BJOG An International Journal of Obstetrics and Gynaecology

DOI: 10.1111/1471-0528.16323
www.bjog.org

Maternal medicine

Glycosylated fibronectin point-of-care test for diagnosis of pre-eclampsia in a low-resource setting: a prospective Southeast Asian population study

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Accepted 11 May 2020. Published Online 16 June 2020.

Objective To determine the performance of a glycosylated fibronectin (GlyFn) point-of-care (POC) test for pre-eclampsia (PE) in a large Southeast Asian cohort (India) in comparison to previously described biomarkers.

Design A total of 798 pregnant women at ≥20 weeks of gestation were enrolled in a prospective case-control study. Study participants included 469 normotensive women with urinary mg protein/mmol creatinine ratio <0.3, 135 with PE (hypertension with urinary mg protein/mmol creatinine ratio ≥0.3) and 194 with gestational hypertension (hypertension with urinary mg protein/mmol creatinine ratio <0.3).

Methods GlyFn levels were determined using a POC device and PIGF, sFlt-1 and PAPPA2 levels were determined by immunoassay. Performance was assessed using logistic regression modelling and receiver-operating characteristic (ROC) curves. Classification performance and positive and negative predictive values are reported at specific thresholds.

Results Increased levels of GlyFn, soluble fms-like tyrosine kinase-1 (sFlt-1) and pregnancy-associated placental protein A2 (PAPPA2), and decreased levels of placental growth factor (PlGF) were significantly associated ($P < 0.01$) with clinically defined PE. Area under the ROC (AUROC) values with 95% confidence intervals were: GlyFn, 0.99 (0.98–0.99); PlGF, 0.96 (0.94–0.98); sFlt-1, 0.86 (0.83–0.89); and PAPPA2, 0.96 (0.94–0.97). Of subjects with GH, 48% were positive for more than two PE biomarkers, and 70% of these delivered preterm.

Conclusions The Lumella™ GlyFn POC test has been validated in a low/middle-income country setting for PE diagnosis and may be a useful adjunctive tool for early identification, appropriate triage, and improved outcomes.

Keywords Gestational hypertension, glycosylated fibronectin, point-of-care test, pre-eclampsia.

Tweetable abstract The Lumella™ point-of-care test had excellent performance in diagnosing PE in a large Southeast Asian cohort.

Linked article This article is commented on by I Herraiz, p. 1695 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.16405

Please cite this paper as: Nagalla SR, Janaki V, Vijayalakshmi AR, Chayadevi K, Pratibha D, Rao PV, Sage KM, Nair-Schaef D, Bean E, Roberts CT Jr, Gravett MG. Glycosylated fibronectin point-of-care test for diagnosis of pre-eclampsia in a low-resource setting: a prospective Southeast Asian population study. BJOG 2020;127:1687–1694.

Introduction

Pre-eclampsia (PE) is a major hypertensive disorder of pregnancy, with serious potential adverse consequences for both mother and child,[1,2] including subsequent cardiovascular disease.[3,4] Worldwide, ~5% of all pregnancies are complicated by PE.[5] PE and hypertensive disorders in pregnancy are the second-most common cause of maternal mortality, resulting in approximately 30 000 maternal deaths each year.[6] PE is a principal cause of maternal, fetal and neonatal mortality in low- and middle-income countries (LMIC),[7–10] where management is hindered by late clinical presentation, which limits the efficacy of medical intervention.[11,12] Thus, early assessment of risk can potentially alleviate the burden of PE in LMIC settings.

The clinical criteria for PE diagnosis have undergone recent revision. Specifically, proteinuria is no longer required, being replaced by a collection of maternal organ

dysfunction categories, including renal insufficiency, hepatic, neurological and haematological complications, uteroplacental dysfunction or fetal growth restriction.[13–15] To some extent, this reflected concerns with assessment of proteinuria using the gold standard of >300 mg/24 hours[16] and the accuracy of protein/creatinine ratio assays.[17] A major factor, however, was the prevailing consensus that PE required the presence of proteinuria, in spite of the occurrence of a significant proportion of PE in the absence of clinically defined proteinuria.[18]

These criteria, which leave hypertension as the principal parameter for PE diagnosis in conjunction with various secondary criteria, have significant consequences for LMIC settings, in that accurate determination of blood pressure requires standardised positioning of the patient and the use of specific instruments validated for PE,[19,20] and evaluation of maternal organ dysfunction that can be subjective, or assessment of intrauterine growth restriction (IUGR) that requires laboratory facilities that may not be available. Thus, detection of non-proteinuric PE may be especially challenging in resource-poor LMIC settings where appropriate clinical facilities may be lacking.

Pre-eclampsia diagnosis can be complemented by appropriate biochemical markers, particularly those that are amenable to testing in a point-of-care (POC) format.[21–25] While POC diagnostic tests are particularly appropriate for LMIC environments, there is an increasing appreciation of the challenges in developing and deploying appropriate diagnostic tools that meet the particular needs of many rural settings.[26–30] We have previously described the association of elevated fibronectin levels in PE[31] and the utility of a specific form of glycosylated fibronectin (GlyFn) in the diagnosis of PE in Finnish[32] and Swiss[33] populations. The association of elevated GlyFn with PE may reflect its differential glycosylation by oxidative stress in PE.[34] To ascertain the performance of this biomarker in a suitable LMIC setting, we evaluated the Lumella™ glycosylated fibronectin (GlyFn) POC test (DiabetOmics, Inc., Beaverton, OR, USA),[32] which uses a fingerstick blood sample and an inexpensive hand-held reader, to determine PE risk in a large prospective Indian case-control cohort. We also compared its performance to s-Flt-1 and PlGF, as well as to pregnancy-associated plasma protein-A2 (PAPPA2), whose levels have previously been reported to be elevated in PE.[35,36]

Methods

Study design and patient characteristics

This was a prospective, case-control study conducted at three institutions in Hyderabad, India. The Institutional Ethics Committees of the participating institutions (Osmania Medical College, Ramdevrao Hospital and Mallareddy Institute of Medical Sciences) approved the study protocol. Written informed consent was obtained from all participants. Three groups of women were enrolled and classified into three groups at enrolment: normotensive women, women with gestational hypertension without proteinuria and women with PE. All women enrolled were >18 years of age with a singleton pregnancy. Exclusion criteria included evidence of major fetal anomaly or chronic hypertension, defined as hypertension prior to pregnancy or onset of hypertension before 20 weeks of gestation, or chronic renal disease, as ascertained by medical chart review. There was no patient involvement in the study design. The study was funded by DiabetOmics, Inc.

Enrolment criteria for normotensive control subjects were systolic blood pressure ≤120 mm Hg and/or diastolic blood pressure ≤80 mm Hg and protein/creatinine ratio <0.3 mg protein/mmol creatinine.[13,14] Subjects with GH or PE were enrolled at the time that hypertension was diagnosed. Criteria for GH were new-onset hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mmHg measured on two occasions at least 6 hours apart) at >20 weeks of gestation without proteinuria (total urinary protein/creatinine ratio <0.3 mg protein/mmol creatinine). Criteria for PE were new-onset hypertension as above and new-onset proteinuria with protein/creatinine ratio ≥0.3 at >20 weeks of gestation, consistent with the definition of PE by the International Society for the Study of Hypertension in Pregnancy (ISSHP).[14] Because the criteria for non-proteinuric PE based upon ISSHP guidelines that require laboratory or ultrasound evidence of maternal end-organ dysfunction or fetal growth restriction[18] are not yet widely adopted in India, we required proteinuria for the diagnosis of PE. Between January 2016 and December 2017, a total of 798 pregnant women meeting the inclusion and exclusion criteria were enrolled in the final study. Blood samples were collected at the time of subject enrolment.

At enrolment, demographic characteristics and medical history were recorded, clinical examination performed and blood obtained by venepuncture for biomarker analysis (GlyFn, PAPPA2, PlGF and sFlt-1). Early-onset PE was defined as PE with onset prior to 34 weeks of gestation. Preterm birth was defined as birth prior to 37 weeks of gestation. The clinical management and timing of delivery were determined by the managing health provider. The results of the biomarker analyses were not available until the end of study and did not, therefore, influence management decisions. The clinical study design workflow is shown in Figure S1.

Assessment of maternal serum levels of GlyFn, PAPPA2, PlGF and sFlt-1

All maternal serum samples were aliquoted and stored at −80°C until analysis. Commercial immunoassay kits for

sFlt-1 and PlGF (R&D Systems, Minneapolis, MN, USA) and PAPPA2 (Ansh Labs, Webster, TX, USA) were used according to the manufacturer's instructions. Inter-assay coefficients of variation ranged from 2 to 8% and the intra-assay coefficients from 2 to 5%.

Point-of-care test (Lumella™ test system)

Serum samples were analysed for GlyFn using a second-generation Lumella™ PE test (DiabetOmics, Inc.) according to the manufacturer's instructions. Test strips were configured with monoclonal antibodies against GlyFn labelled with gold particles for quantification using a hand-held Lumella™ reader system. Briefly, 5 µl of serum was diluted 1:350 in running buffer and 120 µl of diluted serum added to the test strip and inserted into the reader. The GlyFn concentration is displayed on the reader at the end of 10 minutes. Calibration information is supplied by the manufacturer as a lot-specific RFID tag on each test kit. The measurable range of the Lumella™ assay is 50–800 microg/ml. The intra-/inter-assay coefficients of variation at mean concentrations of 50–800 microg/ml were 5–10% and 6–10%, respectively.

Statistical analyses

Analyses were restricted to one sample per woman for a cross-sectional comparison of biomarker values. Baseline maternal characteristics were stratified for women within three groups: controls, cases (mothers who developed PE) and mothers with GH. Overall group differences were compared using a Kruskal–Wallis test for continuous variables; pairwise comparisons were made using a Wilcoxon rank sum test. A Chi-square test was used for comparing categorical variables. Mean biomarker values for women with and without clinical PE were calculated and compared. Final delivery outcomes were also described between groups, including gestational age at delivery, birthweight and preterm birth.

Receiver-operating characteristic (ROC) curves, the area under the curve (AUC) and corresponding 95% confidence intervals (95% CI) for PE were generated using predicted probabilities from simple logistic regression models in the pROC package in RSTUDIO.[37] We estimated and compared the diagnostic accuracy (sensitivity, specificity) using thresholds of ≥263 microg/ml for GlyFn,[31] ≥200 ng/ml for PAPPA2, <100 pg/ml for PlGF and ≥10 000 ng/ml for sFlt-1 for detection of PE. These thresholds for PAPPA2, PIGF and sFlt-1 were based on previous studies,[32,33] and the GlyFn threshold was chosen from a pilot Indian study (unpublished). A level of significance of $P < 0.05$ was used. All statistical analyses were performed using R (3.4.4) via RSTUDIO software version 1.1.447 (www.rstudio.com/products/RStudio/). ROC curves were created using the pROC package.[37]

Results

Patient characteristics

The characteristics of the study groups are shown in Table 1. The average gestational age at collection was slightly lower in the control group ($n = 469$; 29 weeks) than in the PE ($n = 135$; 32 weeks) and GH ($n = 194$; 31 weeks) groups. However, GlyFn levels were not statistically different between second- and third-trimester control samples (second-trimester median, 160.9 microg/ml; third-trimester median, 170.7 microg/ml; P-value of Wilcoxon test = 0.27). Gestational age at delivery was lower in the PE

Table 1. Clinical characteristics of the study groups

	Control	PE	GH
n	469	135	194
Maternal age[a]	23.94 (3.58)	24.30 (3.79)	25.31 (4.30)
Gestational age at collection[b]	28.76 (4.81)	31.71 (3.79)	31.41 (3.85)
Parity[e]			
0	161 (36.0)	6 (4.4)	7 (3.8)
1	163 (36.5)	65 (48.1)	77 (41.6)
2	88 (19.7)	45 (33.3)	54 (29.2)
3	27 (6.0)	17 (12.6)	38 (20.5)
4	5 (1.1)	2 (1.5)	7 (3.8)
5	3 (0.7)	0 (0.0)	2 (1.1)
BMI[e]	24.89 (4.78)	26.19 (4.46)	28.15 (6.08)
Systolic blood pressure	109.15 (12.35)	151.79 (12.34)	145.28 (8.24)
Diastolic blood pressure	72.66 (9.43)	102.86 (10.69)	96.65 (9.07)
Total urinary protein: creatinine ratio[c]	0.15 (0.08)	1.2 (4.4)	0.19 (0.1)
Gestational age at delivery[d]	38.04 (2.70)	33.88 (3.25)	35.63 (2.60)
Preterm = *n* (%)	56/230 (24.3)	92/110 (83.6)	104/154 (67.5)
Birthweight (kg)	2.78 (0.52)	1.93 (0.72)	2.43 (0.57)

Data are presented as mean (SD) unless otherwise noted.
Significant differences between groups are based on P-values (≤0.05) from a pairwise Wilcoxon test for continuous variables and Chi-square test for categorical variables. Except for the instances described below, differences between all pairwise comparisons for each parameter were significant.
[a]Significant differences were only seen between the C versus GH and PE versus GH groups.
[b]Significant differences were only seen between the C versus PE and C versus GH groups.
[c]Data are presented as median (interquartile range).
[d]Birth outcome data were missing for 239 control, 25 PE, and 40 GH mothers.
[e]Parity data were missing for 22 control and 9 GH mothers and maternal BMI data were missing for 35 control and 5 PE mothers.

and GH groups (34 and 36 weeks, respectively, versus 38 weeks in the control group). Among the 135 patients with PE, 71 patients had early-onset PE <34 weeks of gestation (including 45 of 71 with severe PE) and 64 patients had late-onset PE. As expected, the incidence of preterm birth was significantly increased in the PE and GH groups (84 and 68%, respectively, versus 24% in the control group). This likely represents the local practice of early delivery to mitigate the risk of eclampsia, a significant contributor to maternal mortality in India. The incidence of preterm birth among control patients reflects the overall incidence of preterm birth among the Indian referral-based populations at the participating hospitals. The PE and GH groups also exhibited decreased birthweights (1.9 and 2.4 kg, respectively, versus 2.8 kg in the control group). In the total study population, 73 women delivered at <34 weeks of gestation: 11 (4.7%) in the control group, 38 (36%) in the PE group and 24 (15%) in the GH group.

Biomarker performance

All analytes exhibited significant differences between groups (Table 2, Figure S2). The performance characteristics for diagnosis of PE for all biomarkers are shown in Table 3 at 5 and 7% prevalence levels, characteristic of the general population,[6] as well as at the 17% level exhibited in this study cohort. All biomarkers tested exhibited high performance for detection of PE, with GlyFn exhibiting the best overall performance. Figure 1 shows the ROC curves and associated AUCs (control versus PE). AUCs from ROC curves and 95% CI were: GlyFn, AUC = 0.99 (95% CI 0.98–0.99); PAPPA2, 0.96 (95% CI 0.94–0.97); PlGF, 0.96 (95% CI 0.94–0.98); and sFlt-1, 0.86 (95% CI 0.83–0.89).

To determine whether biomarker performance differed between early-onset and late-onset PE, AUCs from ROC curves, 95% CI, and sensitivities and specificities from a comparison of early-onset PE (n = 71) and gestational age-matched controls (n = 371) are shown in Figure S3 and Table S1. The same parameters from a comparison of late-onset PE (n = 64) and gestational age-matched controls (n = 98) are shown in Figure S4 and Table S2. The relative biomarker performance in early-onset (GlyFn, AUC = 0.99 [95% CI 0.98–1.00]; PAPPA2, 0.98 [95% CI 0.95–0.99]; PlGF, 0.96 [95% CI 0.93–0.99]; and sFlt-1, 0.89 [95% CI 0.85–0.93]) and late-onset PE (GlyFn, AUC = 1.0 [95% CI 0.99–1.00]; PAPPA2, 0.92 [95% CI 0.87–0.96]; PlGF, 0.96 [95% CI 0.93–0.99]; and sFlt-1, 0.78 [95% CI 0.71–0.86])

Table 2. Biomarker serum concentrations

	Control	PE	GH	Group difference *P*-value
n	469	135	194	
GlyFn (microg/ml)	169 (86)	412 (212)	266 (136)	<0.001
PAPPA2 (ng/ml)*	50 (47)	316 (276)	127 (191)	<0.001
PlGF (pg/ml)	996 (1153)	0.05 (7)	218.7 (571)	<0.001
sFlt-1 (ng/ml)	11 668 (16 732)	64 452 (117 938)	19 711 (33 422)	<0.001

Data are presented as median (interquartile range).
Group-difference *P*-values are based on a Kruskal–Wallis rank sum test.
Differences in all pairwise comparisons for each parameter from a pairwise Wilcoxon test were significant ($P \leq 0.05$).
*PAPPA2 data were missing for 107 control, 9 PE and 85 GH mothers.

Table 3. Biomarker performance characteristics for diagnosis of PE

	Threshold	Sensitivity	Specificity	NPV*			PPV*		
				5%	7%	17%*	5%	7%	17%
GlyFn (microg/ml)	263	0.985	0.928	0.999	0.999	0.997	0.419	0.507	0.737
PAPPA2 (ng/ml)	200	0.754	0.961	0.987	0.981	0.950	0.506	0.595	0.799
PlGF (pg/ml)	100	0.919	0.921	0.995	0.993	0.983	0.380	0.467	0.823
sFlt-1 (ng/ml)	10 000	0.97	0.437	0.996	0.995	0.986	0.083	0.115	0.261

*Predictive values are based upon the theoretical prevalence of PE of 5% and 7% reported in the published literature and the 17% seen in this study.

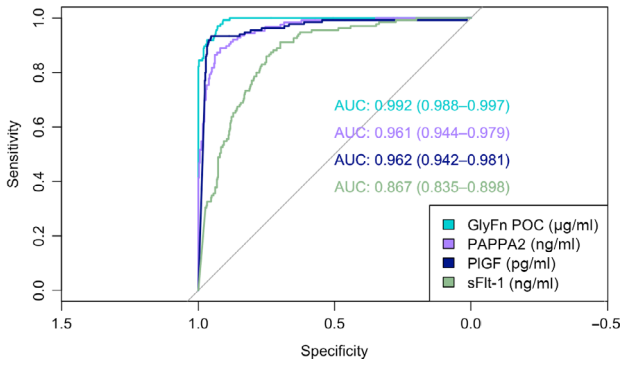

Figure 1. ROC curves and associated AUCs (control versus PE) for GlyFn, PAPPA2, PlGF and sFlt-1.

was similar to the overall analysis; i.e., that GlyFn exhibited the best performance, followed by PAPPA2, PlGF and sFlt-1.

Biomarker concentrations were also evaluated for differences between non-severe PE and severe PE (Table S3), and no significant differences were observed.

Biomarker status in GH subjects

Of the 194 subjects clinically stratified as GH (new-onset hypertension and urinary protein/creatinine ratio <0.3 mg protein/mmol creatinine), 109 subjects had sufficient samples to measure all four PE biomarkers. Of these 109 subjects, 47 (43%) were negative for all biomarkers and 62 (57%) were positive for at least one biomarker. Of these 62, 36 (58%) were positive for GlyFn plus two or three additional PE biomarkers, 17 (27%) were positive for GlyFn and one additional PE biomarker, and nine (15%) were positive for GlyFn alone. All samples with levels above the GlyFn diagnostic threshold (263 microg/ml) were positive for one or more additional biomarkers (Figure S5). Fifty-two GH subjects developed clinical PE (hypertension and proteinuria) and were positive for 3+ PE biomarkers: 34 of these (65%) delivered preterm. The mean birthweight was 2.05 kg (SD = 0.48), lower than the mean birthweight of 2.57 kg (SD = 0.57) in 142 GH subjects who did not develop clinical PE.

Discussion

Main findings

All of the biomarkers studied—GlyFn, PAPPA2, PlGF, and sFlt-1—displayed good test performance for the diagnosis of PE (AUC of 0.99–0.86; Table 3), with GlyFn exhibiting the best overall performance. Overall and relative biomarker performance was not different between early- versus late-onset PE (Figures S3 and S4, Tables S1 and S2), or between non-severe and severe PE (Table S3). Additionally, a significant proportion of subjects clinically stratified as GH were positive for PE biomarkers and also exhibited a higher frequency of preterm birth than control patients (frequently associated with the subsequent development of clinically apparent PE). The findings of this study extend the evaluation of the PE biomarkers GlyFn, PAPP-A2, PlGF and sFlt-1 to a Southeast Asian population and also demonstrate the ability of these biomarkers to predict preterm delivery in GH subjects that may be attributable to non-proteinuric PE or subsequent development of PE with proteinuria among those that were initially classified as GH alone.

Strengths and limitations

Major strengths of this study include a large number of subjects, well-defined inclusion and exclusion criteria, and a direct comparison of the performance of several potential PE biomarkers. One limitation of this study is that all patients enrolled had new-onset hypertension and were being evaluated for PE. Further studies will be necessary to assess the utility of GlyFn as a screening test among asymptomatic women.

Interpretation

The recent revision of guidelines for diagnosis of PE[13–15] reflects the variation in clinical presentation, which makes accurate diagnosis using purely clinical assessment difficult. As a result, a variety of biomarkers have been proposed to complement clinical presentation for PE diagnosis.[21–25] In addition, the results of the recent ASPRE trial support the use of biomarkers to direct aspirin therapy in preterm PE regardless of the particular set of clinical criteria employed.[38] Our studies in this Southeast Asian population demonstrate that several biomarkers—GlyFn, PAPPA2, PlGF and sFlt-1—were effective in detecting PE, with the GlyFn POC test exhibiting the best performance. The concentrations of PLGF were lower and those of sFlt-1 higher than reported by others, perhaps because of ethnic differences between population studies. Asians have been reported to have lower PlGF concentrations than non-Asians.[39] Thus, it is important to validate putative biomarkers in the appropriate context and ethnic setting. We have previously described aspects of fibronectin physiology that may explain its association with PE pathology[32] and that may contribute to its performance as a PE biomarker. One unique additional feature of the GlyFn biomarker is that the baseline concentrations observed do not differ based upon gestational age, in contrast to other biomarker concentrations that do vary with gestational age.[32]

Another finding of this study was the presence of PE biomarker positivity in a proportion of subjects diagnosed as GH without proteinuria and who subsequently delivered preterm. Because we did not ascertain maternal end-organ dysfunction or fetal growth restriction in this study, we cannot exclude the possibility that these patients had non-

proteinuric PE. These data may support the findings of others that nonproteinuric PE patients constitute a significant subclass of those previously thought to have only GH.[18] It will be important to determine whether testing for GlyFn or other PE biomarkers can reproducibly identify this important group, which may be misdiagnosed as classical GH due to the absence of proteinuria.

Application of purely clinical criteria to PE diagnosis is especially difficult in resource-poor LMIC environments such as India that have high rates of PE[40,41] and where accurate determination of standard clinical parameters (hypertension and various maternal organ dysfunction) can be difficult. The biochemical tests for PE diagnosis that are currently commercially available are the Alere Triage (Alere, Inc.) and DELFIA Xpress PlGF 1-2-3 (Perkin Elmer) tests that measure plasma levels of placental growth factor (PlGF),[42,43] and the Roche Elecsys and Thermo Fisher Scientific tests that measure the ratio of serum soluble fms-like tyrosine kinase-1 (sFlt-1) and PlGF.[44,45] The PlGF tests, however, require a blood draw and plasma separation rather than fingerstick whole blood, and a specialised reader, which limits its utility as a true POC test suitable for rural, low-resource environments. Similarly, the sFlt-1/PlGF ratio tests involve two different dilutions of serum and specialised analysis platforms. Thus, the currently available tests are not true POC tests and do not meet the WHO ASSURED criteria for POC tests amenable for use in LMIC settings.[46,47] These criteria, critical for implementation of diagnostics in LMIC, include being affordable, sensitive, specific, user-friendly, reliable and robust, and equipment-free or requiring minimal equipment.

In contrast, the ability of the Lumella™ GlyFn POC test accurately to diagnose PE in patients with a variety of clinical presentations suggests that this biomarker can facilitate PE diagnosis in this and other resource-poor situations. In addition, the Lumella™ GlyFn POC test meets the WHO ASSURED criteria for POC tests to be employed in low-resource settings.[46]

Conclusion

The current study extends our previous demonstration of the utility of GlyFn as a POC-amenable biomarker for PE diagnosis to a large Southeast Asian cohort that represents a major underserved population with significant PE risk. Our results demonstrate that the GlyFn POC test may enable the extension of accurate, rapid, and inexpensive diagnosis of PE in LMIC settings.

Disclosure of interests

SRN, PVR, KMS, DN-S and EB are employees of DiabetOmics, Inc., and SRN, PVR, and CTR are shareholders in DiabetOmics, Inc. The remaining authors report no conflicts of interest. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

SRN, PVR and MGG designed the study. VJ, ARV, KC and DP coordinated patient enrolment and clinical sample collection. KMS, DN-S and EB performed assays and analysed data. SRN, CTR and MGG analysed data and wrote the paper.

Details of ethics approval

Clinical samples were collected under protocols approved by the Institutional Ethics Committees of the participating institutions: Osmania Medical College, reg no. ECR/300/Inst/AP/2013, approved 3-26-2013; Ramdevrao Hospital, reg. no. ECR/709/Inst/TG /2015Dt, approved 2-13-2015; and Mallareddy Institute of Medical Sciences, reg. no. ECR/828/INST/AP/2016, approved 6-29-2016.

Funding

This work was funded by DiabetOmics, Inc.

Acknowledgements

The authors thank R. Suvarna, B. Amulya Keerti, M. Manjula, and D. Ranganath, for assistance with sample collection.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Clinical study design workflow.

Figure S2. Distribution of biomarker levels in control and clinically defined PE subjects.

Figure S3. Analysis of early-onset PE group compared with matching controls.

Figure S4. Analysis of late-onset PE group compared with matching controls.

Figure S5. Distribution of PE biomarkers in GH.

Table S1. Biomarker performance characteristics in early-onset PE versus matching controls.

Table S2. Biomarker performance characteristics in late-onset PE versus matching controls.

Table S3. Biomarker concentrations in non-severe versus severe PE. ■

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