Preterm Birth is Associated with Reduction of Maternal Telomere Length During Pregnancy

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Abstract

Background

Development of methods to detect risk of preterm birth in pregnant women can result in reduction in occurrence of preterm birth. Our objective was to undertake a longitudinal study to investigate the association of telomere length shortening in peripheral blood DNA of women during pregnancy with preterm birth.

Methods

Telomere length reduction in peripheral blood DNA was measured at two time points by Real Time PCR in pregnant women enrolled in a large prospective cohort (GARBH-Ini) in Gurugram, India. Out of 248 women who participated in this study, 60 mothers delivered preterm while 188 mothers delivered at term.

Results

Telomere length was found to be significantly reduced in preterm birth delivering mothers compared to those who delivered at term at less than 20 weeks ($p=0.02$) and at 26-28 weeks ($p=0.84 \times 10^{-15}$) of pregnancy. Predictive modelling by binary logistic regression analysis revealed that reduction of telomere length (T/S ratio) at 26-28 weeks was found to be significantly ($p=7.7181 \times 10^{-06}$) associated with enhanced risk of preterm birth. A mean odds ratio of 8.4 was found for association of T/S ratio with preterm birth outcome. There was no significant difference in maternal age between the mothers who delivered preterm and those who delivered at term.

Conclusions

Our preliminary results indicate that telomere length reduction in maternal peripheral blood DNA might be a potential indicator of risk of preterm delivery.

Background

Telomeres of human chromosomes comprise repeats of TTAGGG sequence that cap chromosomes and play an important role in maintaining genomic integrity [1]. The normal process of cell division reduces the number of repeats in the telomere of a chromosome; i.e., reduces telomere length. The reduction is replenished by the activity of the enzyme Telomerase. Disruptions in this telomere length homeostasis results in telomere length shortening that in turn acts as a sensor of replicative senescence and cellular aging [2]. Associations between inhibition of telomerase activity and a number of physiological outcomes and psychosocial exposures like sustained stress, pregnancy complications, etc. have been previously reported [3-5].

Preterm birth (PTB), defined by the World Health Organization as birth at less than 37 completed weeks of gestation, is estimated to account for 14.9 million or 11.1% of all births worldwide [6,7]. Although survival

rates are improving, recent data suggest that PTB remains a risk factor in half of all neonatal deaths and that 1 million deaths each year are a direct result of complications associated with preterm birth [6,8,9]. Furthermore, immediate and long term morbidity rates among those who survive have remained high and correlates well with the degree of prematurity [6,10,11,12,13]. Development of methods to detect risk of PTB in pregnant women, especially during early pregnancy, can enable subjecting them to appropriate interventions, thereby reducing the risk. This might enable reduction in the incidence of PTB and result in birth of babies at term who thrive in later life.

While telomere length reduction is a biological phenomenon that is associated with many adverse health conditions, including various complications associated with pregnancy, the association between maternal telomere length and preterm birth has not been investigated. We hypothesized that during the period of pregnancy women in whom there is a significant reduction in telomere length will be at an enhanced risk to deliver preterm. In other words, women who have given birth preterm will have a significantly greater mean reduction in telomere length compared to women who have given birth at term. To test this hypothesis, we have studied pregnant women from a large pregnancy cohort in India [14]. We have assayed telomere length in these women at two time points during pregnancy. Our results indicate that telomere length reduction in maternal peripheral blood during pregnancy is associated with enhancement of risk of preterm birth.

Methods

Sample Collection

This prospective observational study was approved by the Ethics Committees of Civil Hospital, Gurugram; Translational Health Science and Technology Institute, Faridabad, and National Institute of Biomedical Genomics, Kalyani. Consecutive pregnant women, recruited in the Interdisciplinary Group for Advanced Research on Birth Outcomes—DBT India Initiative cohort (GARBH-Ini cohort) [14], who provided written informed consent for this study, were enrolled before 20 weeks of gestation, based on self-reported information on last menstrual period and confirmed by a dating ultrasonography. They were followed up until delivery at Gurugram Civil Hospital or at the referral Safdarjung Hospital site, but were excluded from the study if they had an abortion, a miscarriage, or any pregnancy-related health complication. Maternal peripheral blood samples were collected at two time points during pregnancy (<20 weeks and 26-28 weeks). We have analyzed the results obtained for 248 women (approximately in 1:3 ratio of preterm and at term deliveries respectively) which we present in this report. Of them, 60 mothers delivered preterm (spontaneously before 37 weeks of gestation) and 188 mothers delivered at term (on or after 37 weeks, i.e., 259 days).

Telomere length reduction assay

Genomic DNA was extracted from peripheral blood collected in vacutainers containing EDTA (Becton Dickinson) using QIAamp Blood midi kit (Qiagen) and quantitated using Qubit fluorometer (Thermo). Telomere length reduction was measured using Real Time PCR as described previously [15,16]

Amplification of telomere and single copy gene was achieved using SYBR Green PCR Mastermix (Thermo) and 2.50 ng of genomic DNA (both sample and standards) in a total reaction volume of 20µl in QuantStudio-7flex Real Time PCR System (Thermo). Each unknown and standard DNA sample was assayed in duplicate. Relative telomere length was expressed as telomere to single copy gene ratio (T/S ratio) and was calculated using standard curves for both PCR targets.

Statistical Analyses

Test for normality was performed by the Kolmogorov-Smirnov test. To test equality of mean values between women who delivered preterm and at term of age, period of gestation (POG), and log transformed T/S ratio (telomere to single copy gene ratio) at less than 20 weeks and during 26-28 weeks of POG, t tests were performed. For predictive modelling of risk of preterm birth, binary logistic regression analysis was performed with probability of preterm birth outcome as dependent variable and T/S ratio at two times points as independent variables.

$$\log [p/(1-p)] = \alpha + \beta_1 * X_1 + \beta_2 * X_2,$$

where, p denotes the probability that a woman delivers preterm, and X_1 and X_2 denote, respectively, the T/S ratio at or around 20 weeks and during 26-28 weeks. To estimate the enhancement of risk of PTB, and test its statistical significance, with reduction in T/S ratio, we used an empirical resampling approach. We randomly resampled 60 women who delivered pre-term and 60 (out of 188) women who delivered at term. For these 120 women, we estimated the threshold of the T/S distribution below which there were 10% of observations; that is, the lowest decile (t_{10}) of the T/S distribution. We then calculated the risk of PTB for a T/S value below this decile, by calculating the Odds Ratio (OR) as the ratio of the number of women with T/S value below t_{10} who delivered preterm to those who delivered at term. We repeated this resampling and estimation of OR five thousand times, and computed the mean (OR_{10}) and standard deviation (SD_{10}) of OR values. We repeated the same procedure to calculate (OR_{20} , SD_{20}), (OR_{30} , SD_{30}), etc. Final inference on risk of PTB with reduction in T/S ratio was made by examining the trend of mean OR values with increasing deciles.

Results

The distribution of log transformed T/S ratio of study participants at two time points during pregnancy is shown in Figure 1. Inter-experiment variation (%CV) of Real Time PCR assay for T/S ratio was found to be 7%. Maternal age did not differ significantly ($p=0.3$) between these two groups of women. T/S ratio at both time points of assessment during gestation – around 20 weeks and between 26-28 weeks – were found to be significantly lower ($p=0.02$ and $p=0.84 \times 10^{-15}$ respectively) in women who delivered preterm compared to those who delivered at term (Table 1). Results of the predictive model are provided in Table 2. Reduction of telomere length (T/S ratio) at 26-28 weeks was found to be significantly ($p=7.7181 \times 10^{-06}$) associated with enhanced risk of preterm birth. We note that the reduction of telomere length starts early in pregnancy; even before 20 weeks of gestation, there is a trend of telomere shortening among

mothers who delivered preterm, although the extent of the same was not statistically significant ($p=0.4080$). The mean OR values with increasing decile-thresholds of T/S distribution turned out to be $OR_{10}= 8.4$ ($SD_{10}= 4.4$) and $OR_{20}= 5.6$ ($SD_{20}= 4.1$) respectively. Since the value of OR_{20} was much lower than OR_{10} , but both were significantly greater than 1.0, we concluded that the risk of PTB with decrease in T/S ratio was very high and exceeded 5-fold even with a moderate decrease in T/S ratio.

Discussion

The disruption of telomere homeostasis leading to shortening of telomere length is an effective sensor of allostasis and cellular senescence, ultimately resulting in health adversities [17,18]. Although preliminary reports of associations between newborn telomere length and birth outcome or exposure to neonatal stress have been published [19,20], studies on relationship between telomere length in maternal blood during pregnancy and birth outcome have not been undertaken.

We undertook a study embedded in a large prospective pregnancy cohort in India where we investigated telomere length shortening in maternal peripheral blood DNA and reduction in period of gestation resulting in preterm birth. We measured telomere length shortening in DNA at two time points during pregnancy. Our results indicate that telomere length shortening in the pregnant women is associated with preterm birth at both the time points; i.e. less than 20 weeks and 26 – 28 weeks of gestation; the association manifests as significant at later stages of pregnancy (26-28 weeks). Further, the risk of PTB is very high ($OR>5.0$) even with moderate levels of shrinkage of the telomere.

Maternal peripheral blood DNA was used as biospecimen for telomere length analysis. Since blood provides a unique potential window into health [21], our longitudinal study of telomere length shortening in peripheral blood DNA of women during pregnancy provides a valuable opportunity to monitor risk of preterm birth over time. If validated in an independent sample set, telomere length around the middle of pregnancy may serve as a noninvasive predictive biomarker of birth outcome.

While our ability to assess only a small number of women delivering preterm remains a limitation of our study, we have partially mitigated this by three-fold oversampling of women delivering at term. Maternal age did not differ significantly between the two groups of women. Since this was a pilot analysis with a restricted sample size, we were unable to study the effects of possible covariates, such as, socioeconomic status, nutrition and smoking status, etc.

Conclusions

We have analyzed telomere length shortening in pregnant women at less than 20 weeks and 26-28 weeks of gestation and found it to be significantly associated with preterm birth outcome at both time points. The same at 26-28 weeks was also significantly predictive of preterm birth outcome. Our preliminary results indicate that telomere length reduction in maternal peripheral blood DNA might be a potential indicator of risk of preterm delivery. Our findings have a translational potential. The assay of telomere

length reduction can be used as a predictive marker since it is based on Real Time PCR of a noninvasively collected biospecimen, i.e., maternal blood DNA, which can be easily performed in prenatal clinics to monitor pregnancy.

Declarations

Funding

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Conflict of interest

The Authors declare no conflict of interest. None of the Authors have any competing financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

Ethics approval

This prospective observational study was approved by the Ethics Committees of Civil Hospital, Gurugram; Translational Health Science and Technology Institute, Faridabad; National Institute of Biomedical Genomics, Kalyani.

Consent to participate

All participants have provided voluntary informed consent to participate in the study.

Consent to publish

All participants have provided voluntary informed consent to publish their data.

Availability of data and material

All data published in the study are available with the corresponding authors.

Code availability

All codes used in the study are available with the corresponding authors.

Authors' contributions

Study conceptualization and planning: AM, NW, PPM, SB. *Study implementation and management:* AM, NW. *Participant recruitment, collection of biospecimen and clinical data:* KG, BS, NW, SB. *Laboratory*

assays: Sumanta Sarkar, SG, Arpita Maity, AM. *Data analysis:* SS, AM, PPM. *Manuscript preparation:* AM, NW, SS, PPM, SB.

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22. Legends.

Tables

Table 1

Results of t test performed to test for significance of differences of variables between women delivering at term and those delivering preterm.

	Women who delivered term babies [Mean (Standard Deviation)] n = 188	Women who delivered preterm babies [Mean (Standard Deviation)] n = 60	p-Value of t-test for difference in mean values
Maternal Age (years)	22.9 (0.04)	23.4 (0.02)	0.30
Period of Gestation in days	275.2 (8.27)	243.9 (10.34)	0.89×10^{-9}
T/S Ratio (< 20 weeks)	1.47 (0.12)	1.27 (0.17)	0.02
T/S Ratio (26–28 weeks)	1.72 (0.10)	1.33 (0.12)	0.84×10^{-15}

Table 2

Results of predictive modelling by binary logistic regression analysis.

	Estimate	Std. Error	t-value	Pr(> t)
Intercept	2.561	0.734	3.488	0.0005
T/S Ratio (< 20 weeks)	-0.379	0.459	-0.827	0.4080
T/S Ratio (26–28 weeks)	-2.013	0.450	-4.473	7.7181×10^{-06}

Figures

Figure 1

Histogram of log transformed T/S ratio of study participants at (a) less than 20 weeks and at (b) 26-28 weeks