, and consequent health awareness and practices, despite a lower socioeconomic status.

Assessment of dose due to radiation exposure is very important to address the role of chronic low-level radiation in inflicting DNA damage or other indirect effects in the etiology of MCA. Average dose in the area of parental residence due to external gamma radiation was used in the study. As far as exposure to radiation is concerned, most critical and sensitive stages during fetal development are conception, subsequent cell proliferation, and organogenesis spanning first two trimesters of pregnancy (Coggle 1983; Vogel and Motulsky 1997). The study undertaken to assess the overall prevalence pattern of stillbirth and MCA with respect to the prevailing radiation levels at the area of parental residence did not reveal any association with stillbirth, overall MCA or specific anomalies like heart disease, cleft lip/

palate, hypospadias, Down syndrome, or neural tube defect. An exception was statistically significantly higher prevalence of clubfoot in the dose group of 1.51–3.0 mGy/year compared the dose group of 1.5 mGy/year or less, but no clear dose–response was observed.

The frequency of stillbirth in developed countries is around 4 per 1,000 while in less developed countries it is 24 per 1,000 and in the least developed countries it is 31 per 1,000 (Ahman and Zupan 2007). The frequency of about four stillbirths among 1,000 newborns in this coastal community is comparable to that in developed countries. More than 90 % of the mothers were either primi or second gravida and those with higher gravida status were predominantly with previous history of abortion or stillbirth—about 40 % of mothers with gravida three or more had at least one previous abortion or stillbirth and the corresponding figure for second gravida mothers was only 4 %. This could be a probable explanation for the higher frequency of stillbirth among mothers with gravida status of three or more. Consanguinity was also associated with higher frequency of stillbirth and is a known factor (Stoltenberg et al. 1998) owing to higher chance of similar genetic defects running in families.

Overall prevalence of MCA in this population, 1 %, was lower than 2.11 to 4.42 % (Choudhury et al. 1989; Chaturvedi and Banerjee 1989; Ronya et al. 2002; Talukder and Sharma 2006) reported from other parts of India. With a multifactorial origin for congenital anomalies,

it may not be possible to reason out an exact cause for the discrepancy other than the rather unique features of the study population mentioned above.

Clubfoot was the most common specific MCA observed in this study and the prevalence of 2.85/1,000 was generally comparable to that reported from elsewhere in the country—0.4 /1,000 at Kolkata and 2.5 /1,000 Jammu and Kashmir (Gupta et al. 2003). There was a statistically significant increase in prevalence of clubfoot among births from consanguineous marriages and among males. The deformity was highest in the dose group of 1.5–3.0 mGy/year but there was no clear dose–response relationship. The highest frequency of clubfoot as well as overall MCA was among the Hindu ethnic groups of Arayas, a community that practices relatively higher consanguineous marriages (4 %) and generally resides in the coastal area with higher background radiation dose—about 70 % in the dose group of 6 mGy/year or more as against 3 % in the overall sample. But the finding may be an artifact of sample size as Arayas constituted only 1.6 % ($n=2,297$) of the total sample. Of the 404 newborns identified with clubfoot, 380 (94 %) were without any other MCA and it was seen with neural tube defects in nine newborns and with cleft lip/palate, in another five newborns.

All congenital heart diseases (CHD) may not be symptomatic and detectable at birth and hence the observed prevalence at birth will be an under estimate. The reported prevalence of 3.9 ‰ CHD among live newborns in India (Khalil et al. 1994) is higher than the 1.19 ‰ identified in this study. The lower prevalence in the current study may be compatible with lower maternal age at birth as CHD showed increasing trend with maternal age at birth and only less than 10 % of the mothers in the study were older than 30 years at birth. CHD was not statistically associated with any other factors considered in the study, though the prevalence showed wide variation among different ethnic groups and was higher among newborns from consanguineous marriages and among those with a gravida status of four or more. Unlike overall MCA and clubfoot, the prevalence of CHD was marginally higher among females (probably reflecting survival advantage of females over their male counterparts under suboptimal conditions) but the difference was not statistically significant. Among the 168 newborns diagnosed with CHD at birth in the study, 150 had CHD alone and the rest had other MCA as well, the most frequent being Down syndrome in five and clubfoot in another four newborns.

The prevalences of cleft lip/palate and hypospadias in this study were 1.05 and 2.10 ‰ (among males), respectively, and are within the range reported in literature (Radhakrishna et al. 2006; Ghirri et al. 2009; Reddy et al. 2010). The occurrence of both cleft lip/palate and

hypospadias appeared to be sporadic without association with any of the parameters like maternal age at birth, gravida status, consanguinity, ethnicity, or background radiation dose levels. The population based 1:3 age-matched case–control study in the high and normal-level radiation areas of Kerala also did not reveal any association of cleft lip/palate with the prevailing dose levels at parental residences (Koya et al. 2012). More than 95 % (145/152) newborns identified with hypospadias were without any other major anomalies. The proportions of newborns with cleft lip as well as cleft palate, cleft lip alone, and cleft palate alone were 59.7, 21.5, 18.8 %, respectively. Majority (136/149) of the newborns had isolated cleft lip/palate and the most frequent MCA observed among newborns with cleft lip/palate was neural tube defects (four newborns).

Frequency of Down syndrome in the current study was 1 in 1,361 births and the relatively lower prevalence could be explained by lower maternal age at birth (Hecht and Hook 1996; Carothers et al. 1999), which is comparable to that reported in a national level multicentric study of malformation and Down syndrome in India using a common protocol, conducted at New Delhi (Verma et al. 1998), Mumbai (Bharucha 1998), and Baroda (Modi et al. 1998), which had estimated the frequency of Down syndrome to be 1 in 1,230, 1 in 1,511, and 1 in 963, respectively. Association of Down syndrome with maternal age is well known (Gaulden 1992) and the same was seen here also where the prevalence increased sharply after the age of 30 years at the time of delivery. Notwithstanding relatively smaller sample size at higher dose levels, available data on 104 Down syndrome cases detected at birth did not suggest any dose dependence. Comparison of the frequencies of Down syndrome in HLNK (65/86,290) and NLNR (39/55,250) areas yielded an RR of 1.07 (95 % CI, 0.72–1.59) and did not provide evidence to suggest any impact of HLNK on the prevalence of Down syndrome. The findings are in disagreement with an earlier study (Kochupillai et al. 1976) based on an adult population survey that reported higher prevalence of Down syndrome in HLNK with no Down syndrome case having been recorded in the control population.

The prevalence of neural tube defects (0.51 ‰) was comparatively lower as against those from other Indian studies where it ranged from 0.84 to 8.8 ‰ (Verma et al. 1998; Bharucha 1998; Cherian et al. 2005). Like CHD and Down syndrome, the risk of neural tube defects in the present study appears to be higher among female births, although not statistically significant. A similar pattern was seen in other reports (Seller and Opitz 1987). Neither background radiation nor any other

parameters analyzed in this study appear to be risk factor for neural tube defects.

Genetic studies in wild rats based on skeletal and dental variants (Gruneberg 1964; Gruneberg et al. 1966) and a demographic study based on reproductive parameters and infant mortality (Ayengar et al. 1972) in the area did not suggest any difference between high- and normal-level radiation areas. There were reports of a higher prevalence (12/12,918 in HLNRA vs. 0/5,938 in control area) of Down syndrome in HLNRA (Kochupillai et al. 1976) and a higher frequency of mitochondrial DNA mutation (22/595 in HLNRA vs. 1/200 in NLNR; $P < .01$, likelihood ratio chi-square) in saliva samples (Forster et al. 2002). Down syndrome report (Kochupillai et al. 1976) was criticized for design issues (Sundaram 1977). Historic dosimetry and small sample size especially from the control area were some of the weaknesses of the mitochondrial DNA study—the risk of mutation in HLNRA area relative to control area, as estimated from the data was 7.39 with a very wide 95 % CI of 1.003 to 54.5, reflecting lack of precision in the estimate. Our studies on biological and health effects of high-level natural radiation on the inhabitants, conducted at the field laboratory established by Department of Atomic Energy, Government of India, did not find any evidence to implicate high-level natural radiation in any of the outcomes like chromosomal aberrations, karyotype anomalies, micronuclei, overall congenital anomalies, stillbirths, late onset diseases, or telomere length in human adults (Jaikrishan et al. 1999; Cheriyan et al. 1999; Thampi et al. 2002, 2005; Das and Karuppasamy 2009; Das et al. 2009). Studies on cancer incidence among population of the HLNRA area also failed to show any relationship with the high-level natural radiation (Nair et al. 1999; Nair et al. 2009).

Summarizing, the findings are consistent from those noted in other similar studies (Talukder and Sharma 2006). Maternal age at birth is the only incontrovertible factor associated with Down syndrome. Delayed fertilization due to reduced frequency of coitus, relaxed embryo–fetus selection, limited oocyte pool, hormone imbalance, etc. implicated in the etiology of Down syndrome (Gaulden 1992) may also have a role in the frequency of stillbirth and MCA as well. The present findings, however, are in agreement with the studies elsewhere including those on children born to parents of survivors of atomic bombings (Neel et al. 1990), where no statistically significant difference was seen in the frequency of genetic defects between exposed and control groups and at present there is no evidence to suggest any statistically significant association of either stillbirth or MCA with high background radiation. More focused and analytical studies with rigorous dosimetry are being carried out with selected MCAs (Koya et al. 2012) and further nested case–control studies with MCA like hypospadias, clubfoot, and other MCA that are very difficult to identify/diagnose in the field are being planned.

Strengths and limitations of the study

This study is based on monitoring about 142,000 consecutive newborns at birth and the large sample size is the major strength of this study. The chance of under ascertainment of major congenital anomalies detectable at birth was remote in the study since all newborns were examined by the pediatricians of the collaborating hospitals and their observations were verified by medical doctors of LLRRL trained in detecting congenital anomalies. Since all the relevant information was recorded in pre-coded proforma during the stay of the woman in the hospital by staff nurses and medical doctors of the hospital and the data were scrutinized for completeness and consistency by medical and paramedical staff of LLRRL who made regular visits for the purpose to the participating hospitals, missing data was not an issue in the study. The clinical diagnosis of Down syndrome was confirmed cytologically. Etiology of different MCA, though not well understood, could be different and pooled analysis might mask small differences between HLNRA and NLNRA. Hence, most frequent MCA were analyzed separately to find out association, if any, with the elevated levels of natural radiation. The uniqueness of the study population—lower maternal age at birth, acceptance of small family norm, universal antenatal folic acid supplementation, higher literacy, and consequent health awareness and practices—may have influence in reducing the prevalence of stillbirth and MCA and the spectrum of observed anomalies. Radiation exposure profile in the natural high-level radiation scenario is quite complex due to local movement of the population coupled with the general Indian pattern of shift of residence of female to her husband's place after marriage. One limitation of the study is that the average dose at the area of residence of the parents was used in the study as a surrogate for the actual dose. Since the radiation level in the region under study is purely due to primordial radioactivity, drastic change in radiation levels over the years was unlikely. It may also be appreciated that reconstruction of past radiation dose, taking into consideration the complex biological processes underlying gametogenesis/fetal development, is near impossible. Smaller sample size from areas with higher radiation levels was another limitation of this observational study.

Acknowledgments The co-operation and unfailing support of the medical, nursing, and paramedical staff of the Directorate of Health Services, Government of Kerala and the staff of the Ministry of Social Welfare is gratefully acknowledged. Without their dedication and hard work, these investigations would not have been possible. The contribution of Dr. PS Chauhan and Mr. MV Thampi at different stages of the study is also gratefully acknowledged. The authors also acknowledge the services of the cytogeneticists of the laboratory who did the karyotyping for cytological confirmation of suspected chromosomal anomalies including Down syndrome.

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 3 Congenital anomalies observed in the study

Achondroplasia
Acromelia
Albinism
Amelia
Anal atresia
Anencephaly
Ankylosis major joints
Anophthalmia
Apert syndrome
Aplasia external auditory meatus
Aplasia forearm
Arthrogryposis
Bicornuate uterus with vaginal septum
Brain cyst multiple pathology
Cataract
Congenital heart disease
Cleft lip and/or palate
Clubfoot
Collodion baby
Coloboma eye
Corneal opacity
Craniosynostosis
Cretinism
Cyst urachus
Cystic hygroma
Diaphragmatic hernia
Digital aplasia
Dislocation of hip
Down syndrome
Duodinal atresia
Ectopic anus
Ectopic vesicae
Ectropion eyelid
Edward syndrome
Encephalocele
Epispadias
Exomphalos
Fetal ascitis
Fetal monster
Gastroschisis
Hemophilia
Hepatomegaly
Hepatosplenomegaly
Hirschsprung disease
Holoprosencephaly
Holt–Oram syndrome
Hydrocephalus

Table 3 (continued)

Hydronephrosis
Hypertrophic pyloric stenosis
Hypoplasia testis and scrotum
Hypospadias
Ileal atresia
Imperforate anus
Intersex/ambiguous genitalia
Laryngomalacia
Mandibular aplasia
Megacolon
Meningocele
Meningomyelocele
Mesomelia
Micro penis
Microcephaly
Microphthalmia
Microtia
Mucopolysaccharrodosis
Myelocele
Oesophageal atresia
Omphalocele
Osteogenesis imperfecta
Papyraceous baby
Patau syndrome
Phocomelia
Pierrie Robin syndrome
Polycystic kidney
Prune belly syndrome
Recto vaginal fistula
Rhizomelia
Sacroccocygeal teratoma
Spina bifida
Thoracophagus
Tracheo esophageal fistula
Treacher Collin's syndrome
Turner syndrome

References

- Ahman E, Zupan J (2007) Neonatal and perinatal mortality—country, regional and global estimates 2004. World Health Organisation, Geneva
- Ayengar ARG, Sundaram K, Mistry KB, Suntha CM, Nambi CSV, Kaithuria SP et al (1972) Evaluation of the long term effects of high background radiation on selected population groups of Kerala coasts. In: Proceedings of 4th UN International Conference on Peaceful Uses of Atomic Energy. United Nations, New York, pp 31–51
- Bharatwal DS, Vaze GH (1959) Radiation dose measurements in the monazite areas of Kerala State in India. In: Proceedings of the 2nd

- UN International Conference on Peaceful Uses of Atomic Energy. United Nations, New York, p 156
- Bharucha BA (1998) Study of malformation and Down syndrome in India (SOMDI): Bombay region. *Indian J Hum Genet* 4:88–92
- Breslow NE, Day NE (1987) Statistical methods in cancer research, vol II: The design and analysis of cohort studies. Oxford University Press, Oxford
- Carothers AD, Boyd E, Lowther G, Ellis PM, Couzin DA, Faed MJ et al (1999) Trends in prenatal diagnosis of Down syndrome and other autosomal trisomies in Scotland 1990 to 1994, with associated cytogenetic and epidemiological findings. *Genet Epidemiol* 16(2):179–190
- Chaturvedi P, Banerjee KS (1989) Spectrum of congenital malformations in newborns from rural Maharashtra. *Indian J Pediatr* 56(4):501–507
- Cherian A, Seena S, Bullock RK, Antony AC (2005) Incidence of neural tube defects in the least-developed area of India: a population-based study. *Lancet* 366:930–931
- Cheriyian VD, Kurien CJ, Das B, Ramachandran EN, Karuppasamy CV, Thampi MV et al (1999) Genetic monitoring of the human population from high level natural radiation areas of Kerala on the southwest coast of India. II. Incidence of numerical and structural chromosomal aberrations in the lymphocytes of newborns. *Radiat Res* 152((Suppl)):S154–S158
- Choudhury AR, Mukherjee M, Sharma A, Talukder G, Goash PK (1989) Study of 1,26,266 consecutive births for major congenital defects. *Ind J Pediatr* 56(4):493–499
- Coggle JE (1983) Biological effects of radiation, 2nd edn. Taylor and Francis Inc, New York
- Das B, Karuppasamy CV (2009) Spontaneous frequency of micronuclei among the newborns from high level natural radiation areas of Kerala in the southwest coast of India. *Int J Radiat Biol* 85(3):272–280. doi:10.1080/09553000902751462
- Das B, Saini D, Seshadri M (2009) Telomere length in human adults and high level natural background radiation. *PLoS One* 4(12):e8440
- Forster L, Forster P, Lutz-Bonengel S, Willkomm H, Brinkmann B (2002) Natural radioactivity and human mitochondrial DNA mutations. *Proc Natl Acad Sci USA* 99:13950–13954
- Gaulden ME (1992) Maternal age effect: the enigma of Down syndrome and other trisomic conditions. *Mutat Res* 296(1–2):69–88
- Ghirri P, Scaramuzza RT, Bertelloni S, Pardi D, Celandroni A, Cocchi G et al (2009) Prevalence of hypospadias in Italy according to severity, gestational age and birthweight: an epidemiological study. *Ital J Pediatr* 35:18. doi:10.1186/1824-7288-35-18
- Gruneberg H (1964) Genetic research in an area of high natural radioactivity in South India. *Nature* 204:222–224
- Gruneberg H, Bains GS, Berry RJ, Riles LL, Smith CAB, Weiss RA (1966) A search for genetic effects of high natural radioactivity in South India. Report No. SRS 307. London: Medical Research Council, Her Majesty's Stationary Office
- Gupta RK, Gupta CR, Singh D (2003) Incidence of congenital malformations of the musculoskeletal system in new live borns in Jammu. *JK Sci* 5(4):157–160
- Hecht CA, Hook EB (1996) Rates of Down syndrome at live birth by one-year maternal age intervals in studies with apparent close to complete ascertainment in populations of European origin: a proposed revised rate schedule for use in genetic and prenatal screening. *Am J Med Genet* 62(4):376–385
- Jaikrishan G, Andrews VJ, Thampi MV, Koya PKM, Chauhan PS (1999) Genetic monitoring of the human population from high level natural radiation areas of Kerala on the Southwest Coast of India. I. Prevalence of congenital malformations in newborns. *Radiat Res* 152((Suppl)):S149–S153
- Khalil A, Aggarwal R, Thirupuram S, Arora R (1994) Incidence of congenital heart disease among hospital live births in India. *Indian Pediatr* 31(5):519–527
- Khan HA, Sempos CT (1989) Statistical methods in epidemiology. Oxford University Press, Oxford
- Kochupillai N, Verma IC, Grewal MS, Ramalingaswamy V (1976) Down syndrome and related abnormalities in an area of high background radiation in coastal Kerala. *Nature* 262:60–61
- Koya PKM, Chougankar MP, Predeep P, Jojo PJ, Cheriyian VD, Mayya YS et al (2012) Effect of low and chronic radiation exposure: a case-control study of mental retardation and cleft lip/palate in the monazite bearing coastal areas of Southern Kerala. *Radiat Res* 177:109–116
- Modi UJ, Nayak U, Aiyer S, Bharani S (1998) Study of malformation and Down syndrome in India (SOMDI): Baroda region. *Indian J Hum Genet* 4:93–98
- Nair MK, Nambi KSV, Amma SN, Gangadharan P, Jayalekshmi P, Jayadevan S et al (1999) Population study in the high natural background radiation area in Kerala, India. *Radiat Res* 152((Suppl)):S145–S148
- Nair RRR, Rajan B, Akiba S, Jayalekshmi P, Nair MK, Gangadharan P et al (2009) Background radiation and cancer incidence in Kerala, India-Karunagapally cohort study. *Health Phys* 96(1):55–66
- Neel JV, Schull WJ, Awa AA, Satoh C, Kato H, Otake M et al (1990) The children of parents exposed to atomic bombs: estimates of the genetic doubling dose of radiation for humans. *Am J Hum Genet* 46:1053–1072
- Radhakrishna U, Ratnamala U, Gaines M, Beiraghi S, Hutchings D, Golla J et al (2006) Genomewide scan for nonsyndromic cleft lip and palate in multigenerational Indian families reveals significant evidence of Linkage at 13q33.1–34. *Am J Hum Genet* 79:580–585
- Reddy SG, Reddy RR, Bronkhorst EM, Prasad R, Ettema AM, Sailer HF et al (2010) Incidence of cleft lip and palate in the state of Andhra Pradesh, South India. *Indian J Plast Surg* 43(2):184–189
- Ronyra R, Gupta D, Ghosh SK, Narang R, Jain KB (2002) Spectrum of congenital surgical malformations in newborns. *J Indian Med Assoc* 100(9):565–566
- Seller MJ, Opitz JM (1987) Neural tube defects and sex ratios. *Am J Med Genet* 26(3):699–707
- Singh M (1985) Early diagnosis of congenital malformations. In: Care of newborn. 3rd edn. Sagar Publications, New Delhi
- StatSoft, Inc. (2010). STATISTICA (data analysis software system), version 9.1. www.statsoft.com.
- Stoltenberg C, Magnus P, Lie RT, Daltveit AK, Irgens LM (1998) Influence of consanguinity and maternal education on risk of stillbirth and infant death in Norway, 1967–1993. *Am J Epidemiol* 148:452–459
- Sundaram K (1977) Down's syndrome in Kerala [letter]. *Nature* 267:728
- Sunta CM (1993) A review of the studies of high background areas of the S–W coast of India. In: Sohrabi M, Ahmed JU, Durani SA (eds) Proceedings of the international conference on high levels of natural radiation. Atomic Energy Organization of Iran, Tehran, p 71
- Talukder G, Sharma A (2006) Genetic causes of congenital malformation in India. *Int J Hum Genet* 6(1):15–25
- Thampi MV, Cheriyian VD, Kurien CJ, Ramachandran EN, Karuppasamy CV, Koya PKM et al (2002) Cytogenetic studies in the high level natural radiation areas of Kerala. *Int Congress Ser* 1225:207–211
- Thampi MV, Cheriyian VD, Jaikrishan G, Das B, Kurien CJ, Ramachandran EN et al (2005) Investigations on the health effects of human population residing in the high-level natural radiation areas in Kerala in the southwest coast of India. *Int Congr Ser* 1276:8–12

- United Nations Scientific Committee on the Effects of Atomic Radiation (1993) Report to the general assembly. I. Sources, effects and risks of ionizing radiation. United Nations, New York
- United Nations Scientific Committee on the Effects of Atomic Radiation (2000) Report to the general assembly with scientific annexes. Vol I. Annex B: exposures from natural radiation sources. United Nations, New York
- Verma IC, Anand NK, Kabra M, Menon PSN, Sharma N (1998) Study of malformation and Down syndrome in India (SOMDI): Delhi region. *Indian J Hum Genet* 4:84–87
- Vogel F, Motulsky AG (1997) Human genetics—problems and approaches, 3rd edn. Springer, New York
- World Health Organization (1959) Effects of radiation on human heredity: investigations of areas of high natural radiation. WHO Technical Report Series No. 166, Geneva