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Altered level of salivary placental growth factor is associated with preeclampsia

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Abstract

A pilot, case-control study was conducted to compare the concentrations of placental growth factor (PIGF) and placental alkaline phosphatase (PLAP) in saliva of preeclampsia (PE) patients with normotensive controls in the second and third trimesters. Measured by ELISA assays, levels of salivary PIGF were significantly lower in PE patients (n=13) compared to controls (n=15) (two-way ANOVA, $p = 0.0208$) independent of gestational age at time of collection ($p = 0.49$). Salivary PLAP differences between PE and controls were not statistically significant. Placenta-specific proteins are detectable in maternal saliva and may serve as noninvasive biomarkers to monitor placenta health and disease during pregnancy.

INTRODUCTION

Preeclampsia (PE) remains the leading cause of maternal and infant morbidity and mortality worldwide (1). The heterogeneity of PE has resulted in challenges in both predicting disease course and identifying at-risk women. While clinical factors and maternal demographics may help stratify at-risk women during gestation, there is currently no predictive screening tool that allows personalized treatment strategies (2–4). An overall imbalance in anti-angiogenic and proangiogenic biomarkers is believed to play a defining role in the pathogenesis of PE (5–7). Serum levels of PIGF, sFlt-1 and their ratio can be used to predict PE development (8). Recently, the diagnostic potential of placental-specific exosomes detected by PLAP has been discovered (9, 10). Saliva contains biomarkers that may be used for the early detection and monitoring of systemic diseases (11, 12) and pregnancy complications (13), and may hold great potential as a noninvasive biofluid for PE screening. The first step towards developing such a screening tool is to identify and validate salivary biomarkers associated with PE. We aimed to quantify the placenta-specific salivary biomarkers, PIGF and PLAP, in pregnant women and perform a comparative analysis of

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quantitative biomarker levels between normotensive pregnancies and women with PE throughout the second and third trimesters.

METHODS

An IRB-approved, prospective, nested, case-control, pilot study was conducted to compare the salivary levels of PIGF and PLAP in normal pregnant women (n=15) and PE patients (n=13) admitted to Tufts Medical Center (Boston, MA). Participants provided written informed consent. Preeclampsia was diagnosed based on new onset hypertension (systolic blood pressure ≥ 140 mm Hg; diastolic blood pressure of ≥ 90 mm Hg) and substantial proteinuria (≥ 300 mg in 24h) at ≥ 20 weeks of gestation. PE with severe features was based upon the definition described by the American College of Obstetrics and Gynecology (14). Namely, severe PE patients present with one of the following: thrombocytopenia (platelet count $< 100,000 \times 10^9/L$), elevated liver enzymes, persistent right upper quadrant or epigastric pain, renal insufficiency, pulmonary edema, and/or new onset headache. The control group consisted of normotensive women with singleton pregnancies matched according to trimester at time of sample collection. Subject demographic characteristics are in Table 1. Women carrying multiple gestations, known placental pathology or gestational hypertension were excluded.

Maternal subjects provided free flowing saliva into 50 mL conical tubes placed on ice. Salivary supernatant was prepared from 5 mL whole saliva by centrifugation at $2600 \times g$ for 15 min at $4^\circ C$ and transferred to a 15 mL conical tube. Proteins were stabilized with a stock solution of $1 \mu L$ of 10 mg/mL aprotinin (A1153; Sigma-Aldrich, St. Louis, MO), $13.2 \mu L$ of 400 mM stock of sodium orthovanadate (Na_3OV_4) (13721-39-6, Sigma-Aldrich), and $10 \mu L$ of 10 mL/mg stock of phenylmethanesulfonyl fluoride (PMSF) (392-98-6, Sigma-Aldrich) (15). Salivary supernatant was diluted 1:1 with the buffer solution.

Salivary PLAP and PIGF concentrations were quantified using commercially available ELISA kits according to manufacturer's instructions (Human PLAP Elisa Kit, MyBioSource, MBS701995, Human PLGF Elisa Kit, Abcam, ab100629). Positive and negative controls were run on each plate. Non-pregnant subjects served as additional negative controls. To account for varying gestational ages at the time of collection, data were analyzed with a two-way ANOVA with replication for unequal sample sizes. We further analyzed the data by performing a multi-linear regression analysis with gestational age and preeclampsia status as the independent variables. A nominal P value < 0.05 indicated statistical significance. Analyses were performed using Prism software (GraphPad Inc, La Jolla, CA).

RESULTS

Clinical data are in Supplemental Table 1. There were nine women who developed early onset PE (< 34 weeks' GA); 5 women were diagnosed with late onset PE (≥ 34 weeks' GA). Seven women (47%) developed severe preeclampsia. Seven healthy pregnant women served as controls for the early onset PE comparison; eight healthy pregnant women served as controls for the late onset PE comparison. Overall, the median salivary PIGF concentration

in the PE cohort was 6.6 pg/ml, IQR 5.9–7.8 pg/ml; the median salivary PIGF in the control cohort was 8.6 pg/ml, IQR 7.3–8.9 pg/ml (Fig. 1). The median salivary PLAP concentration in the PE group was 451.9 pg/ml, IQR 279.2–969.3 pg/ml; the median salivary PLAP concentration in the control group was 733.17 pg/ml, IQR 653.7–948.9 pg/ml (Fig 2). Results of the two-way ANOVA revealed that PIGF levels differed significantly between cases and controls ($p = 0.0208$) with no impact of GA on salivary levels ($p = 0.49$). Salivary PLAP levels were not significantly different between cases and controls ($p = 0.23$). A multi-linear regression analysis revealed that PIGF was significantly associated with PE status after adjusting for GA ($p=0.0209$). PLAP levels were not significantly associated with PE status in this model.

DISCUSSION

Studies have shown that serum levels of PIGF are significantly decreased in PE patients, even before the onset of clinical disease (16, 17). Further, second and third trimester serum and urinary levels of PIGF appear to be lower in EOPE than in LOPE (18–20). Our pilot study is consistent with previous findings in maternal blood and urine, demonstrating that saliva may be a useful and noninvasive biofluid to monitor disease onset and progression.

While a recent study reported no significant differences in salivary PIGF or PLAP levels between PE patients and controls (13), we found decreased levels of PIGF in saliva of PE patients. The reasons for the discrepancy could be twofold. First, unlike Chaparro *et al.*, we did not process saliva with ultracentrifugation before analysis. Second, we collected saliva samples across the second and third trimesters. The Chaparro *et al.* group presented results only from third trimester subjects. These differences in both processing and timing of collection could have resulted in the discrepancy seen between reports.

Further investigation should include a more comprehensive measurement of angiogenic/anti-angiogenic factors in saliva throughout gestation with a larger sample size. Overall, we took the first step in identifying the presence of salivary placenta biomarkers and explored their potential associations with PE. These results pave the way for the future development of noninvasive screening tests to monitor placenta health and disease risk during pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- PIGF and PLAP are quantifiable in maternal saliva.
- Preeclampsia patients showed reduced salivary PIGF in second and third trimesters.
- Placental-specific salivary proteins may serve as noninvasive biomarkers of pregnancy complications.

Figure 1:
Box and whisker plot representing median salivary PLGF concentrations (pg/mL), 25th and 75th percentiles and extreme values (whiskers) between PE and controls

Figure 2:
Box and whisker plot representing median salivary PLAP concentrations (pg/mL), 25th and 75th percentiles and extreme values (whiskers) between PE and controls

Table 1.
Demographic and clinical characteristics of subjects.

Characteristic	Preeclampsia Patients (n = 13)	Controls (n= 15)	P Value (t test)
Gestational Age (weeks) *	32 (26 3/7 – 36 3/7)	32 5/7 (17 – 40 4/7)	0.77
Maternal Age (years) *	31.7 (25 – 42)	32.1 (22 – 43)	0.85
Preeclampsia with severe features (%)	54	Not applicable	---
Maternal Ethnicity (%)			
• Caucasian	62	33	
• African American	23	27	
• Asian	--	14	
• Hispanic	15	13	
• Other	--	13	
Smoker (%)	0	0	1
Primigravida (%)	38	47	0.68

* Mean and ranges provided