ORIGINAL ARTICLE

ASSOCIATION OF MOTHER'S BLOOD GROUP WITH CORD BLOOD RELATIVE TELOMERE LENGTH

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ABSTRACT

Background: Cord blood relative telomere length(RTL), the biological chronometer which can determine life span, has been found highly variable at birth due to fetal programming under the influence of different risk factors and markers of biological aging. This study was designed to compare RTL of maternal and new born and its association with blood groups of mothers as well as other related maternal and new born parameters.

Methods: Maternal and cord RTL's were measured in 250 samples using a quantitative real-time PCR method. Correlation was used to check the association of maternal and cord blood RTL. Kruskal Wallis was used to examine the comparison of cord blood RTL with maternal demographics.

Results: Maternal mean age (Mean±S.D) was 27.17± 5.11 and paternal mean age was 34.03 ± 4.90. Maternal and cord RTL showed 0.01 level of significance at 95% confidence level. Cord blood RLT kilo base pair (kbp) (6.76± 1.35) was higher than maternal RTL (6.43± 1.35).Maternal blood groups were distributed highest (n=84, 33%) as B+ve and the lowest (n=1, 0.4%) as B-ve. Regarding maternal telomere length the longest was (6.68 ±1.34) in A^{+ve} group and A^{-ve} had the smallest length 5.32 ±0.95, however the results were not statistically significant (0.134). On contrary cord blood RTL was longest in O^{-ve} blood group, 6.95±0.56and smallest in B^{-ve} 5.41 RTL with p-value 0.159.

Conclusion: Cord Blood RLT was higher than in maternal blood in target population of Karachi, Pakistan. The longest maternal RTL was in A^{+ve} and smallest in O^{+ve} cord blood. Majority of mothers were B^{+ve} followed by O^{+ve}.

Key words: Telomere; Cord Blood; Blood groups; Parity; Gravidity.

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INTRODUCTION

Human telomeres, long non-coding tandem repeat sequences (TTAGGG), comprise less than 1% of the total genome measuring almost 150 million base pairs[1].Being markers of biological aging, they are viewed as a 'mitotic clock', which shortens during every cell division in somatic cells (not in stem cell, germ cell and cancerous cells) due to the failure of DNA polymerase to complete synthesis of lagging strand, this is termed as "end replication problem". Prenatal stress, considered as one of the exposome and modified environmental factors, cause telomeres reduction and play a significant role in determining the quality of life[2].Among other factors are energy intake, obesity, sleep quality, emotional health, intrauterine tobacco exposure, and sedentary lifestyle all of which can also influence pregnancy and subsequently the fetus.

Intrauterine period, the time of fetal cell proliferation, maturation, differentiation responds drastically to external or internal environmental insults. Maternal Epigenetics under the pressures of environmental exposure, qualification and health status can influence the phenotype of the growing child by genetic programming of fetus. Placental functionality can prompt fetal gene expression modifications due to endocrine disorders, toxins, infectious agents, maternal nourishment status. Hence, decreased telomere length will increase risk of cardiovascular, hypertension, COPD, type 2 Diabetes, hyperglycemia and metabolic diseases[3]especially during the first two years of child

life which are critical period for developing brain and immune system. This can increase the possibility of associated cancers and mortality in their later life. Shorter telomeres are also observed in the daughters of depressive mothers because of greater cortisol response to stress[4].

Significant association between blood group antigens and risk of various diseases such as ischemic stroke, myocardial infarction, vascular disorders, arterial thromboembolism, coronary heart disease and sickle cell anemia due to blood groups has been reported[5]. Spontaneous abortion is also one of the most common risk factors for new born due to their blood antigens. Hemolytic diseases in newborn are due to the molecular disturbances in the Rh (Rhesus) (RHD and RHCE) genes. Rh proteins (D, CcEe) formed from these genes are expressed in RBC and are involved in the formation and stability of erythrocyte membrane. Assessment of RhD-negative individuals through PCR during pregnancy revealed deletion of RhD gene[6].

The relationship of blood groups with pregnancy outcome, illustrates the involvement of telomeres and this investigation could add blood groups genetics to the data of risk factors. This study was designed to compare RTL of maternal and new born infants and its association with blood groups of mothers and evaluate other parameters related to maternal and new born that would influence the cord blood RTL.

METHODS

Pregnant females and their newborns (n= 250) were included in the study from Ziauddin Medical Hospital, Karachi from May to October, 2018. It was a cross sectional study and samples were collected through consecutive sampling technique after approval from Ethics Review Committee (ERC) of Ziauddin University and Hospital. Females with age 18-35 years and gestational age 37 weeks or above were included in the study. Questionnaire was filled after taking the informed consent from patients or their attendants'. Detailed information of maternal characteristics, birth weight, gestational age, infant sex and mode of delivery was obtained from hospital record registers. Females with any known malignancies or known diabetes mellitus were not included in the study.

Firstly, the 5 reference DNA(adults) were used to draw a standard curve with concentrations ranging from 0.1 to 12 ng/µl. Venous blood (3-5 ml) samples from pregnant females were collected in ethylenediaminetetraacetic acid (EDTA) tubes before delivery. Umbilical cord blood (3-5 ml) samples were collected immediately postpartum into EDTA tubes from the cord when it was still in contact with maternal placenta. Samples were then transported to laboratory and stored at -20°C.DNA extraction was done by EZ-10 spin column genomic DNA kit(BioBasic Canada Inc.). The concentration of each DNA sample was confirmed by using Qubit dsDNA HS Assay kit at 260 nm and 280 nm (Qubit 2.0 Invitrogen life technologies USA) on Qubit spectrophotometer. Gradient SimpliAmp conventional thermal cycler (Applied Biosystem) was used to optimize temperature before the assay was moved onto quantitative analysis by real time PCR. Primer sequences for telomere and single-copy gene (β globin) amplification were:

Tel F, 5'GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT3';TelR,5'TCCCGA-CTATC-CCTATCCCTATCCCTATCCCTATCCCTA3';HBG F, 5'GCTTCTGACACAAC-TGTGTTCACTAGC 3';HBG R,5'CACCAACTTCATCCACGTTCACC 3'7. All PCR products were analyzed on 2% agarose gel electrophoresis. Real time PCR or Quantitative PCR (qPCR) was done by using Platinum SYBR Green qPCR SuperMix-UDG, (Invitrogen). In all runs, the reference DNA was loaded to control the inter-plate variation. All samples were assayed in duplicate wells and average values of two measurements were used for the statistical analyses.Each reaction well contained 30 µl reaction mixture:15µl of master mix, 2.5 µl of 10uM forward primers, 2.5 µl 10uM reverse primers, 1 µl ROX dye 10 µl DNA (10 ng) template was used. This method was an extension of basic PCR including relative quantification of target DNA by using (cycle threshold) ct(Step One software(v2.3)) values. Temperatures for telomere PCR: first holding stage for 50 °C for 2 min and 95 °C for 2 min, then 40 cycles were set at denaturation 95°C for 15 sec, annealing at 68°C for 40 sec and extension 75°C for 3 min and second holding for 72 °C for 40 sec. For β globin reference gene PCR: first holding stage for 50 °C for 2 min and 95 °C for 2 min, then 40 cycles were set at denaturation 95°C for 15 sec, annealing at 56°C for 40 sec and extension 75°C for 3 min and second holding for 72 °C for 40 sec. To measure relative telomere length of maternal and cord blood samples T/S ratio was calculated.

T/S= ct telomere/ ct single copy gene (reference gene)

To convert T/S ratio to base pairs (bp) by using another formula8

(3,274 + 2,413 * (T/S))

Statistical analysis: SPSS version 20 was used for data analysis. Quantitative variables considered in study were presented by Mean± SD and qualitative variables were presented by frequency and percentages. Spearmen Correlation was used to check the correlation of maternal and cord blood RTL at 95% confidence level. Kruskal Wallis test was used to examine the comparison of cord blood RTL with maternal demographics. Mann Whitney U test was also used to check the comparison between two groups.

RESULTS

Demographic characteristics of 250 mother-new-

born pairs are reported in Table 1. Maternal mean age (Mean±S.D.)was27.17± 5.11and paternal mean age was 34.03 ± 4.90. Females (n=73, 29 %) had single gravida whereas (n= 61, 24%) had 2 gravida and (n= 60, 14%) females reported more than 6 gravida. Negative correlation (-0.123) was seen between gravida and cord blood RTL having insignificant p-value (0.059).Decrease in RTL of newborns was found with increase in gravida (Table 2).

Table 1: Characteristics of female-newborn and relative telomere length (RTL) of maternal and cord blood.

Variables									
	Age in years of parents (Mean±SD)		Gestational Age in weeks		Mode of delivery n%		Newborn gender n%		Newborn weight (kg) (Mean±SD)
	Maternal	Paternal	Range	n (%)	SVD	LSCS	Girl	Boy	
Assessment values	27.17 ±5.11	34.03 ±4.90	36-38	80(32)	150 (60)	100 (40)	133 (53)	117 (47)	2.99 ±0.48
			39-42	170(68)					
p-value	0.217	0.846	0.946		0.054		0.74		0.11

Table 2: Mean difference of RTL of maternal and cord blood between gravida, parity and miscarriages.

Gravidity	Frequency n(%)	Maternal Blood RTL (Mean ±SD)	*p*-value	Cord Blood RTL (Mean ±SD)	*p*-value
1	73(29)	6.59±1.04		6.9±1.41	
2	61(24)	6.54±1.26		6.8±1.6	
3	49(19)	6.45±1.26	0.22	6.7±1.36	0.522
4	27(11)	6.45 ±1.0		6.3±0.7	
>5	40(16)	6.01±0.85		6.4±1.06	
Parity					
1	84(33)	6.56±1.01		6.89±1.4	
2	60(24)	6.54 ±1.14		6.85±1.4	
3	54(21)	6.54±1.12	0.185	6.68±1.26	0.641
4	20(8)	6.14±1.20		6.51±0.81	
>5	31(12)	5.5±0.86		5.61±0.4	
Miscarriages					
0	197(79)	6.46 ±1.1		6.78±1.42	
1	35(14)	6.30 ±0.9		6.70±1.22	
2	11(4)	6.27 ±1.07		6.79±0.69	
3	4(1.6)	6.57 ±0.7	0.341	6.24±0.81	0.978
4	3(1)	5.6 ±0.7		6.58±0.42	

Females with single parity (n=84, 33%) and(n=31,12%) had more than 6 children. Mean difference among parity and newborn RTL was also not significant (0.641) but as in gravida with increase in parity decrease RTL was seen in newborns (Table 2).No miscarriages were seen in (n=197, 79%) study females (Table 2).New born characteristics had revealed greater than 38-week gestational age babies (n=170, 68 %) with cord blood RTL 6.72±1.37 and (35-37) weeks babies (n=80,32%) had RTL 6.82±1.36.The mean birth weight of the 250 newborns was 2.99 ±0.480 indicating the normal weight of the newborns. Most of the delivery cases were spontaneous vaginal delivery (SVD) (n=150, 60%) with RTL 6.62±1.12 and (n=100,40%) cases were lower segment cessation section (LSCS) having RTL 6.97±1.63 with mean difference <0.05 (Table 1). On comparison among newborns, girls(n=133, 53%) were greater in number than boys (n=117, 47%), having longer RTL 6.77±1.39 as compared to boys 6.75±1.32. The mean RLT(kbp) in cord blood 6.76± 1.35 was higher than maternal blood 6.43± 1.35 at 95% confidence level. The association of maternal cord RTL had co-efficient of Correlation of 0.34and statistically significant P-value <0.05 (Figure 1).

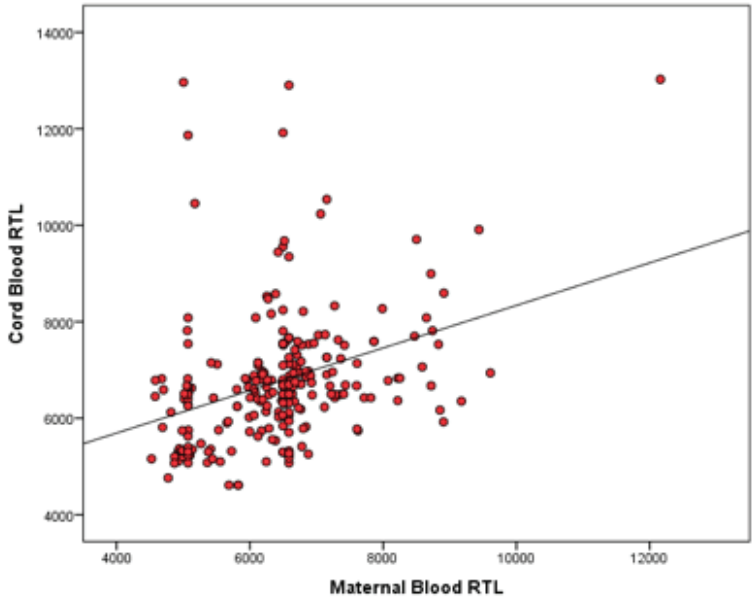

Figure 1: Scatter plot between cord RTL and maternal RTL present the relationship between maternal and cord RTL. There was a significant correlation with the <0.05 level of significance.

In this study we found B+ve blood group females (n=84, 33 %) were predominant. O+ve(n=69, 27%) was second highest and only a single participant (n=1) had B-vegroup. Taking into account of maternal blood RTL A+ve (n=60, 24%) had 6.68 ±1.34 longest telomeres among all groups followed byB+ve (n= 84, 33.6%) 6.40 ±0.93 and O–ve (n=4,1%) 6.40 ±0.70. Females (n=7, 3%) having A-ve had the smallest length 5.320±0.95 and there was no significant difference among RTL and blood groups as the p value was >0.05. Whereas in cord blood RTL O–ve blood group 6.93±0.56 had maximum length among all the groups and B-ve 5.41 RTL was smallest and also revealed no significance, p value >0.05. (Table 3)

Table 3: Maternal and cord blood RTL association with blood group

Blood group	n(%)	Maternal RTL(kbp) (Mean ±SD)	*p*-value	Cord Blood RTL(kbp) (Mean ±SD)	*p*-value
A +ve	60 (24)	6.68±1.34		6.79±1.49	
A -ve	7 (3)	5.32 ±9.50		5.65±7.04	
B +ve	84(33.6)	6.40±9.34		6.75±1.27	
B -ve	1 (0.4)	NA	0.134	5.41	0.159
O +ve	69 (28)	6.36 ±1.01		6.83±1.49	
O -ve	4 (1.6)	6.40 ±7.02		6.95±0.56	
AB +ve	25 (10)	6.36 ±9.67		6.87±1.09	

***NA: not available**

On computing ct values we found maternal blood average delta ct(cycle threshold) was 2.40 ±3.66 which indicated that telomere gene require 2.4 more cycles of qPCR to produce the same fluorescence as by single copy gene (b-globin). For cord blood average delta ct 3.57 ± 4.51specified that telomere gene required 3.5 more cycles (Figure 2).

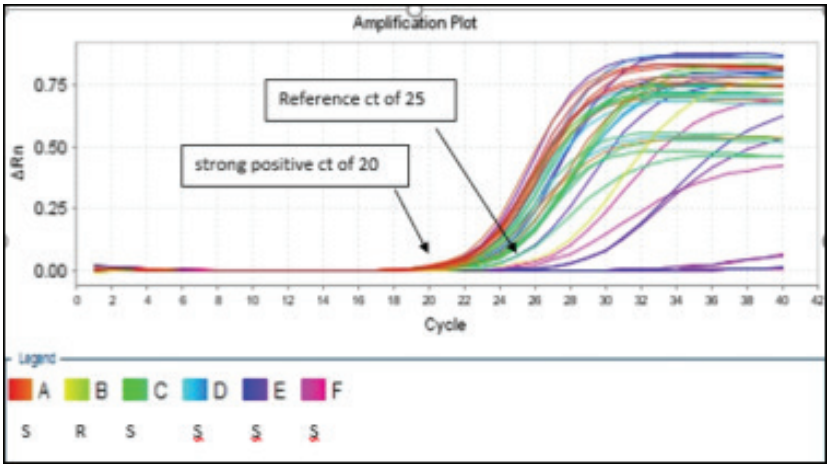

Figure 2: Amplification plot of telomere.

Reference DNA sample ct of 25 and ct values of telomere ranging from minimum 20 to maximum 28 with average ct 25.6 ± 3.7 and variance 14%.

DISCUSSION

To best of our knowledge, this is the novel study that showed the comparison between blood groups and RTL of mother and cord blood. The study statistics highlighted blood group B^{+ve} as the most prevalent in the targeted population. Regarding maternal telomere length the longest was 6.68 ±1.34in A^{+ve} group and the smallest length was 5.32 ±0.95 in A^{-ve} whereas in cord blood RTL O^{-ve} had longest 6.93±0.56 length and B^{-ve} had shortest5.41 RTL. Mostly researchers have studied the association of blood groups with different diseases. Fagherazzi et al. found disease (type 2 diabetes mellitus) risk association with blood groups especially B^{+ve} and AB^{+ve} mothers[9]. Therefore, mostly females of this study had B^{+ve} and could be more prone to risk of diseases as compared to O^{+ve} which had lower risk[10]. On contrary, in our study Gestational Diabetic patients [n=18(7%)] showed no risk association(0.246) with blood groups and RTL. Although strong relationship between diseases like uterine cancer, heart diseases, type 2 diabetes etc. with blood groups has been highlighted by multiple studies[11, 12].

The maternal and cord blood RTL had moderate association and significant results gave the wide range of maternal blood RTL(kbp)4.52-12.1whereas, cord blood RTL was 4.61- 13kbp in Karachi population. This is in accordance to the data which gave the range of 10 to 15 kb in general population[13]. Study by Weng Q et al. had also discovered 15 % longer telomeres in cord than maternal which was similar to our study[14].

Maternal and paternal age had no association with newborn cord RTL and the results were statistically insignificant (0.217, 0.846). These findings could be supported by multiple researches on mother age and decrease telomerase activity in mature ova and reduction in primordial follicle in female with age. However, studies have reported (p-value = 0.001) influence of paternal age on cord blood RTL due to presence of telomerase activity in sperms[15]. In our respective study shorter RTL was perceived in parous and multi-parous compared to nulliparous females as was observed in another research, a 4.2% shorter T/S ratio in parous compared to nulliparous women. Similar study gave positive association with 116 fewer base pair on average[16]with parity but our study didn't have shown any association and insignificant results (0.159). However, null parity had been found affiliated with decreased risk of morbidity and mortality due to less exposure to the risk factors involved in pregnancies leading to stable telomere biology.

When cellular division slows, it undergoes a progressive deterioration known as senescence, which is commonly referred as aging. A study reported 25 % decrease in placental RTL during the third trimester of gestation. This exposed the effect of gestational age on RTL and possibly role of aging started before birth[17]. Nevertheless, there was no significant mean difference(0.946) seen among different gestational ages in this research. Although decrease of RTL (7.20-6.72) with increase in gestation (36-42) was observed. Studies reported results of longer telomeres in preterm than newborns with large gestational age(LGA)[18]. Our study had a support of research done previously on low birth weight infants and rapid telomere deletion of 240 bp per week during pregnancy[19]. LGA in

newborns may perhaps increase the risk of different diseases: polycythemia, hypoglycemia, diabetes and obesity after birth and later in adulthood[20]. Due to disturbance in RTL fetal programming takes place which can cause metabolic disturbance and modified tissue development. Further at molecular level it could damage DNA, protein and lipids and might leads to new altered genetics which can result in adverse outcomes. In this study we found negative correlation (-0.101) with newborn birth weight and RTL, however, p value 0.119 was not significant. In accordance to above, Vasu et al. results also revealed negatively correlation (p = 0.001)[21].

Comparison between SVD and LSCS showed smaller RTL (6.62±1.12) in 60% SVD compared to 40% LSCS (6.97±1.63) deliveries having no noteworthy(0.74) results. A previous study has also found no significant association between modes of delivery[14]and detected the telomere length of 7.85 kb in normal delivery newborns and 7.61 kb in cesarean section which are not consistent with our results[22].Almanzar, research on stress-associated with delivery modes in 2015 showed an increase in immune cells (neutrophils, leukocytes, and NK cells) in SVD, whereas, LSCS, a more stress-associated delivery modes have less circulating T cells with high Interleukin-7.Such babies could be more prone to neonatal infectious diseases due to changes in immune system and decrease in the protective effect of RTL[23].

In our study positive ΔC_t results were observed and indicated that reference gene is more compared to target gene unlike Cawthon results had negative results (–9.05) which revealed that their reference gene PCR requires nine more of PCR cycles than the telomere PCR cycles[7].Females involved in smoking and other risk factors like gutka, chalia, which can be confounders to reduce RTL, were not spotted in the target population.

Moreover, the relationship between newborn RTL and mother blood groups had been evaluated for the first time in Karachi population but no significant association was foundin our current study. More in depth studies are still required to search the relationship between maternal factors and newborn RTL.

CONCLUSION

We have used quantitative PCR for measurement of RTL of maternal and cord blood and calculated telomere lengths in kilo base pair from T/S ratio. This study identified the telomere genetics in Pakistani females-newborns and their role in the molecular genetics of multiple diseases and aging. Cord blood RTL was longer than maternal intargeted population and had significant association. Our study did not support the idea of association between maternal blood groups and RTL of mother and cord blood.

LIMITATIONS

Mean difference between mother- newborn variables and cord blood RTL had insignificant results which can be due to the small sample size. We were not able to take father blood samples so we could not further calculate the association of father-newborn RTL, which could be important for the detection of inheritance pattern of telomeres. Further investigations on maternal health condition could also be helpful in future data.

FUTURE RECOMMENDATIONS

Longitudinal cohort studies should be done to monitor all the risk factors and evaluating the telomerase level in the blood. Southern blotting should be done to validate the results.

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REFERENCES

1. Blackburn EH, Epel ES, Lin J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. Science 2015;350(6265):1193-8.
2. Steptoe A, Hamer M, Lin J, Blackburn EH, Erusalimsky JD. The longitudinal relationship between cortisol responses to mental stress and leukocyte telomere attrition. J Clin Endocrinol Metabol 2016;102(3):962-9.
3. Barker DJ. Adult consequences of fetal growth restriction. Clin Obstet Gynecol 2006;49(2):270-83.
4. Gotlib IH, LeMoult J, Colich NL, Foland-Ross LC, Hallmayer J, Joormann J, et al. Telomere length and cortisol reactivity in children of depressed mothers. Mol Psychiatry 2015;20(5):615-20.
5. Zakai NA, Judd SE, Alexander K, McClure LA, Kissela BM, Howard G, et al. ABO blood type and stroke risk: the RE asons for Geographic And Racial Differences in Stroke Study. J Thromb Haemost 2014;12(4):564-70.
6. Le Van Kim C, Colin Y, Cartron JP. Rh proteins: key structural and functional components of the red cell membrane. Blood Rev 2006;20(2):93-110.
7. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30(10):e47.
8. Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, et al. Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999–2002. Soc Sci Med 2013;85:1-8.
9. Fagherazzi G, Gusto G, Clavel-Chapelon F, Balkau B, Bonnet F. ABO and Rhesus blood groups and risk of type 2 diabetes: evidence from the large

E3N cohort study. Diabetologia 2015;58(3):519-22.
10. Franchini M, Favaloro EJ, Targher G, Lippi G. ABO blood group, hypercoagulability, and cardiovascular and cancer risk. Crit Rev Clin Lab Sci 2012;49(4):137-49.
11. Sun X, Nichols HB, Tse CK, Bell MB, Robinson WR, Sherman ME, et al. Association of parity and time since last birth with breast cancer prognosis by intrinsic subtype. Cancer Epidemiol Prev Biomarkers 2016;25(1):60-7.
12. Mueller NT, Mueller NJ, Odegaard AO, Gross MD, Koh WP, Yuan JM, et al. Higher parity is associated with an increased risk of type-II diabetes in Chinese women: the Singapore Chinese Health Study. BJOG 2013;120(12):1483-9.
13. Zhu X, Han W, Xue W, Zou Y, Xie C, Du J, et al. The association between telomere length and cancer risk in population studies. Sci Rep 2016;6:22243.
14. Weng Q, Du J, Yu F, Huang T, Chen M, Lv H, et al. The known genetic loci for telomere length may be involved in the modification of telomeres length after birth. Sci Rep 2016;6:387296.
15. Saretzki G, Von Zglinicki T. Replicative aging, telomeres, and oxidative stress. Ann N Y Acad Sci 2002;959(1):24-9.
16. Pollack AZ, Rivers K, Ahrens KA. Parity associated with telomere length among US reproductive age women. Hum Reprod 2018;33(4):736-44.
17. Gielen M, Hageman G, Pachen DM, Derom C, Vlietinck R, Zeegers MP. Placental telomere length decreases with gestational age and is influenced by parity: a study of third trimester live-born twins. Placenta 2014;35(10):791-6.
18. Friedrich U, Schwab M, Griese EU, Fritz P, Klotz U. Telomeres in neonates: new insights in fetal hematopoiesis. Pediatr Res 2001;49(2):252.
19. Lee SP, Hande P, Yeo GS, Tan EC. Correlation of cord blood telomere length with birth weight. BMC Res Notes 2017;10(1):469.
20. Kirley K, Shalowitz M. Early growth patterns associated with cardiovascular disease. Curr Cardio Risk Rep 2013;7(2):147-53.
21. Vasu V, Turner KJ, George S, Greenall J, Slijepcevic P, Griffin DK. Preterm infants have significantly longer telomeres than their term born counterparts. PloS one 2017;12(6):e0180082.
22. Garcia-Martin I, Janssen AB, Jones RE, Grimstead JW, Penketh RJ, Baird DM, et al. Telomere length heterogeneity in placenta revealed with high-resolution telomere length analysis. Placenta 2017;59:61-8.
23. Almanzar G, Schönlaub J, Hammerer-Lercher A, Koppelstaetter C, Bernhard D, Prelog M. Influence of the delivery modus on subpopulations and replication of lymphocytes in mothers and newborns. Early Hum Dev 2015;91(12):663-70.