Association of birth outcomes and postnatal growth with adult leukocyte telomere length: Data from New Delhi Birth Cohort

Mohamad Tarik¹ | Lakshmy Ramakrishnan¹ | Sikha Sinha² | Harsh Pal Singh Sachdev² | Nikhil Tandon³ | Ambuj Roy⁴ | Santosh Kumar Bhargava⁵

¹Department of Cardiac Biochemistry, All India Institute of Medical Sciences, New Delhi, India

²Department of Pediatrics and Clinical Epidemiology, Sitaram Bhartia Institute of Science and Research, New Delhi, India

³Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India

⁴Department of Cardiology, All India Institute of Medical Sciences, New Delhi, India

⁵Department of Pediatrics, Sunder Lal Jain Hospital, New Delhi, India

Correspondence

Prof. Lakshmy Ramakrishnan, R-61, Department of Cardiac Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India.
Email: lakshmy_ram@yahoo.com

Funding information

Department of Biotechnology, Ministry of Science and Technology, India, Grant/Award Number: BT/PR5317/FNS/20/552/2012

Abstract

Born small for gestational age due to undernutrition in utero and subsequent catch-up growth is associated with risk of developing chronic diseases in adulthood. Telomere length has been shown to be a predictor of these age-related diseases and may be a link between birth size, a surrogate for foetal undernutrition, and adult chronic diseases. We assessed the relationship of leukocyte telomere length in adult life with birth outcomes and serial change in body mass index (BMI) from birth to adulthood. Leukocyte relative telomere length (RTL) was measured by MMqPCR in 1,309 subjects from New Delhi Birth Cohort who participated in two phases of the study between 2006–2009 (Phase 6) and 2012–2015 (Phase 7) at a mean age of 39.08 (± 3.29), and its association with birth outcomes and conditional BMI gain at 2, 11, and 29 years was assessed in a mixed regression model. We did not find any significant association of RTL with body size at birth including birthweight, birth length, and birth BMI. Gestational age was positively associated with RTL ($P = .017$, multivariate model: $P = .039$). Conditional BMI gain at 2 and 11 years was not associated with RTL. BMI gain at 29 year was negatively associated with RTL in multivariate model ($P = .015$). Born small for gestational age was not associated with RTL in adulthood. Leukocyte telomere attrition was observed in those born before 37 weeks of gestational age as well as in those who gained weight as adults, which may predispose to chronic diseases.

KEYWORDS

Barker hypothesis, birth cohort, cardiovascular disease, child growth, childhood obesity, chronic disease

1 | INTRODUCTION

The prevalence of noncommunicable disease is rising in India (WHO, 2018). Barker et al. reported an association between low birthweight (LBW) or small for gestational age (SGA; birthweight < 2,500 gm), a surrogate of intrauterine growth, and mortality due to cardiac disease (Barker et al., 1993; Barker, Winter, Osmond, Margetts, & Simmonds, 1989). Subsequent research has demonstrated correlation between variations in birthweight and blood pressure (Barker, Osmond, Golding, Kuh, & Wadsworth, 1989; Curhan et al., 1996), cholesterol

concentration (Barker, Martyn, Osmond, Hales, & Fall, 1993), carotid atherosclerosis (Martyn, Gale, Jespersen, & Sherriff, 1998), fibrinogen (Martyn, Meade, Stirling, & Barker, 1995), obesity (Stettler, Kumanyika, Katz, Zemel, & Stallings, 2003; Stettler & Stallings, 2002), and metabolic syndrome (Curhan et al., 1996; Hofman et al., 1997) in adulthood. Approximately 27% of babies born in India are low birthweight, with nearly half of them being full term babies (Radha et al., 2015), a consequence of inadequate nutrient intake by mothers during pregnancy, and this may be contributing to the rising trend of chronic diseases in this part of the world. Several plausible mechanisms have been

proposed to explain the in utero origin of adult diseases (Edwards, Coulter, Symonds, & McMillen, 2001; McTernan et al., 2001; Seckl, Cleasby, & Nyirenda, 2000), but the underlying mechanism is yet not completely elucidated. Telomere length has been linked to adult chronic diseases, and it is likely that adverse nutritional conditions during foetal growth increases oxidative stress, which in turn may affect the telomeres. Animal studies have shown that LBW due to intrauterine growth restriction during gestation and subsequent catch-up growth results in shorter telomere length (Jennings, Ozanne, Dorling, & Hales, 1999; Tarry-Adkins et al., 2009; Tarry-Adkins, Martin-Gronert, Chen, Cripps, & Ozanne, 2008). Studies in humans have been equivocal with some studies showing an association between telomere length and birthweight (Entringer et al., 2011; Raqib et al., 2007), whereas others have shown no effect of birthweight on telomere length in young adults (Kajantie et al., 2012; Smeets, Codd, Samani, & Hokken-Koelega, 2015). Earlier reports have not even addressed the issue of postnatal catch-up growth subsequent to being born SGA on telomere length. Retarded growth during intrauterine phase as well as during infancy, childhood, and adolescence has been reported to increase risk of coronary heart disease irrespective of size at birth (Eriksson, Forsén, Tuomilehto, Osmond, & Barker, 2001). Studies have also shown ill effects of catch-up growth during infancy and childhood on risk of development of chronic diseases later in life in children born SGA.

The New Delhi Birth Cohort (NDBC) in which the present study is carried out has information on birthweight and six monthly changes in height and weight till 21 years (Bhargava et al., 2004) in a cohort of more than 1,000 subjects. Frequent anthropometric measurements of the cohort during infancy, childhood, adolescence, and adulthood provided a unique opportunity to study the relative importance of prenatal and postnatal growth on RTL in adulthood. The association of birth outcomes (birth size and gestational age) and body mass index (BMI) gain at 2, 11, and 29 years was studied with RTL at adulthood.

2 | METHODS

The present study is carried out in the subjects of NDBC, which was established in 1969 to study pregnancy outcomes and child growth. Details of the cohort are given in our previous publication (Bhargava et al., 2004). Briefly, newborn offspring's weight and length were recorded within 72 hr and six monthly intervals (± 15 days) until 21 years of age in the first four phases during 1969–1990. After a break of 8 years, in 1998 (Phase 5), the subjects who were by now around 29 years of age were retraced to assess metabolic and cardiovascular risk factors. Data on socio-economic status, education, family history of diseases, tobacco and alcohol consumption, diet, and physical activity were collected using standard questionnaires from 1,583 retraced subjects. Anthropometry, blood pressure, and ECG were recorded. Blood was taken for analysis of plasma glucose, insulin lipids, and proinflammatory markers. In Phase 6 (2006–2009), 1,100 subjects participated, all the measurements except proinflammatory markers were repeated, and in addition, cIMT, brachial artery endothelial

Key messages

- Gestational age is associated with shorter telomere in adult life in the NDBC cohort.
- Small for gestational age (SGA) is not associated with leukocyte's telomere attrition at the mean age of 39.08 (± 3.29) years.
- Conditional BMI gain at 2 and 11 years is not associated with telomere attrition in adult life, although BMI gain at 29 years was associated with shorter telomere.

function, and body composition using dual X-ray absorptiometry were performed. In Phase 7 conducted between 2012 and 2015, all anthropometric, clinical, and biochemical measurements (except proinflammatory markers) as mentioned for Phase 5 were repeated (Figure 1).

2.1 | Current study

In the present study, 1,353 subjects were included, whose blood samples were collected in Phases 6 and 7 of the NDBC. Of the 1,353 subjects, 44 subjects were excluded due to poor DNA quality, leaving 1,309. Therefore, the current study was carried out in stored blood available from 655 subjects from Phase 6 from which good quality DNA could be extracted as well as from freshly collected blood samples from 654 subjects recruited in Phase 7 of the study. The mean age of the participants from Phase 6 who were included in the present study was 35.98 (± 1.03) years and that of participants recruited in Phase 7 and included in the present study was 42.19 (± 1.13) years and the mean age of total subjects (1,309) was 39.08 (± 3.29) years. The first adult follow-up phase (1998–2002) was conducted on 1,526 participants. In comparison with the original cohort of 8,181 subjects, these participants had 7% more male subjects, the rate of maternal literacy was 6% higher, the mean birthweight was 32 g higher, and the mean birth length was 2 mm longer. The height, weight, and BMI in childhood and adolescence were approximately 0.1 SD lower than in the original cohort (Bhargava et al., 2004). In the subsequent adult phases during which this study was conducted, in comparison with the original cohort, the participants were comparable for birthweight, and paternal education and occupation but there were marginal or small differences in mean birth length (1 mm higher), maternal literacy (6% higher), nuclear families (7% higher), household income, type of housing, utilization of health services, water supply, and sanitation. Ethical clearance was obtained from institution ethical committee, and informed consent was obtained from all subjects. In both the phases, 10 ml of blood was obtained from subjects after an overnight fast. Plasma (fluoride and EDTA) and serum samples were separated, and plasma glucose (by glucose oxidase method on Beckman autoanalyser) and serum lipids (total cholesterol and triglycerides by enzymatic method and HDL by immune-inhibition method) were

In Phase 4, recruitment was restricted to the subjects who would have reached 20 years of age during this period. The minimal, and maximal ages of the subjects at each year of follow-up are shown by the error bars.

FIGURE 1 Description of the various phases of the New Delhi Birth Cohort study

measured. The rest of the plasma and serum were aliquoted and preserved at -70°C till further analysis. High sensitive C-reactive protein (hs-CRP) was determined in serum by solid phase ELISA method using commercially available kit (catalogue number: 11190; Biocheck, USA) in only in fresh serum samples collected in Phase 7. RTL was determined by qPCR after extracting DNA from stored samples from Phase 6 and freshly collected samples from Phase 7. To validate the qPCR method, absolute telomere length (aTL) was measured by Southern blot assay in a subset of 94 samples as previously described (Tarik et al., 2018).

2.2 | Measurement of telomere length by qPCR

DNA was isolated from whole blood using Dneasy blood and tissue kit (Qiagen, Germany). Leukocyte telomere length was measured by monochrome multiplex quantitative PCR (MMqPCR) method described by Cawthon (2009) with minor modifications. Reference DNA at concentrations 120, 40, 13.33, 4.44, and $1.48\text{ ng }\mu\text{L}^{-1}$ were prepared from standard genomic DNA. Duplicate samples of $2\text{ }\mu\text{L}$ of reference DNA and DNA extracted from blood samples ($\sim 20\text{ ng }\mu\text{L}^{-1}$) were taken in $1\times$ master mix consisting of $0.5\times$ SYBR Green I, 10 mM Tris-HCl, 50 mM KCl, 3 mM MgCl_2 , 0.2 mM each dNTP, 1 mM DTT, 1 M betaine, and 0.625 U AmpliTaq Gold DNA polymerase and nuclease free water in a final volume of $25\text{ }\mu\text{L}$ and added into reaction wells of 96-well PCR

plate (Axygen, USA) compatible with real-time PCR detection system (IQ5 software, Bio-Rad) and amplified. One nontemplate control (NTC) and two positive controls were also amplified in duplicates in each run. For the multiplex qPCR, the telomere primers (forward: AC ACTAAGGGTTTGGGTTTGGGTTAGTGT and reverse: TGTTAGGTAT CCTCATCCCTATCCCTATCCCTATCCCTAACA, final concentration of 900 nM) and single copy gene, albumin primers (forward: GGCGGCGGGCGGGGCTGGGCGGAAATGCTGCACAGAATCCTTG and reverse: GCGCGGCGGGCGGGGCTGGGCGGAAATGCTGCACAGAATCCTTG, final concentration 500 nM) were added in each reaction, and product was amplified using real-time PCR (Bio-Rad iQ5 software 2.0 version). After the run was completed, the Ct values of telomere and albumin gene was transferred to spreadsheet separately and the ratio of the copy number of telomere (T), and the single copy gene (S) was determined, which is predicted as the proportional to the average telomere length (i.e., the T/S ratio) per cell.

2.3 | Statistical analysis

Data were checked for normality, and variables with skewed distributions were log transformed. Mean and SD was computed for describing the characteristics of study subjects. Univariate and multivariate regression models were used for analysing the relationship between RTL and anthropometric variable from birth to adulthood

and biochemical variables. We used all recorded data from the NDBC (not just those for subjects recruited for this study) to derive SD scores for height, weight, and BMI for each subject at age 6 months and at birthdays from age 1 to 21 years as previously described (Bhargava et al., 2004). We also examined the associations of RTL with growth (conditional BMI gain) during infancy (birth to 2 years), childhood (2–11 years), and adolescence and adulthood (11–29 years). To measure growth during a time interval (e.g., between the ages of 2 and 11 years), we regressed the value at the end of the interval (age 11 years) on the value at the beginning of the interval (age 2 years) and at earlier time points (birth) and expressed the residual as an SD score (Adair et al., 2009). The conditional BMI gain is useful in assessment of association with growth during specific time period as this method overcomes the highly correlated measurements of height and weight in an individual. Age 29 was the age at which first retracing and follow up happened in the cohort. BMI gain as adult was taken as the conditional BMI gain from adolescence age (11 years) to age 29.

3 | RESULTS

Of the 1,353 samples available for the study, genomic DNA of good integrity on 0.8% agarose gel could be obtained from 1,309 samples, and these were used for telomere length analysis. The mean age of the total participants was 39.08 (± 3.29) year. A total of 655 subjects with mean age of 35.98 (± 1.03) years were taken from Phase 6 and 654 subjects with mean age of 42.19 (± 1.13) years from Phase 7. Leukocyte RTL ranged from 0.401 to 2.344 with mean values of 0.984 (± 0.29) in 1,309 subjects, 0.95 (± 0.31) in 655 subjects during Phase 6, and 1.02 (± 0.32) in 654 subjects from Phase 7.

Mean RTL was comparable between males and females in Phase 6, whereas in Phase 7, females had significantly higher RTL as compared with males. The characteristics of the study subjects included in Phases 6 and 7 are depicted in Table 1. Birth measurements were comparable between males and females in Phase 6, whereas males were heavier and taller at birth as compared with females in Phase 7. As adults, females were shorter and weighed less as compared with males in both the phases. As compared with males, waist circumference was lower in females in both phases, but BMI was higher in Phase 7.

3.1 | Association of telomere length with anthropometric variables from birth to adulthood in New Delhi Cohort subjects

The data from Phases 6 and 7 of the study were analysed together in mixed univariate and multivariate regression models (Table 2). In the univariate model, birthweight and birth BMI were not associated with RTL at the age of ~39 years. There was a significant positive association of gestational age with telomere length ($P = .017$), and association remained significant after adjusting for confounding factors in multivariate regression Model 2 ($P = .039$) and in Model 3 ($P = .05$). Conditional BMI gain at 2 and 11 year was not significantly associated with telomere length. Conditional BMI gain at adulthood (29 years) was negatively associated with RTL after adjusting for confounders in Model 2 ($P = .015$) and Model 3 ($P = .044$). Additionally, we have assessed the association of telomere length with conditional BMI gain from 29 to current age, but the association was not significant in univariate and multivariate regression model ($B = -0.026$, 95% CI $[-0.128, 0.076]$; $P = .612$). There was no significant association of RTL with birthweight,

TABLE 1 Body measurements at birth and as adults

Variable	Phase 6					Phase 7				
	Males		Females		P value ^b	Males		Females		P value ^b
	N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)	
Birth										
Birthweight (kg)	352	2.88 (0.43)	237	2.82 (0.37)	.059	385	2.89 (0.45)	233	2.79 (0.38)	.005
Birth length (cm)	336	48.79 (2.03)	236	48.55 (1.86)	.147	375	48.72 (2.03)	232	48.30 (1.91)	.013
Birth BMI	336	12.04 (1.25)	236	11.93 (1.17)	.273	375	12.11 (1.27)	232	11.92 (1.22)	.079
Gestational age (weeks)	340	38.69 (2.69)	247	39.06 (2.39)	.087	389	38.78 (2.58)	233	39.04 (2.64)	.226
Adult										
Age (years)	388	35.95 (1.01)	269	36.03 (1.07)	.324	434	42.14 (1.14)	262	42.17 (1.10)	.770
Height (cm)	387	170.07 (6.30)	268	155.46 (5.52)	<.001	428	170.11 (6.16)	259	155.04 (5.79)	<.001
Weight (kg)	388	76.03 (14.46)	269	64.96 (14.06)	<.001	428	78.81 (13.26)	260	70.74 (14.14)	<.001
BMI (kg m ⁻²)	387	26.26 (4.47)	268	26.81 (5.34)	.155	428	27.20 (4.15)	259	29.42 (5.51)	<.001
Waist circumference (cm)	388	95.0 (11.8)	268	86.0 (12.4)	<.001	429	98.2 (11.1)	259	94.4 (11.5)	<.001
Telomere length (relative) ^a	387	0.95 (0.31)	268	0.95 (0.31)	.910	407	1.00 (0.31)	247	1.05 (0.34)	.028

^aGeometric mean after natural log transformation.

^bP value for gender difference.

Bold values indicate statistical significance at the $p \leq 0.05$ level).

TABLE 2 Association of telomere length with birth outcome and conditional BMI gain from birth to adulthood in New Delhi Cohort subjects

Variable	Univariate regression		Multivariate regression	
	Model 1		Model 2	Model 3
	N	Coefficients (P value)	Coefficients (P value) N = 822	Coefficients (P value) ^a N = 819
Birthweight (kg)	1,166	0.029 (.178)		
Birth length (cm)	1,139	0.003 (.503)		
Birth BMI (kg m ⁻²)	1,139	0.010 (.155)	0.002 (.854)	0.003 (.678)
Gestational age (weeks)	1,168	0.008 (.017)	0.009 (.039)	0.008 (.050)
BMI gain at 2 years	1,140	-0.003 (.693)	-0.009 (.445)	-0.005 (.633)
BMI gain at 11 years	1,089	0.0102 (.538)	-0.021 (.175)	-0.012 (.423)
BMI gain at 29 year	1,014	-0.018 (.072)	-0.042 (.015)	-0.036 (.044)
Age (years)	1,309	-0.126 (.106)	0.001 (.891)	0.003 (.711)
Gender	1,309	0.023 (.169)	0.061 (.062)	0.044 (.094)
Adult body weight (kg)	1,303	-0.0005 (.399)		
Adult body height (cm)	1,300	-0.0009 (.484)		
Adult BMI (kg m ⁻²)	1,300	-0.001 (.330)		
Hs-CRP ^b	580	-0.007 (.021)		

^aAppropriate adjustment for age, gender, and phase.^bAdjusted for age, gender, phase, birth BMI, BMI gain at 2, 11, and adulthood, tobacco use, alcohol intake, gestational age, current BMI, systolic blood pressure, triglyceride, and occupational status.^cAdjusted for age, gender, phase, birth BMI, BMI gain at 2, 11, and adulthood, tobacco use, alcohol intake, gestational age, current BMI, and socio-economic status score.^dAppropriate adjustment for age and gender (hs-CRP measured in Phase 7 only). $p \leq 0.05$ are reported in bold.

gestational age, birth BMI, BMI gain at 2, 11, and adulthood in univariate and multivariate regression analysis when males and females were separately analysed. Hs-CRP was inversely associated with RTL in univariate regression model (regression coefficient $B = -0.007$, $P = .021$) adjusted for age and gender.

We have also assessed the association of birth outcomes with aTL measured in 94 subjects by Southern blot. The mean aTL was $7,391.12 \pm 892.79$ bp (ranged: 5,588.76–10,050.00 bp). aTL was not associated with birthweight ($n = 76$, $B = 0.132$; 95% CI $[-0.372, 0.635]$; $P = .604$), birth BMI ($n = 69$; $B = 99.28$; 95% CI $[-109.87, 308.42]$; $P = .347$) and gestational age ($n = 72$, $B = 6.855$, 95% CI $[-76.62, 62.91]$; $P = .845$). Conditional BMI gain at 2, 11, and 29 years ($n = 45$) was also not associated with aTL.

4 | DISCUSSION

4.1 | Telomere length and birth outcomes

The study did not find any significant association between birthweight, birth length, and birth BMI and RTL in adult life at the mean age of $39.08 (\pm 3.29)$ years. Evidence of the association of telomere length with birthweight is inconsistent. aTL measured by Southern blot in a subset of samples ($n = 76$) also did not show association with birth parameters. Similar to our study, Kajantie et al. (2012) and Song et al. (2015) as well as Dutch famine birth cohort study (de Rooij et al., 2015) did not find any association between telomere length and birthweight. Kajantie et al. (2012) used data from three cohorts to study the association of telomere length with birthweight: The Helsinki Birth Cohort (HSBC) of 1,894 subjects aged 56–69, the Helsinki study of the very low birthweight adult of 164 subjects aged 18–27, and the FinnTwin16 cohort aged 23–31 years. The authors did not find any association between telomere length and birthweight in any of the three cohorts and the findings are similar to that of our study. In the Newcastle Thousand Family Study, 318 individual born in Newcastle in 1947 were followed up at age 49–51 for measurement of telomere length. This study also found no association between birthweight and later telomere length corroborating our results (Pearce et al., 2012). Song et al. studied leukocyte telomere length in 62-year-old women to assess Type 2 diabetes risk factors between low birthweight and normal birthweight women. The authors did not observe any significant difference in telomere length between low birthweight subjects and normal birthweight subjects. Although, low birthweight (SGA) was significantly associated with increased risk of Type 2 diabetes in later life, leukocyte telomere length did not appear to mediate significant proportion of the total effect of LBW on Type 2 diabetes in this cohort of women (Song et al., 2015). In a Dutch famine birth cohort study of 131 subjects, de Rooij et al. observed that leukocyte telomere length at the age of 68 years did not differ between those who were exposed compared with those who were unexposed to famine during gestation. The leukocyte telomere length was not associated with birthweight in this Dutch cohort (de Rooij et al., 2015).

As against the abovementioned studies, Strohmaier et al. (2015) reported association of telomere length with birthweight in 16-year-old monozygotic twins but not seen in dizygotic twins. Raqib et al. (2007) reported significantly shorter lymphocyte TL in 5 years old preschool children ($n = 132$) born with low birthweight compared with normal birthweight and suggested that this may be due to persistent immune activation because of high infection rate leading to higher T cell turnover. Entringer et al. (2011) reported that LBW due to severe maternal stress during gestation ($n = 45$) is associated with significantly shorter telomere length. All the three studies showing significant association of birthweight with telomere length have been conducted in children, adolescence, or young adults. The positive association seen in young subjects as compared with no association seen in older age groups indicates that the association may change

throughout life. This could be due to changing telomere dynamics with relatively rapid loss of telomere during early childhood and subsequently a slow linear decline in adulthood (Frenck, Blackburn, & Shan-non, 1998).

Laganović et al. (2014) paradoxically reported longer telomere in young men (age 21) born SGA as compared with those born appropriate for gestational age. The authors suggest that slower telomere attrition due to a decline in stem cell turnover in IUGR/preterm may be the reason for the longer telomere length in SGA.

The results of our study do not support the hypothesis that telomere length may be a marker linking foetal adverse exposure with adult diseases and does not corroborate findings of animal studies where intrauterine growth restriction during the foetal period was found to associate with shorter telomeres in cells of specific organs such as kidney (Jennings et al., 1999), aortic cells (Tarry-Adkins et al., 2008), and pancreatic islets cells (Tarry-Adkins et al., 2009). In the present study, the telomere length was measured in leukocytes unlike in animal studies where telomere length was measured in specific tissues. There is disagreement on whether leukocyte telomere would be a reflection of telomere length in tissues with recent studies reporting that leukocyte telomere length does not strongly correlate with telomere length in other organs (Daniali et al., 2013; Dlouha, Maluskova, Kralova Lesna, Lanska, & Hubacek, 2014). Rate of telomere attrition differ between tissues (Ulaner, Hu, Vu, Giudice, & Hoffman, 2001) due to differences in the rate of cell division as well as differences in susceptibility to oxidative stress.

4.2 | Impact of gestational age on leukocyte telomere length at adult life

A significant positive association of RTL with gestational age was observed in the present study after adjusting for age and gender and the association remained significant after adjusting with all possible confounding factors in multivariate regression models, which suggests that gestational age has independent effect on leukocyte telomere length. aTL measured by Southern blot in subset of samples ($n = 72$) did not show an association with gestational age possibly due to small sample size. The association of telomere length with gestational age has been studied earlier and the reports are contradictory. A recent study by Smeets et al. (2015) reported a positive association of gestational age with leukocyte telomere length at 21 years of age, a finding similar to that in our study. Kajantie et al. (2012) on the other hand reported no association between gestational age and leukocyte telomere length in three cohorts with different age ranges. An inverse association was reported by Pearce et al. in the Newcastle birth cohort between gestational age and telomere length possibly a chance finding due to number of confounding variables (Pearce et al., 2012).

This effect of gestational age on telomere length can be ascribed to stress in utero. Babies born prior to 37 weeks of gestation is often associated with stressful exposure, which is likely to increase oxidative stress burden and thereby telomere shortening. Oxidative stress risk factors including malnutrition, antioxidant deficient diets, physiological

and psychological stressors, behavioural factors (cigarette smoking, alcohol intake, and drug use), obesity, environmental pollutants, and genotoxic agents are well-studied risk factors of preterm birth (Goldenberg, Culhane, Iams, & Romero, 2008; Romero et al., 2006), and these stressors induce foetal cell senescence signals, which is likely to trigger premature labour (Menon, 2014). Another reason for a shorter telomere in subjects born prior to 37 weeks of gestation could be catch-up growth seen in preterm babies. Most preterm babies would undergo an accelerated growth near term and this can result in replicative stress.

4.3 | Conditional BMI gain and telomere length

Conditional BMI gain at 2 and 11 years was not significantly associated with RTL, but conditional BMI gain at 29 year was significantly associated with shorter telomere. We came across only one study, which has studied the association between BMI z-score change from childhood to adulthood and telomere length. Buxton et al. (2014) reported an inverse association of BMI changes from age at adiposity rebound (which was around 5 years) to 31 years with telomere length in female subjects from the Northern Finland Birth Cohort. A one unit z-score increase in BMI score change was associated with 3.08% decrease in telomere length in the study.

The mechanism responsible for the association of change in BMI with telomere length has not been elucidated. It can be speculated that increased adiposity due to larger gain in weight would lead to increased oxidative stress (Sankhla et al., 2012; Savini, Catani, Evangelista, Gasperi, & Avigliano, 2013) and inflammation (Kiecolt-Glaser et al., 2003), which may accelerate telomere attrition (Goldenberg et al., 2008). A significant inverse association was observed between hs-CRP and telomere length by us ($P = .05$), which suggests that low-grade inflammation may be accelerating leukocyte telomere attrition.

We observed a shorter telomere length in participants from Phase 6 at a mean age of 36 years compared with participants from Phase 7 at a mean age of 42. This is contrary to the negative association between age and telomere length reported in literature. This could be attributed to various reasons. First, the samples from Phase 6 were stored upto 3–6 years prior to extraction of DNA for telomere length analysis and the MMqPCR method used to assess RTL is known to be sensitive to differences in DNA quality (Dlouha et al., 2014). To minimize this effect, we only used good quality DNA for RTL analysis. Second, diet, stress, physical activity, and lifestyle changes can also influence telomere length via improving telomerase activation (Boccardi, Paolisso, & Mecocci, 2016; Cassidy et al., 2010). We however did not assess these factors in our study. In a study, Weischer et al. (2014) reported that 44% individuals during 10 years follow up showed an elongation in telomere length, possible explanation given by the authors is underestimated regenerative capacity of nonstem cells.

One weakness of our study is that, we measured RTL by RT-PCR and not aTL by Southern blotting, which is the gold standard but difficult to perform in large number of samples. We did carry out a

comparative analysis of RTL by RT-PCR and Southern blot in around 100 samples to validate our measurement and found a good agreement between the two methods (Tarik et al., 2018). Our study had several strengths. Trained personnel collected anthropometric data at several times from birth till adulthood providing us with unique opportunity to study association of adiposity and weight gain at different phases of life with telomere length.

In conclusion, our finding suggests that birth size is not associated with leukocyte telomere length. Leukocyte telomere attrition was observed in those born prior to 37 weeks of gestational age possibly predisposing to chronic diseases.

ACKNOWLEDGMENT

We acknowledge Bhaskar Singh and Rajeshwari Verma for maintaining liaison with the cohort and NDBC team for recruitment of participant. The current study was funded by Department of Biotechnology, Ministry of science and technology, India.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

MT and LR was involved in the design of experiment, data analysis, and the writing of the manuscript. SKB contributed towards recruitment of the participants for the study. SS helped in statistical analysis. HPSS, NT, and AR contributed in the designing of the study and evaluation of manuscript.

ORCID

Mohamad Tarik <https://orcid.org/0000-0002-6385-3460>

REFERENCES

- Adair, L. S., Martorell, R., Stein, A. D., Hallal, P. C., Sachdev, H. S., Prabhakaran, D., ... Victora, C. G. (2009). Size at birth, weight gain in infancy and childhood, and adult blood pressure in 5 low- and middle-income-country cohorts: When does weight gain matter? *The American Journal of Clinical Nutrition*, 89(5), 1383–1392. <https://doi.org/10.3945/ajcn.2008.27139>
- Barker, D. J., Hales, C. N., Fall, C. H., Osmond, C., Phipps, K., & Clark, P. M. (1993). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): Relation to reduced fetal growth. *Diabetologia*, 36(1), 62–67. <https://doi.org/10.1007/BF00399095>
- Barker, D. J., Martyn, C. N., Osmond, C., Hales, C. N., & Fall, C. H. (1993). Growth in utero and serum cholesterol concentrations in adult life. *BMJ (Clinical Research Ed.)*, 307(6918), 1524–1527. <https://doi.org/10.1136/bmj.307.6918.1524>
- Barker, D. J., Osmond, C., Golding, J., Kuh, D., & Wadsworth, M. E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ (Clinical Research Ed.)*, 298(6673), 564–567. <https://doi.org/10.1136/bmj.298.6673.564>
- Barker, D. J., Winter, P. D., Osmond, C., Margetts, B., & Simmonds, S. J. (1989). Weight in infancy and death from ischaemic heart disease. *Lancet (London, England)*, 2(8663), 577–580.
- Bhargava, S. K., Sachdev, H. S., Fall, C. H. D., Osmond, C., Lakshmy, R., Barker, D. J. P., ... Reddy, K. S. (2004). Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *The New England Journal of Medicine*, 350(9), 865–875. <https://doi.org/10.1056/NEJMoa035698>
- Boccardi, V., Paolisso, G., & Mecocci, P. (2016). Nutrition and lifestyle in healthy aging: The telomerase challenge. *Aging*, 8(1), 12–15. <https://doi.org/10.18632/aging.100886>
- Buxton, J. L., Das, S., Rodriguez, A., Kaakinen, M., Couto Alves, A., Sebert, S., ... Blakemore, A. I. (2014). Multiple measures of adiposity are associated with mean leukocyte telomere length in the northern Finland birth cohort 1966. *PLoS ONE*, 9(6), e99133. <https://doi.org/10.1371/journal.pone.0099133>
- Cassidy, A., De Vivo, I., Liu, Y., Han, J., Prescott, J., Hunter, D. J., & Rimm, E. B. (2010). Associations between diet, lifestyle factors, and telomere length in women. *The American Journal of Clinical Nutrition*, 91(5), 1273–1280. <https://doi.org/10.3945/ajcn.2009.28947>
- Cawthon, R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Research*, 37(3), e21. <https://doi.org/10.1093/nar/gkn1027>
- Curhan, G. C., Willett, W. C., Rimm, E. B., Spiegelman, D., Ascherio, A. L., & Stampfer, M. J. (1996). Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*, 94(12), 3246–3250. <https://doi.org/10.1161/01.CIR.94.12.3246>
- Daniali, L., Benetos, A., Susser, E., Kark, J. D., Labat, C., Kimura, M., ... Aviv, A. (2013). Telomeres shorten at equivalent rates in somatic tissues of adults. *Nature Communications*, 4, 1597. <https://doi.org/10.1038/ncomms2602>
- de Rooij, S. R., van Pelt, A. M. M., Ozanne, S. E., Korver, C. M., van Daalen, S. K. M., Painter, R. C., ... Roseboom, T. J. (2015). Prenatal undernutrition and leukocyte telomere length in late adulthood: The Dutch famine birth cohort study. *The American Journal of Clinical Nutrition*, 102(3), 655–660. <https://doi.org/10.3945/ajcn.115.112326>
- Dlouha, D., Maluskova, J., Kralova Lesna, I., Lanska, V., & Hubacek, J. A. (2014). Comparison of the relative telomere length measured in leukocytes and eleven different human tissues. *Physiological Research*, 63(Suppl 3), S343–S350.
- Edwards, L. J., Coulter, C. L., Symonds, M. E., & McMillen, I. C. (2001). Prenatal undernutrition, glucocorticoids and the programming of adult hypertension. *Clinical and Experimental Pharmacology & Physiology*, 28(11), 938–941. <https://doi.org/10.1046/j.1440-1681.2001.03553.x>
- Entringer, S., Epel, E. S., Kumsta, R., Lin, J., Hellhammer, D. H., Blackburn, E. H., ... Wadhwa, P. D. (2011). Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, 108(33), E513–E518. <https://doi.org/10.1073/pnas.1107759108>
- Eriksson, J. G., Forsén, T., Tuomilehto, J., Osmond, C., & Barker, D. J. (2001). Early growth and coronary heart disease in later life: Longitudinal study. *BMJ (Clinical Research Ed.)*, 322(7292), 949–953. <https://doi.org/10.1136/bmj.322.7292.949>
- Frenck, R. W., Blackburn, E. H., & Shannon, K. M. (1998). The rate of telomere sequence loss in human leukocytes varies with age. *Proceedings of the National Academy of Sciences of the United States of America*, 95(10), 5607–5610. <https://doi.org/10.1073/pnas.95.10.5607>

- Goldenberg, R. L., Culhane, J. F., Iams, J. D., & Romero, R. (2008). Epidemiology and causes of preterm birth. *Lancet (London, England)*, 371(9606), 75–84. [https://doi.org/10.1016/S0140-6736\(08\)60074-4](https://doi.org/10.1016/S0140-6736(08)60074-4)
- Hofman, P. L., Cutfield, W. S., Robinson, E. M., Bergman, R. N., Menon, R. K., Sperling, M. A., & Gluckman, P. D. (1997). Insulin resistance in short children with intrauterine growth retardation. *The Journal of Clinical Endocrinology and Metabolism*, 82(2), 402–406. <https://doi.org/10.1210/jcem.82.2.3752>
- Jennings, B. J., Ozanne, S. E., Dorling, M. W., & Hales, C. N. (1999). Early growth determines longevity in male rats and may be related to telomere shortening in the kidney. *FEBS Letters*, 448(1), 4–8. [https://doi.org/10.1016/S0014-5793\(99\)00336-1](https://doi.org/10.1016/S0014-5793(99)00336-1)
- Kajantie, E., Pietiläinen, K. H., Wehkalampi, K., Kananen, L., Räikkönen, K., Rissanen, A., ... Hovatta, I. (2012). No association between body size at birth and leukocyte telomere length in adult life—Evidence from three cohort studies. *International Journal of Epidemiology*, 41(5), 1400–1408. <https://doi.org/10.1093/ije/dys127>
- Kiecolt-Glaser, J. K., Preacher, K. J., MacCallum, R. C., Atkinson, C., Malarkey, W. B., & Glaser, R. (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proceedings of the National Academy of Sciences of the United States of America*, 100(15), 9090–9095. <https://doi.org/10.1073/pnas.1531903100>
- Laganović, M., Bendix, L., Rubelj, I., Kirhmajer, M. V., Slade, N., Lela, I. V., ... Jelaković, B. (2014). Reduced telomere length is not associated with early signs of vascular aging in young men born after intrauterine growth restriction: A paradox? *Journal of Hypertension*, 32(8), 1613–1619; discussion 1619–1620. <https://doi.org/10.1097/HJH.0000000000000217>
- Martyn, C. N., Gale, C. R., Jespersen, S., & Sherriff, S. B. (1998). Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. *Lancet (London, England)*, 352(9123), 173–178. [https://doi.org/10.1016/S0140-6736\(97\)10404-4](https://doi.org/10.1016/S0140-6736(97)10404-4)
- Martyn, C. N., Meade, T. W., Stirling, Y., & Barker, D. J. (1995). Plasma concentrations of fibrinogen and factor VII in adult life and their relation to intra-uterine growth. *British Journal of Haematology*, 89(1), 142–146. <https://doi.org/10.1111/j.1365-2141.1995.tb08920.x>
- McTernan, C. L., Draper, N., Nicholson, H., Chalder, S. M., Driver, P., Hewison, M., ... Stewart, P. M. (2001). Reduced placental 11 β -hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: An analysis of possible mechanisms. *The Journal of Clinical Endocrinology and Metabolism*, 86(10), 4979–4983. <https://doi.org/10.1210/jcem.86.10.7893>
- Menon, R. (2014). Oxidative stress damage as a detrimental factor in preterm birth pathology. *Frontiers in Immunology*, 5(567). <https://doi.org/10.3389/fimmu.2014.00567>
- Pearce, M. S., Mann, K. D., Martin-Ruiz, C., Parker, L., White, M., von Zglinicki, T., & Adams, J. (2012). Childhood growth, IQ and education as predictors of white blood cell telomere length at age 49–51 years: The Newcastle Thousand Families Study. *PLoS ONE*, 7(7), e40116. <https://doi.org/10.1371/journal.pone.0040116>
- Radha, K. P., Ganga, B. G., & Venkateswarulu (2015). A study on maternal factors affecting low birth weight in institutional deliveries. *Journal of Dental and Medical Sciences*, 14(1), 45–48.
- Raqib, R., Alam, D. S., Sarker, P., Ahmad, S. M., Ara, G., Yunus, M., ... Fuchs, G. (2007). Low birth weight is associated with altered immune function in rural Bangladeshi children: A birth cohort study. *The American Journal of Clinical Nutrition*, 85(3), 845–852. <https://doi.org/10.1093/ajcn/85.3.845>
- Romero, R., Espinoza, J., Gonçalves, L. F., Kusanovic, J. P., Friel, L. A., & Nien, J. K. (2006). Inflammation in preterm and term labour and delivery. *Seminars in Fetal & Neonatal Medicine*, 11(5), 317–326. <https://doi.org/10.1016/j.siny.2006.05.001>
- Sankhla, M., Sharma, T. K., Mathur, K., Rathor, J. S., Butolia, V., Gadhop, A. K., ... Kaushik, G. G. (2012). Relationship of oxidative stress with obesity and its role in obesity induced metabolic syndrome. *Clinical Laboratory*, 58(5–6), 385–392.
- Savini, I., Catani, M. V., Evangelista, D., Gasperi, V., & Avigliano, L. (2013). Obesity-associated oxidative stress: Strategies finalized to improve redox state. *International Journal of Molecular Sciences*, 14(5), 10497–10538. <https://doi.org/10.3390/ijms140510497>
- Seckl, J. R., Cleasby, M., & Nyirenda, M. J. (2000). Glucocorticoids, 11 β -hydroxysteroid dehydrogenase, and fetal programming. *Kidney International*, 57(4), 1412–1417. <https://doi.org/10.1046/j.1523-1755.2000.00984.x>
- Smeets, C. C. J., Codd, V., Samani, N. J., & Hokken-Koelega, A. C. S. (2015). Leukocyte telomere length in young adults born preterm: Support for accelerated biological ageing. *PLoS ONE*, 10(11), e0143951. <https://doi.org/10.1371/journal.pone.0143951>
- Song, Y., Huang, Y.-T., Song, Y., Hevener, A. L., Ryckman, K. K., Qi, L., ... Liu, S. (2015). Birthweight, mediating biomarkers and the development of type 2 diabetes later in life: A prospective study of multi-ethnic women. *Diabetologia*, 58(6), 1220–1230. <https://doi.org/10.1007/s00125-014-3479-2>
- Stettler, N., Kumanyika, S. K., Katz, S. H., Zemel, B. S., & Stallings, V. A. (2003). Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. *The American Journal of Clinical Nutrition*, 77(6), 1374–1378. <https://doi.org/10.1093/ajcn/77.6.1374>
- Stettler, N., & Stallings, V. A. (2002). Adult obesity and growth in childhood. Association of birth weight with adult weight is confounded by maternal body mass index. *BMJ (Clinical Research Ed.)*, 324(7338), 674.
- Strohmaier, J., van Dongen, J., Willemsen, G., Nyholt, D. R., Zhu, G., Codd, V., ... Martin, N. G. (2015). Low birth weight in MZ twins discordant for birth weight is associated with shorter telomere length and lower IQ, but not anxiety/depression in later life. *Twin Research and Human Genetics: The Official Journal of the International Society for Twin Studies*, 18(2), 198–209. <https://doi.org/10.1017/thg.2015.3>
- Tarik, M., Ramakrishnan, L., Sachdev, H. S., Tandon, N., Roy, A., Bhargava, S. K., & Pandey, R. M. (2018). Validation of quantitative polymerase chain reaction with southern blot method for telomere length analysis. *Future Science OA*, 4(4), FSO282. <https://doi.org/10.4155/fsoa-2017-0115>
- Tarry-Adkins, J. L., Chen, J. H., Smith, N. S., Jones, R. H., Cherif, H., & Ozanne, S. E. (2009). Poor maternal nutrition followed by accelerated postnatal growth leads to telomere shortening and increased markers of cell senescence in rat islets. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 23(5), 1521–1528. <https://doi.org/10.1096/fj.08-122796>
- Tarry-Adkins, J. L., Martin-Gronert, M. S., Chen, J. H., Cripps, R. L., & Ozanne, S. E. (2008). Maternal diet influences DNA damage, aortic telomere length, oxidative stress, and antioxidant defense capacity in rats. *The FASEB Journal*, 22(6), 2037–2044. <https://doi.org/10.1096/fj.07-099523>
- Ulaner, G. A., Hu, J. F., Vu, T. H., Giudice, L. C., & Hoffman, A. R. (2001). Tissue-specific alternate splicing of human telomerase reverse transcriptase (hTERT) influences telomere lengths during human development. *International Journal of Cancer*, 91(5), 644–649. [https://doi.org/10.1002/1097-0215\(200002\)9999:9999::AID-IJC1103>3.0.CO;2-V](https://doi.org/10.1002/1097-0215(200002)9999:9999::AID-IJC1103>3.0.CO;2-V)

- Weischer, M., Bojesen, S. E., & Nordestgaard, B. G. (2014). Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. *PLoS Genetics*, 10(3), e1004191. <https://doi.org/10.1371/journal.pgen.1004191>
- World Health Organization (WHO). (2018). Noncommunicable diseases country profiles 2018, (online). <https://apps.who.int/iris/bitstream/handle/10665/274512/9789241514620-eng.pdf?ua=1>

How to cite this article: Tarik M, Ramakrishnan L, Sinha S, et al. Association of birth outcomes and postnatal growth with adult leukocyte telomere length. *Matern Child Nutr.* 2019; e12857. <https://doi.org/10.1111/mcn.12857>