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Gingival crevicular fluid prostaglandin E₂ level as a predictor of preterm low birth weight: a pilot investigation

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Abstract: Periodontal infections, which serve as a reservoir of inflammatory mediators such as prostaglandin E₂ (PGE₂), may pose a threat to the fetal-placental unit and cause preterm delivery. This study was conducted to estimate the levels of PGE₂ in gingival crevicular fluid (GCF) and serum to explore the possible use of the GCF-PGE₂ level as a risk predictor of preterm low birth weight (PLBW). Twenty-two pregnant female patients were selected for the study. Samples of GCF and serum were collected from each patient, and sampling was repeated at one month after parturition. The level of PGE₂ in GCF and serum was estimated using a commercially available ELISA kit (Neogen™). The mean serum PGE₂ level was 4.4 ng/ml and 1.64 ng/ml before and after parturition, respectively, and the difference was statistically significant ($P < 0.001$). The mean GCF-PGE₂ level was 5.8 ng/ml and 5.5 ng/ml before and after parturition, respectively, but the difference was not significant. There was positive correlation between the serum-PGE₂ and GCF-PGE₂ levels, and there was a negative correlation between PGE₂ level and gestational age. The present findings suggest that there is a weak correlation between maternal GCF-PGE₂ level and birth outcome. Further clinical trials with a larger sample size are warranted for further investigation of the association between GCF-PGE₂ level and PLBW. (J Oral Sci 53, 293-300, 2011)

Keywords: gingival crevicular fluid; prostaglandins; preterm low birth weight; periodontal diseases; serum.

Introduction

Preterm birth is a major public health problem and is the leading cause of neonatal morbidity and mortality. The cost of neonatal care for preterm infants is a major burden on the health care budgets of many countries. Identification of women at risk of preterm delivery is difficult, even though it is desirable from a management viewpoint. Current methods of detecting such high-risk patients rely on obstetric history, demographic factors, or premonitory symptoms that are neither sensitive nor specific. Biochemical markers are also currently being developed for this purpose.

There is now compelling evidence that infection is not only associated with preterm delivery but is also a causative factor (1,2). Infections affecting the mother during pregnancy may perturb the normal regulation of gestation by cytokines and hormones, resulting in preterm labor and preterm birth. Given the close relationship between inflammation and infection, it seems likely that alterations in the levels of inflammatory mediators resulting from a response to an infectious agent may represent the key mechanism through which infection is linked to preterm low birth weight (PLBW) (3).

Periodontal diseases are a group of infectious diseases initiated and sustained by predominantly gram-negative, anaerobic bacteria that colonize the sub-gingival area. Tissue destruction in periodontitis is due mainly to activation of immune cells by components of microorganisms, which stimulate production of pro-inflammatory mediators (4). There is ample evidence for an association

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between maternal periodontal disease and PLBW, and it has been postulated that the pathogenetic mechanisms involved may be similar to those of other maternal infections (5,6).

Current understanding of the biological events surrounding normal labor and preterm labor strongly suggests that prostaglandins play a pivotal role in the initiation process. Some reports have suggested that the serum level of prostaglandin E_2 (PGE_2) increases at onset of labor (7). Periodontal infections, which serve as a reservoir of inflammatory mediators such as PGE_2 , may pose a threat to the fetal-placental unit. A study conducted at the University of North Carolina (8) has demonstrated that the levels of PGE_2 and interleukin-1 β (IL-1 β) in gingival crevicular fluid (GCF) are correlated with those in amniotic fluid. Another case control study has suggested that mothers of low-birth-weight (LBW) infants have a higher mean GCF- PGE_2 level than mothers of normal-birth-weight (NBW) infants, and that mothers with an elevated level of GCF- PGE_2 are 9 times more likely to bear PLBW infants (9).

In the light of the above facts, the present study was conducted to estimate the maternal PGE_2 levels in GCF and serum before and after parturition, and to explore the possibility that the level of PGE_2 in GCF could be applicable as a risk predictor of PLBW.

Material and Methods

Study population

The study sample was selected among pregnant women presenting for prenatal check-ups at the Department of Obstetrics and Gynecology, Dr. B. R. Ambedkar Medical College and Hospital, Bangalore, India. Women who planned to deliver at the same hospital were invited to participate. Patients were enrolled after obtaining institutional ethical approval and informed consent for collection of biological fluids and periodontal intervention, and the study conformed to the provisions of the Declaration of Helsinki. Figure 1 shows a flow chart of the patient recruitment procedure and follow-up. The subjects were recruited on the basis of the following criteria.

Inclusion Criteria:

1. Pregnant women aged 18 to 35 years with clinical presentation of normal delivery, reporting with either a first or a second pregnancy.
2. Single gestation 28-32 weeks at the time of recruitment.
3. Subjects with ≥ 20 completely erupted teeth, excluding the third molars
4. Subjects with ≥ 3 mm attachment loss at not more

than 30% of sites examined (moderate periodontitis)

5. Patients who presented with clinically healthy gingiva and complied with oral hygiene instructions.

Exclusion Criteria:

1. Mothers with diabetes, asthma, glomerulonephritis, hyperthyroidism, or a history of congenital heart disease.
2. Use of corticosteroids/antibiotics during pregnancy.
3. Mothers with habits such as tobacco smoking, tobacco chewing or alcohol use.
4. Any clinically evident systemic infection.

Measurement of periodontal status

Periodontal parameters measured were the Oral Hygiene Index-Simplified (OHI-S) (10), Bleeding Index (11) and clinical attachment level. The full-mouth periodontal clinical attachment level (CAL) was measured at six sites per tooth with a UNC-15 (University of North Carolina) graduated probe, using the cemento-enamel junction as a reference point.

Oral hygiene instructions

Oral hygiene instructions included reinforcement of plaque control instructions [brushing and rinsing (0.2% chlorhexidine) twice daily]. Only patients who complied with oral hygiene instructions and reported an OHI-S of ≤ 0.5 and a BI of $\leq 5\%$ were considered for collection of biological fluids.

Collection of GCF and serum

Samples of GCF and serum were collected from each patient upon admission with onset of labor, and sampling was repeated one month after parturition. A 5- μ l sample of GCF was collected from sites with clinically healthy periodontium after isolation by placing micro-capillary pipettes (Sigma Chemical Company, St Louis, MO, USA) at the entrance of the gingival crevice. A 2-ml sample of peripheral venous blood was collected by vein puncture. The blood samples were centrifuged and the serum was separated. The samples of GCF and serum were stored in plastic vials at -70°C until analyzed for PGE_2 level.

Method of PGE_2 estimation

The level of PGE_2 was estimated using a commercially available ELISA kit (NeogenTM) in accordance with the manufacturer's instructions. The sensitivity of the assay at 80% absorbance is 0.2 ng/ml and that at 50% absorbance is 1.4 ng/ml. The assay is capable of detecting PGE_2 levels within the range 0.1-10 ng/ml. The cross-reactivity of the assay with similar molecules such as other prostaglandins (PGI, PGF etc.) is negligible.

Fig. 1 Flow chart showing selection of the study population and loss to follow-up.

Assessment of pregnancy outcomes

Primary outcomes measured were preterm birth (PTB) and low birth weight (LBW). Preterm birth was defined as spontaneous delivery at less than 37 completed weeks of gestation, and full-term birth (FTB) was defined as spontaneous delivery at more than 37 completed weeks of gestation. Low birth weight was assessed as positive when the infant had a birth weight of less than 2,500 grams, and normal birth weight (NBW) was assessed as positive when the infant had a birth weight of 2,500 grams or more. Estimation of gestational age was based on the last menstrual period, ultrasound examinations, sequential physical examinations and postnatal examinations.

Table 1 Incidence of PTB and LBW infants

Pregnancy outcomes (Total <i>n</i> = 22) 100%	PTB (<i>n</i> = 14) 63.64%	FTB (<i>n</i> = 8) 36.36%
NBW (<i>n</i> = 15) 68.2%	7	8
LBW (<i>n</i> = 7) 31.8%	7	nil

Power analysis

A sample size of 25 subjects was estimated to be necessary to detect a difference in the concentration of PGE₂ of 1 ng/ml. For the sample size calculation, 80% power at a statistical significance of 0.05 was considered. An attrition rate of 15% was assumed and a sample size of 30 subjects was decided. There was a 27% attrition rate, and the reasons are explained in the flow chart shown in Fig. 1. Post hoc power analysis showed that the power of the test to detect the difference in serum PGE₂ levels was 91% with a minimum detectable difference of 1.64 ng/ml, whereas for GCF it was 60% with a minimum detectable difference of 0.35 ng/ml.

Statistical methods

The data of patients who completed the follow-up were analyzed statistically. The significance of differences between the mean serum-PGE₂ and GCF-PGE₂ levels before and after parturition was evaluated using Student's *t* test (paired "*t*" test). The association between serum-PGE₂ and GCF-PGE₂, and the correlation between PGE₂ levels and birth characteristics were evaluated using the Pearson correlation coefficient. Multiple linear regression analysis was utilized to identify significant linear relationships between birth outcome and other variables. Differences were considered to be statistically significant at *P* < 0.05.

Results

The sample population investigated in this study ranged in age from 21 to 31 years, with a mean of 25.72 ± 2.64 years. All the patients were of low socio-economic status. Among 22 mothers, 7 delivered LBW infants and 15 delivered NBW infants. Table 1 shows the number of infants with LBW and preterm birth. There were 15 NBW and 7 LBW infants. All the LBW infants were preterm. Among the NBW infants, 7 were born preterm and 8 at full term.

Table 2 shows the mean PGE₂ levels in GCF and serum before and after parturition. Mean serum PGE₂ levels were 4.40 ± 1.98 ng/ml and 1.64 ± 0.54 ng/ml before

Table 2 Mean PGE₂ levels in maternal GCF and serum before and after parturition

PGE ₂ (ng/ml) (Mean ± SD)	Serum			GCF		
	Before parturition	After parturition	<i>P</i> value	Before parturition	After parturition	<i>P</i> value
All mothers (<i>n</i> = 22)	4.40 ± 1.98	1.64 ± 0.54	< 0.001*	5.80 ± 2.75	5.50 ± 2.55	0.089
PTB mothers (<i>n</i> = 14)	4.89 ± 2.01	1.72 ± 0.57	< 0.001*	6.21 ± 3.53	5.93 ± 3.38	0.091
FTB mothers (<i>n</i> = 8)	3.54 ± 1.71	1.51 ± 0.49	< 0.001*	5.58 ± 2.32	5.25 ± 2.05	0.166
LBW mothers (<i>n</i> = 7)	5.85 ± 2.28	1.80 ± 0.71	< 0.001*	6.15 ± 1.69	5.64 ± 1.56	0.096
NBW mothers (<i>n</i> = 15)	3.73 ± 1.46	1.57 ± 0.45	< 0.001*	5.64 ± 3.17	5.43 ± 2.96	0.150

Table 3 Mean PGE₂ levels in maternal GCF and serum among mothers of LBW and PTB infants

PGE ₂ (ng/ml) Before parturition (Mean ± SD)	FTB mothers	PTB mothers	<i>P</i> value	NBW mothers	LBW mothers	<i>P</i> value
Serum	3.54 ± 1.71	4.89 ± 2.01	0.05*	3.73 ± 1.46	5.85 ± 2.28	0.007*
GCF	5.58 ± 2.32	6.21 ± 3.53	0.6	5.64 ± 3.17	6.15 ± 1.69	0.34

Table 4 Pearson correlation of PGE₂ levels with birth outcome

Correlation parameters	Pearson <i>r</i>	<i>P</i> value
Serum PGE ₂ vs GCF	0.356	0.104
Serum PGE ₂ vs gestational age	-0.634	0.002*
Serum PGE ₂ vs birth weight	-0.574	0.005*
GCF PGE ₂ vs gestational age	-0.305	0.168
GCF PGE ₂ vs birth weight	-0.417	0.05*

and after parturition, respectively, and the difference was statistically significant at $P < 0.001$. Mean GCF-PGE₂ levels were 5.80 ± 2.75 ng/ml and 5.50 ± 2.55 ng/ml before and after parturition, respectively, but the difference was not significant. Serum and GCF levels of PGE₂ among the LBW and PTB mothers were higher before parturition than after. The difference was significant for serum but not for GCF.

Table 3 shows mean PGE₂ levels among the mothers of LBW and PTB infants. Both serum and GCF PGE₂ levels were lower in mothers of NBW and FTB infants than in mothers with LBW or PTB infants. The mean serum PGE₂ level was 3.73 ± 1.46 ng/ml in mothers of NBW infants and 5.85 ± 2.28 ng/ml in mothers of LBW infants. The difference was statistically significant ($P = 0.007$). Mean GCF-PGE₂ was 5.64 ± 3.17 ng/ml in mothers of NBW infants and 6.15 ± 1.69 ng/ml in mothers of LBW infants, but the difference was not significant. Mean serum PGE₂ was 3.54 ± 1.71 ng/ml in mothers of FTB infants and 4.89 ± 2.01 ng/ml in mothers of PTB infants. The difference was statistically significant ($P = 0.05$).

Mean GCF-PGE₂ was 5.58 ± 2.32 ng/ml in mothers of FTB infants and 6.21 ± 3.53 ng/ml in mothers of PTB infants, but the difference was not significant.

Table 4 shows the correlation between serum-PGE₂ and GCF-PGE₂ levels and also that between PGE₂ levels and birth outcome. There was a positive correlation between the level of PGE₂ in serum and that in GCF ($r = 0.356$). Higher values of serum PGE₂ were associated with higher values of GCF-PGE₂. There was a negative correlation between serum PGE₂ and birth characteristics. Higher values of serum PGE₂ were associated with lower gestational age and birth weight. There was negative correlation between GCF-PGE₂ and birth outcome. Higher values of GCF-PGE₂ were associated with lower gestational age and birth weight. Figure 2 shows a graphical representation of the correlation analysis.

Table 5 shows the *P* values for multiple linear regression analysis of birth outcome in association with serum PGE₂ and GCF-PGE₂ levels. There was a significant linear relationship between birth outcome and serum PGE₂ level. Significance was demonstrated for both birth weight and gestational age. When considered together, serum and GCF-PGE₂ levels had a statistically significant effect on birth outcome.

Discussion

Biochemical markers have been developed with the aim of providing new tools for predicting and preventing preterm deliveries. Such markers can be grouped into different categories. Higher levels of cytokines such as IL-1, IL-6 and TNF- α , PGE₂, fibronectin and α -fetoprotein in amniotic fluid have been associated with an increased

Table 5 Multivariate linear regression analysis

Predictors	Outcome variable					
	Birth weight			Term		
	Regression co-efficient	Student <i>t</i>	<i>P</i> value	Regression co-efficient	Student <i>t</i>	<i>P</i> value
Serum PGE ₂	-105.01	2.393	0.028*	-0.872	2.971	0.008*
GCF PGE ₂	-46.95	-0.827	0.419	-0.366	0.964	0.348
Multiple correlation	R = 0.619; <i>P</i> = 0.031*			R = 0.656; <i>P</i> = 0.025*

Fig. 2 Graphical representation of correlation analysis.

risk of preterm delivery. Other biochemical markers that have been evaluated are matrix metalloproteinases, estriol, elastase, protease, phospholipase and prolactin (12).

The role of prostaglandins in human labor has been well documented. Increased concentrations of PGE₂ in amniotic fluid have been demonstrated in women undergoing term and preterm labor. Levels of PGE₂ in amniotic fluid rise steadily throughout pregnancy until a critical threshold level is reached to induce labor, cervical dilation and delivery (13). PGE₂ is a major

arachidonic acid metabolite released locally, and has many pro-inflammatory effects on periodontal tissues. Macrophages are believed to be a major source of PGE_2 in periodontal tissues (14).

GCF is a complex mixture of substances derived from serum, leukocytes, cells of the periodontium and oral bacteria. GCF is a unique window for analysis, and determination of its constituents has practical diagnostic utility. Since protocols for collection of GCF are non-invasive, previous attempts have been made to correlate changes in the constituents of GCF with those occurring in serum (15).

The association between periodontal disease and preterm births has gained increasing attention in the past two decades. Hence the present study was designed to explore the possibility of utilizing the level of GCF- PGE_2 as a risk predictor for PLBW.

Patients with known risk factors for PLBW were excluded from the study. This included women above 35 and below 18 years of age, and those presenting with multiple gestation or systemic infections, as all these are established risk factors for PLBW (16). In Indian women, although the prevalence of smoking is low, the use of tobacco as an ingredient in pan chewing is common. The use of alcohol is also not uncommon among groups with low socioeconomic status. Subjects with a history of, or current use of tobacco (smoking and non-smoking) and alcohol were excluded, as these are also known risk factors for PLBW (17). Since inflamed periodontium is known to have higher levels of biochemical markers, the presence of which may mask the effects of changes induced by pregnancy, all of the study patients presented with clinically healthy periodontium.

Our study results showed that the mean levels of serum PGE_2 and GCF- PGE_2 were higher before parturition than after. Serum PGE_2 levels, which peaked during onset of labor, were reduced significantly after parturition. This is in accord with earlier studies demonstrating that the serum PGE_2 level increases at onset of labor (7,18). The results of different studies should be interpreted considering the characteristics of the study population, the methods of sample collection, the clinical status of patients at the time of sample collection, the time point at which the samples were collected, and the method used for estimation of PGE_2 .

The GCF- PGE_2 level decreased after parturition, but not to a significant degree. Changes in the constituents of GCF may reflect changes in the systemic circulation and also local tissues. Since the oral cavity is colonized by a number of facultative and anaerobic bacteria, there is an active host defense mechanism at the gingival interface,

irrespective of whether disease is clinically evident (19). The local host response in periodontal tissues is also responsible for the production of inflammatory mediators, but the systemic circulation may also contribute to inflammatory mediators present in GCF. Thus inflammatory mediators present in periodontal tissues may mask changes in the components of GCF induced by pregnancy.

The increase in the serum PGE_2 level at onset of labor is thought to originate from various intrauterine tissues. High levels of cyclooxygenase-2 are expressed in fetal membranes prior to labor (7). After parturition, involution of various intrauterine tissues occurs, thus restoring the serum PGE_2 level to normal. On the other hand, in periodontal tissues, apart from any impact of pregnancy, local inflammatory and host immune responses appear to be critical determinants of PGE_2 concentration. Hence the difference in the level of PGE_2 before and after parturition is appreciable in serum, but not in GCF.

Although not statistically significant, our study demonstrated a positive correlation between the GCF and serum levels of PGE_2 . This is in agreement with the results of a study conducted at the University of North Carolina (8), which showed that the level of PGE_2 in GCF was highly correlated with that in amniotic fluid. Since collection of serum is easier than collection of amniotic fluid, our study attempted to evaluate the correlation between PGE_2 levels in GCF and serum. Another study has demonstrated a positive correlation between IL-6 levels in maternal serum and amniotic fluid during the second trimester (20), suggesting that changes in the levels of inflammatory mediators in amniotic fluid reflect those in serum. As there are no published reports of the correlation between levels of PGE_2 in serum and GCF during pregnancy, our present results were compared with those of investigations that had assessed the correlation between GCF- PGE_2 and amniotic PGE_2 levels. Some reports have indicated that increases in the serum levels of inflammatory mediators correspond to increases in their levels in GCF (21,22).

Our present study showed that the mean serum PGE_2 level was higher in mothers of PTB infants than in mothers of FTB infants, and was also higher in mothers of LBW infants than in mothers of NBW infants. A negative correlation between PGE_2 levels and birth outcome was evident. Higher values of PGE_2 were associated with lower gestational age and lower birth weight. Some studies have indicated that preterm delivery may be caused by a premature increase in the serum level of PGE_2 (18,21). The relationship between systemic levels of PGE_2 and onset of labor has been well established. Our

results also showed that the mean level of PGE₂ in GCF was higher in mothers of LBW infants than in mothers of NBW infants, and was higher in mothers of PTB infants than in mothers of FTB infants. These results are similar to those of a study conducted by Offenbacher, which suggested that mothers of PLBW infants had a higher mean GCF-PGE₂ level than mothers of NBW infants (9).

Our study differed from previous investigations of GCF-PGE₂ in a few aspects. The Offenbacher study (9) was a cross-sectional one that evaluated GCF-PGE₂ levels at only one time point, whereas our study evaluated PGE₂ levels in GCF and serum before and after parturition. The North Carolina study (8) evaluated the correlation between the PGE₂ level in GCF and that in amniotic fluid, whereas our study attempted to evaluate the correlation between the PGE₂ level in GCF and that in serum.

Higher levels of inflammatory markers are present in the lower genital tract of women during full-term and preterm pregnancies. The pathophysiology of spontaneous preterm delivery is poorly understood, and further clarification of this issue would lead to substantive improvements in the clinical management of preterm labor. In order to understand the complex changes that occur during preterm delivery, investigations of biochemical markers are necessary. However, none of the biochemical markers studied to date has proven to be reliable for predicting preterm delivery.

The present investigation was designed to explore the possible utility of the GCF-PGE₂ level for prediction of PLBW. However, this objective was not met due to certain limitations, which were as follows: 1) The study design did not include a non-pregnant control group. 2) PGE₂ levels before conception were not recorded. Comparison of the PGE₂ levels with a control non-pregnant group, and with the levels before pregnancy, would have given a clearer idea of the effects of pregnancy and local inflammation on the levels of PGE₂. 3) The sample size was too small to allow significant statistical comparisons to be drawn.

However, the results of our study allowed us to conclude that the mean GCF-PGE₂ level was higher in mothers of LBW and PTB infants than in mothers of NBW and FTB infants. The increase in the levels of PGE₂ in GCF and serum was associated with PLBW. There was decrease in both of these parameters after parturition, but the change in the GCF-PGE₂ level was not significant. There was positive correlation between the serum and GCF levels of PGE₂, but the correlation was not statistically significant, due to the small study sample size. These results suggest that the changes in the level of PGE₂ in serum

reflect those in GCF, but because local inflammatory and host immune responses in periodontal tissues appear to be critical determinants of PGE₂ concentration, separate from the impact of pregnancy, the correlation may not be clinically significant. Our study has thus provided evidence for a weak correlation between birth outcome and GCF-PGE₂ level, but it is premature to conclude that the GCF-PGE₂ level can be used as a predictor of pregnancy risk. Further clinical trials with a large sample size will be required to investigate the use of the GCF-PGE₂ level as a predictor of PLBW.

Hence in the light of the present findings, the use of the GCF-PGE₂ level as an index of the serum level of PGE₂, and as an indicator of pregnancy risk, is questionable.

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