ORIGINAL RESEARCH

Correlation of maternal serum glycosylated fibronectin in pre-eclampsia

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Received: 15 December, 2022 Accepted: 13 January, 2022

ABSTRACT

Objective: To assess the correlation of maternal serum glycosylated fibronectin level in preeclamptic pregnant women with severity of pre-eclampsia.

Methods: An 18-month prospective study was conducted at the Rajarajeshwari Medical College and Hospital (RRMCH), Bengaluru, in the department of obstetrics and gynaecology (Jan 2021 – May 2022). Twenty-five preeclamptic pregnant women ≥20 weeks gestation made up the anticipated sample size. The Lumella™ PE test gadget was used to perform the maternal serum GlyFN test using the GlyFN POC device (from DiabetOmics, Inc.).

Results: There was statistically significant difference in severe pre-eclampsia between <=350 and >350 serum fibronectin values (P value <0.05). There were 93.75% (15 out of 16) severe pre-eclampsia cases in >350 serum fibronectin group where it was only 6.25% in <=350 group.

Conclusion: GlyFn POC test is a promising biochemical marker for severity of preeclampsia, and may be a useful adjunctive tool for rapid and accurate triage and intervention.

Key words: biomarker, glycosylated fibronectin, point-of-care, preeclampsia

INTRODUCTION

Preeclampsia is a pregnancy-specific condition that has the potential to be fatal. [1] Preeclampsia and other hypertensive disorders account for 10 to 25% of all maternal deaths and are the second greatest cause of maternal mortality globally [2]. Preeclampsia clinical signs, which can happen later in the course of the condition and have detrimental effects on both the mother and the unborn child, are regrettably possible. To properly manage preeclampsia and to lessen negative outcomes, robust biomarkers for screening, diagnosis, and monitoring are required, especially with regard to severe preeclampsia. [3,4]

This is especially true in underdeveloped nations where the disease burden is highest and medical care is frequently ineffective because of late presentation. [5,6] Additionally, since 1990, the prevalence of preeclampsia has been rising, which may be directly linked to the rise in obesity. [7] In order to manage pre-eclampsia and ensure appropriate triage to expert medical institutions, early and efficient diagnostic tests are critically required.

Although several angiogenic/antiangiogenic markers, including soluble vascular endothelial growth factor receptor 1 (sFlt1), placental growth factor (PlGF), and soluble endoglin, have been used to develop single- or multi-analyte tests that may exhibit more robust performance,

no currently available biomarkers perform sufficiently well to justify replacing the current clinical diagnosis of preeclampsia. [8-16]
Biochemical markers such as soluble endoglin, placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF), pregnancy-associated plasma protein A-2 (PAPPA2), glycosylated fibronectin (GlyFn), vasopressin, and copeptin can be used to support the diagnosis of pre-eclampsia [17-21] We outline a point-of-care (POC) platform for GlyFn level analysis in blood that will enable quick evaluation of preeclampsia progression and has the potential to be used as a screening tool.
Hence, a study was done to assess the correlation of maternal serum glycosylated fibronectin level in preeclamptic pregnant women with severity of pre-eclampsia.

METHODS

An 18-month prospective study was conducted at the Rajarajeshwari Medical College and Hospital (RRMCH), Bengaluru, in the department of obstetrics and gynaecology (Jan 2021 – May 2022). Twenty-five preeclamptic pregnant women at ≥20 weeks of gestation made up the anticipated sample size. The Lumella^{TM} PE test gadget was used to perform the maternal serum GlyFN test using the GlyFN POC device (from DiabetOmics, Inc.). The test can be completed at a doctor's office or clinic and just involves a quick finger stick.
Pregnant Women with preeclampsia at ≥20 weeks of gestation attending our OP/ IP department fulfilling inclusion and exclusion criteria and counselled for undergoing the test. Patients of age 18 to 35 with Pregnancies complicated by preeclampsia and Singleton pregnancies more than 20 weeks POG and those who were willing to give consent for the study were included in the study. Patients with multiple pregnancies, and other medical disorders complicating pregnancy were excluded from the study.
A single-use lancet is used to clean and pierce the finger. At the puncture site, a tiny drop of blood is allowed to accumulate. Without pressing the bulb, the micropipette is gently touched to the blood and allowed to fill to the designated line. The micropipette used for blood collection has its tip placed inside the buffer. Press the bulb twice to release the blood. Inverting the sample eight to ten times mixes it. The test cartridge is inserted for the example application, and the reader is turned on. 120 µl of the diluted sample is added to the test strip at the application port of the inserted cartridge after 5 µl of the sample is diluted 1:350 in the running buffer. At the end of 10 minutes, the reader will show the GlyFn concentration.

The GlyFn concentration will be displayed on the reader as per the below grouping.

Risk Stratification	GlyFn values (µg/ml)
Normal	<250
Abnormal	251-350
Positive	351-600
High positive	>600

Quantitative characteristics are summarised by mean and SD while qualitative data is summarised by frequency and percentage. Chi-square test is used to analyse the parameters. A p-value of 0.05 or lower will be regarded as significant after conducting all necessary statistical analyses. Utilizing coGuide software V.1.0, data was analysed. (India: BDSS Corp., 2020. coGuide statistics software, Version 1.0)

RESULTS

A total of 25 subjects included in the final study.

Table 1: Descriptive analysis of Age in the study population (N=25)

	Mean ± S.D	Median	Minimum	Maximum	95% CI	
					Lower CI	Upper CI
Age	27.16 ± 3.91	27.00	20.00	35.00	25.63	28.69

Table 2: Descriptive analysis of study population (N=25)

	Frequency	Percentage
Gravida		
PRIMI	14	56
MULTI	11	44
Preeclampsia		
Non-severe Preeclampsia	9	36
Severe Preeclampsia	16	64
Mode of delivery		
NVD	8	32
LSCS (Emergency)	17	68
Serum Fibronectin		
High positive	8	32
Positive	9	36
Normal	2	8
Abnormal	6	24
Serum Fibronectin		
> 350	17	68
<= 350	8	32
Gestational age (Term/Preterm)		
Term	10	40
Very preterm	4	16
Moderate preterm	4	16
Late preterm	7	28

Table 3: Descriptive analysis of Indication for LSCS in the study population (N=16)

Indication for LSCS	Frequency	Percentage
Severe pre-eclampsia with imminent signs	7	43.75%
Severe pre-eclampsia	7	43.75%
Thick MSL	1	6.25%
Non progression of labour	1	6.25%

Table 4: Descriptive analysis of Gestational age at termination (weeks) in the study population (N=25)

Name	Mean ± S.D	Median	Minimum	Maximum	95% CI	
					Lower CI	Upper CI
Gestational age at termination (weeks)	35.64 ± 2.93	36.29	28.86	40.29	34.49	36.79

Table 5: Comparison of Serum fibronectin with Age, Gravida, Mode of delivery, Gestational age at termination (weeks)(N=25)

Parameter	Serum Fibronectin		Chi square value	P value
	>350 (N=17)	<=350 (N=8)		
Age	27.06 ± 3.45	27.38 ± 5.01	-	0.8551£
Gravida				
MULTI	7 (41.18%)	4 (50.00%)	0.17	1.0000*
PRIMI	10(58.82%)	4 (50.00%)		
Mode of delivery				
NVD	4 (23.53%)	4 (50.00%)	1.75	0.3592*
LSCS (Emergency)	13 (76.47%)	4 (50.00%)		
Gestational age at termination (weeks)	34.58± 2.88	37.89± 1.38	-	0.0054£

Table 6: Comparison of Mode of delivery across Preeclampsia

Mode of delivery	Severity of Preeclampsia		Chi square value	P value
	Non-severe Preeclampsia (N=9)	Severe Preeclampsia (N=16)		
NVD	5 (55.56%)	3 (18.75%)	3.59	0.0870*
LSCS (Emergency)	4 (44.44%)	13 (81.25%)		
Serum fibronectin For <=350 (N=8)				
NVD	4 (57.14%)	0 (0.00%)	-	*
LSCS (Emergency)	3 (42.86%)	1 (100.00%)		
Serum fibronectin For >350 (N=17)				
NVD	1 (50.00%)	3 (20.00%)	0.88	0.4265 *

LSCS (Emergency)	1 (50.00%)	12 (80.00%)		

Table 7: Comparison of Serum Fibronectin with severity of Preeclampsia (N=20)

Severity of Preeclampsia	Serum Fibronectin		Chi square value	P value
	>350	<=350		
Non-severe Preeclampsia (N = 9)	2 (22.22%)	7 (77.78%)	13.54	<0.001*
Severe Preeclampsia (N = 16)	15 (93.75%)	1 (6.25%)		

£ = IST P-value; *=Chi-square test value

DISCUSSION

Out of the total 25 preeclamptic patients in our study population, 17 patients (68%) had GlyFn value >350 and only 8 (32%) had Glyfn value <=350. Given the substantial correlation between total serum Fn and preeclampsia that others have previously reported, the relationship of increased GlyFn with preeclampsia is not wholly unexpected. [22-26] The greater engagement of a particular fraction of Fn with the pathological processes that cause preeclampsia is likely the cause of this Fn fraction's better performance. [1] This in turn may indicate a specific role for Fn splice variants or proteolytic fragments with distinctive glycosylation patterns in the development of preeclampsia. Given that both plasma fibronectin (pFn) and cellular fibronecetin (cFn) are glycosylated and present in the circulation, we are unsure whether this particular Fn species is produced from one of them or from both, but an earlier study [24] noted a link between preeclampsia and higher cFn. It is noteworthy that core-1 O-glycan Galb1-3GalNac epitope expression in the human placenta has recently been shown to be regulated by oxygen levels; as a result, placental insufficiency may also play a role in the altered glycoprotein levels seen in preeclampsia.

One unique additional feature of the GlyFn bio-marker is that GlyFn levels remain constant in normotensive patients throughout pregnancy, in contrast to other bio-marker concentrations which vary with gestational age of patient. Thus, GlyFn is a particularly helpful analyte for monitoring preeclampsia. [27][28]

In our study, the mean gestational age at termination for the study population was 35.64 weeks. The mean gestational age at termination was 34.58 weeks in GlyFn >350 group and 37.89 weeks in Glyfn <= 350 group with P value 0.0054, which is statistically significant as P value <0.01. Similar to our results, another study by Rasanen and others found that GlyFn values had a significant linear relationship with gestational age at delivery [27]. This study also showed that for every 100mg/mL increase in GlyFn, there was a predicted decrease in gestational age at delivery of 0.59 weeks (4 days).

Given that both preeclampsia and gestational diabetes are linked to inflammation and endothelial dysfunction [29–37], it is possible that these connections explain why GlyFn is linked to both diseases. Thus, elevated levels of a particular type of GlyFn may be associated to first-trimester inflammation and endothelial dysfunction caused by disturbed spiral artery remodelling. The different patterns of GlyFn abundance in these 2 related conditions (i.e., consistently elevated in all trimesters in gestational diabetes but a progressive increase during the course of preeclampsia) are not fully understood, but they may indicate that the conditions that cause gestational diabetes are established early in pregnancy and remain at a constant level, whereas the conditions that cause preeclampsia are continuously increased during its initiation and development.

In our study, there was statistically significant difference in severe pre-eclampsia between <=350 and >350 serum fibronectin value groups (P value <0.001). There were 93.75% (15

out of 16) severe pre-eclampsia cases in >350 serum fibronectin group whereas there were only 6.25% in <=350 group. The study by Rasanen and others analysed the difference in weekly GlyFn change in the third trimester through repeated-measures analysis and the results showed that mild preeclampsia was associated with a weekly change of 81.7 mg/mL (SE 94.1) vs 195.2 mg/mL (SE 88.2) for severe preeclampsia [27]. A study by Huhn, et al. found that GlyFn positivity was highest in severe forms of PE and eclampsia. The positivity declined in less severe disease. [38]

CONCLUSION

There was statistically significant difference in severe pre-eclampsia between <=350 and >350 serum fibronectin values (P value<0.05). There were 93.75% (15 out of 16) severe pre-eclampsia cases in >350 serum fibronectin group where it was only 6.25% in <=350 group. GlyFn POC test is a promising biochemical marker for severity of preeclampsia, and may be a useful adjunctive tool for rapid and accurate triage and intervention.

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