Original Article

Diagnostic Accuracy of Serum Glycosylated Fibronectin in Prediction of Preeclampsia: A Nested Case–Control Study

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Abstract

Background: Preeclampsia is a life-threatening complication of pregnancy that occurs in approximately 7% of all pregnancies. In India, the incidence of preeclampsia is 8%–10% and the prevalence is 5.4%, whereas the prevalence of hypertensive disorders of pregnancy is 7.8%. **Aim and Objectives:** This study was aimed at evaluating the diagnostic accuracy of serum glycosylated fibronectin (S. GlyFn) in the prediction of preeclampsia. **Methods:** A nested case–control study was carried out for 16 months in the department of obstetrics and gynecology. A total of 240 women were recruited and followed after written consent and ethical clearance. Six were lost to follow-up, 15 had second-trimester abortions (excluded from the study), and 32 women developed hypertensive disorders of pregnancy (cases), out of which 1 woman developed antepartum eclampsia, 10 women developed preeclampsia with severe features, and 21 women developed preeclampsia without severe features. One hundred and eighty-seven women remained normotensive throughout the pregnancy until 6 weeks postpartum. After randomization, out of these samples, 54 were analyzed and considered controls. Levels of S. GlyFn were estimated using an ELISA kit using the ELISA technique. **Results:** The mean S. GlyFn level was significantly higher at the time of enrollment among those women who later developed preeclampsia (127.59 ± 27.68 ng/m) as compared to controls (107.79–53.51 ng/mL). GlyFn at a cutoff value of 126.70 ng/mL significantly (P = 0.034) discriminates cases of preeclampsia with severe features from healthy controls with a sensitivity of 90.00%, a specificity of 63.00%, a 31.03% positive predictive value, and 97.14% negative predictive value. **Conclusion:** S. GlyFn, at a cutoff value of 126.70 ng/mL, had good sensitivity to discriminate PE from normotensive and was also a good prognostic marker.

Keywords: Diagnostic accuracy, hypertensive disorder of pregnancy, preeclampsia, serum glycosylated fibronectin

Résumé

Contexte: La prééclampsie est une complication potentiellement mortelle de la grossesse qui survient dans environ 7 % de toutes les grossesses. En Inde, l'incidence de la prééclampsie est de 8 % à 10 % et la prévalence est de 5,4 %, alors que la prévalence des troubles hypertensifs de la grossesse est 7,8 %. But et objectifs : Cette étude visait à évaluer la précision diagnostique de la fibronectine sérique glycosylée (S. GlyFn) chez la prédiction de la prééclampsie. **Méthodes:** Une étude cas-témoin nichée a été menée pendant 16 mois dans le service d'obstétrique et gynécologie. Au total, 240 femmes ont été recrutées et suivies après consentement écrit et autorisation éthique. Six ont été perdus de vue, 15 avaient avortements au deuxième trimestre (exclus de l'étude), et 32 femmes ont développé des troubles hypertensifs de la grossesse (cas), dont 1 femme a développé une éclampsie antepartum, 10 femmes ont développé une prééclampsie avec des caractéristiques sévères et 21 femmes ont développé une prééclampsie sans traits sévères. Cent quatre-vingt sept femmes sont restées normotendues tout au long de la grossesse jusqu'à 6 semaines après l'accouchement. Après randomisation, sur ces échantillons, 54 ont été analysés et considérés comme témoins. Les niveaux de S. GlyFn ont été estimés à l'aide d'un kit ELISA en utilisant la technique ELISA. **Résultats:** Le niveau moyen de S. GlyFn était significativement plus élevé au moment de l'inscription chez les femmes qui ont développé plus tard une prééclampsie (127,59 ± 27,68 ng/m) par rapport aux témoins (107,79–53,51 ng/mL). GlyFn à une valeur seuil de 126,70 ng/mL de manière significative (P = 0,034)

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discrimine les cas de prééclampsie avec des caractéristiques sévères des témoins sains avec une sensibilité de 90,00 %, un spécificité de 63,00 %, une valeur prédictive positive de 31,03 % et une valeur prédictive négative de 97,14 %. **Conclusion:** S. GlyFn, à une valeur seuil de 126,70 ng/mL, avait une bonne sensibilité pour distinguer l'EP du normotendu et était également un bon marqueur pronostique.

Mots-clés: Précision diagnostique, trouble hypertensif de la grossesse, prééclampsie, fibronectine glycosylée sérique

Introduction

Preeclampsia is a life-threatening medical complication of pregnancy that occurs in approximately 7% of all pregnancies.[1] In India, the incidence of preeclampsia is 8%–10% among pregnant women. As per studies, the prevalence of hypertensive disorders in pregnancy is 7.8%, whereas the prevalence of preeclampsia is 5.4%.[2] About 5%–8% of women with preeclampsia may fall into the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count).[3] In preeclampsia, there is multisystem involvement, leading to deleterious effects on the kidneys, liver, brain, and clotting system. Preeclampsia if left unattended it might be converted into a more serious condition known as eclampsia. Preeclampsia poses an increased risk to the mother and fetus. Preeclampsia is defined as per the ACOG guideline.[4,5] Gestational hypertension is defined as a systolic blood pressure of 140 mmHg or greater or a diastolic blood pressure of 90 mmHg or greater that occurs on two occasions 4 h apart after 20 weeks of gestation in a woman with previously normal blood pressure. Preeclampsia with severe features defined as systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on one occasion or one of the following**:** proteinuria ≥300 mg in 24 h, persistent +1, or more on dipstick random samples, or a protein/creatinine ratio >0.3 mg/dL. In the absence of proteinuria, new-onset hypertension with any one of the following, thrombocytopenia (platelet/ml <100,000), serum creatinine >1.1 mg/dl, elevated liver enzymes transaminases twice the upper limits of normal, pulmonary edema, new-onset headache, or visual disturbances.

There is a need of hour to discover a novel biomarker for the screening and diagnosis of preeclampsia.[3,6] In an attempt to diagnose preeclampsia early and effectively, various biomarkers have been searched, but no suitable biomarker has been found that is good enough to predict the current clinical diagnosis of preeclampsia at an early stage.[7,8]

GlyFn levels are elevated in metabolic complications of pregnancy. In a few studies, elevated maternal serum GlyFn levels were observed in preeclampsia, but further research is required. Cellular fibronectin has been found to be associated with many other pathological conditions such as diabetes, preterm birth, and inflammation.[9-12] The hypothesis behind this rise in serum glycosylated fibronectin (S. GlyFn) level is that it is associated with vascular endothelial damage even much earlier as compared to the appearance of symptoms and this vascular endothelial damage is prime feature of preeclampsia. Thus, the estimation of S. GlyFn level in the first or early second trimester might be helpful in the prediction of preeclampsia. The aim of this study was to assess the role of S. GlyFn as a predictor of preeclampsia.

Methods

This was a nested case–control study conducted in the obstetrics and gynecology department in collaboration with pathology and medicine. The duration of the study was 16 months from September 2020 to December 2021.

Sample size

The sample size was calculated on the basis of 80% power of the study, error rate, usually set at 0.05 level is four, and the incidence of preeclampsia in developing countries.[13,14] The incidence of preeclampsia is 12.3% maximum (4.0%–12.3%).[13-15]

$$n = Z^2P\,(1 - P)/d^2$$

Where,

- n = sample size,
- Z = Z statistics for a level of confidence, for the level of confidence of 95%, which is conventional, Z value is 1.96.
- P = expected prevalence or proportion (in proportion of one; if 12.3%, P = 0.123)
- d = precision (in proportion of one; if 5%, d = 0.05)

$$n = 1.96 \times 1.96 \times 0.123 \times 0.8/0.05^2$$

$$=240$$

After attaining written informed consent and ethical clearance from the institutional ethics committee, a total of 240 normotensive pregnant women with a gestational period between 14 and 20 weeks of gestation were recruited. Women with antiphospholipid antibody syndrome, systemic lupus erythematosus, diabetes, and chronic hypertension were excluded from the study.

All enrolled women were subjected to a detailed history, demographic profile, general examination, physical examination, systemic examination, and obstetric examination. 2 mL of blood samples were withdrawn from the antecubital vein and collected in a plain vial.

A second sample was collected after the development of preeclampsia without severe features, with severe features, or eclampsia, defined as per updated ACOG guidelines 2013.[4,5] Samples were centrifuged at 3000 rpm for 15 min. The clear supernatant (serum) was transferred to an Eppendorf vial and stored at − 40°C in a deep freezer until analyzed.

All women were followed; 6 women lost to follow-up; 15 pregnant women who had second-trimester abortions were excluded from the study. Thirty-two women developed hypertensive disorders of pregnancy (cases); out of these, 1

woman developed antepartum eclampsia, 10 women developed preeclampsia with severe features, and 21 women developed preeclampsia without severe features [Flow Diagram 1].

Fifty-four control samples were taken after randomization from those 187 samples of women who remained normotensive throughout the pregnancy until 6 weeks postpartum. The institutional ethics committee gave approval for this study (Letter number 811/Ethics/2020, ref. code 101st ECMII B-Thesis/P76).

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 23.0 (Armonk, NY: IBM Corp). The categorical variables were presented as numbers and percentages (%) and continuous variables were presented as mean ± standard deviation and median. Quantitative variables were compared using an unpaired *t*-test between the two groups. Qualitative variables were compared using the Chi-square test or Fisher's exact test. Receiver-operating characteristic (ROC) was used for diagnostic testing of parameters. P <0.05 was considered statistically significant.

Results

In the present study, the majority of cases and controls were from urban areas (64.5% and 68.5%, respectively). The majority of cases (38.7%) were from the lower-middle socioeconomic status. The majority of the controls were of middle socioeconomic status (38.9%). The majority of the women from both groups were literate (100% and 97.64%). The majority of women in cases (74.2%) and controls (81.5%) were primigravida [Table 1].

In this study, the mean body mass index (BMI) of cases was higher as compared to controls. The mean BMI of cases 25.54 ± 2.51 kg/m^2 and of controls was 23.44 ± 2.43 kg/m^2 [Table 2].

The mean S. GlyFn level was significantly higher at the time of enrollment among those women who later developed preeclampsia (127.59 ± 27.68 ng/mL) as compared to controls (107.79 ± 53.51 ng/mL) who remained normotensive throughout the pregnancy [Table 3].

The mean level of S. GlyFn was significantly higher in preeclampsia with severe features (144.28 ± 24.53) as compared to preeclampsia without severe features (119.64 ± 25.94 ng/ml) at the time of enrollment [<20 weeks of gestation; Table 4].

After the development of PE, overall levels decreased, but the mean level of S. GlyFn in preeclampsia with severe features (87.84 ± 24.25 ng/ml) was significantly higher as compared to preeclampsia without severe features 73.09 ± 14.62 ng/ml.

ROC curve analysis

After ROC analysis, the diagnostic accuracy of S. GlyFn at a cutoff value of 92.80 ng/ml discriminates the cases (PE) and controls at AUC = 0.614 (P = 0.080) with 100.00% sensitivity, 48.10% specificity, 52.54% positive predictive value (PPV), and 100% negative predictive value (NPV) [Figure 1].

Diagnostic accuracy of S. GlyFn to discriminate the cases of preeclampsia with severe features from controls at a cutoff value of 126.70 ng/ml and an AUC of 0.712 had 90.00% sensitivity, 63.000% specificity, 31.03% PPV, and 97.14% NPV [Figure 2].

At cutoff value of 92.80 ng/ml S., GlyFn discriminates the cases of preeclampsia without severe features from controls at AUC = 0.568 (P = 0.364) with 100.00% sensitivity, 48.10% specificity, 42.86% PPV, and 100% NPV [Figure 3].

Table 1: Demographic distribution of women (n=85)

	Groups			*P*
	Case (n=31), *n* (%)	Controls (Group I) (n=54), *n* (%)	Total (n=85), *n* (%)	
Locality				
Urban	20 (64.5)	37 (68.5)	57 (67.1)	0.705
Rural	11 (35.5)	17 (31.5)	28 (32.9)	
Socioeconomic status				
Lower	7 (22.6)	7 (13.0)	14 (16.5)	0.714
lower middle	12 (38.7)	20 (37.0)	32 (37.6)	
Middle	10 (32.3)	21 (38.9)	31 (36.5)	
middle upper	2 (6.5)	5 (9.3)	7 (8.2)	
Upper	0	1 (1.9)	1 (1.2)	
Educational status				
Illiterate	0	2 (3.7)	2 (2.4)	0.428
Literate	32 (100)	52 (96.3)	83 (97.64)	
Parity				
Primigravida	23 (74.2)	44 (81.5)	67 (78.8)	0.706
Second gravid	6 (19.4)	8 (14.8)	14 (16.5)	
Multigravida	2 (6.5)	2 (3.7)	4 (4.7)	

Applied Chi-square test for significance

Table 2: Comparison of body mass index in cases and control

	Groups, mean±SD			*P*
	Cases (n=31)	Controls (n=54)	Total (n=85)	
Weight (kg)	60.84±5.79	55.37±5.10	57.36±5.95	<0.001
Height (cm)	154.40±4.09	153.84±5.08	154.05±4.72	0.603
BMI (kg/m^2)	25.54±2.51	23.44±2.43	24.21±2.65	<0.001

Applied unpaired *t*-test for significance. BMI=Body mass index, SD=Standard deviation

Table 3: Serum glycosylated fibronectin level at the time of enrollment (<20 weeks of gestation) among cases and controls (n=85)

	Groups, mean±SD			*P*
	Cases (n=31)	Controls (n=54)	Total (n=85)	
Serum GlyFn level (ng/mL) <20 weeks of gestation	127.59±27.68	107.79±53.51	115.01±46.60	0.059

Applied unpaired *t*-test for significance. SD=Standard deviation, GlyFn=Glycosylated fibronectin

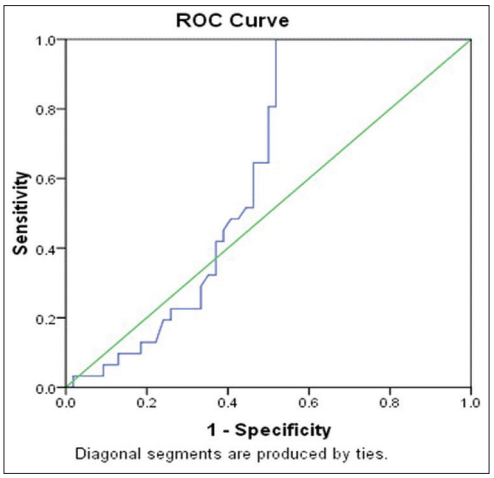


Figure 1: Diagnostic accuracy of serum glycosylated fibronectin to discriminate the cases of preeclampsia and normotensive controls at a cutoff value of 92.80 ng/mL had 100.00% sensitivity, 48.10% specificity

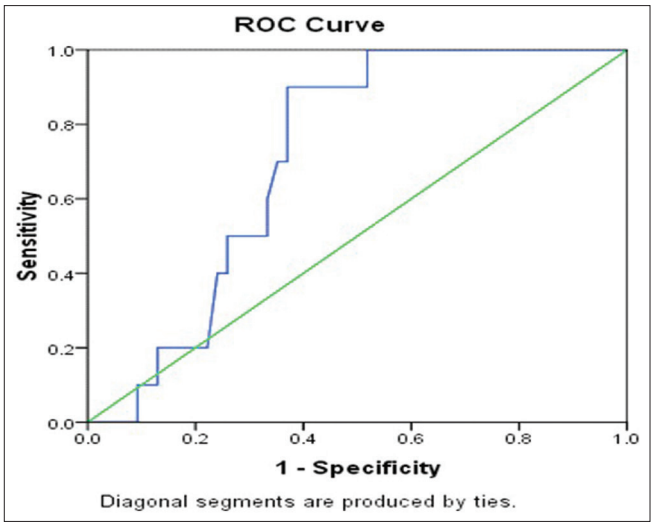


Figure 2: Diagnostic accuracy of serum glycosylated fibronectin to discriminate the cases of preeclampsia with severe features and normotensive control at cutoff value of 126.70 ng/ml, AUC = 0.712 had 90.00% sensitivity, 63.00% specificity

Discussion

In the present study, the mean age for preeclampsia was high as compared to the control group. Various other studies reported high mean ages in the preeclampsia group: 28.3 years, 30.43 ± 5.77 years, and 26.4 years.[16-18] The risk of preeclampsia increases with age.

In our study, the majority of cases of preeclampsia were from lower-middle socioeconomic status (as per the B.G. Prasad scale).[19] The majority of patients with preeclampsia were literate; various other authors reported the same.[16,20,21]

There were certain factors that stood out as obstacles for women seeking antenatal advice during pregnancy, including difficulty approaching health-care centers, inconvenient transport, a lack of knowledge, economic constraints, and much more. Therefore, more attention should be given to pregnant women living in villages or remote areas.[17]

One meta-analysis reported that the mean BMI was higher in severe preeclampsia than in nonsevere PE and concluded that an increase in BMI increases the risk of preeclampsia.[22] In our study, the mean BMI was higher (26.50 kg/m^2) in preeclampsia with severe features than in preeclampsia without severe features (25.09 kg/m^2). Other authors had reported almost similar findings.[17,23] In the present study, the majority of patients were from urban areas and were registered patients; this might be because our institute is a tertiary care center situated in an urban region. The majority of the cases were primigravida; various other authors reported preeclampsia in primigravida.[8,24,25]

Primigravida is six to eight times more susceptible to preeclampsia than multigravida. The odds of developing preeclampsia were 2.68 times higher in primigravida women as compared to multigravida women.[26] As observed in various studies, preeclampsia is a disease of first pregnancy.[27,28] The mechanism to explain this association of nulliparity with preeclampsia is immune maladaptation adaptation.[27] First exposure to chorionic villi, which are of fetal origin, and a strong maternal immunological response is more likely during the first pregnancy, and hence, there is an increased risk of preeclampsia in the first pregnancy.[29]

S. GlyFn might be a prognostic marker in preeclampsia because the mean S. GlyFn level was significantly higher at the time of enrollment (<20 weeks of gestation) among those women who later developed preeclampsia (127.59 ± 27.68 ng/ml) as compared to controls (107.79 ± 53.51 ng/mL) who remain normotensive throughout the pregnancy. In preeclampsia with severe features, the mean level of S. GlyFn was significantly higher as compared to preeclampsia without severe features.

One of the studies determined the performance of GlyFn levels using a POC device (Lumella test) for preeclampsia in low- and middle-income countries and found a significant association ($P < 0.01$) between increased levels of GlyFn in preeclampsia.[30] This rise in fibronectin is due to vascular injury and the release of fibronectin. This release is due to enzymatic degradation or increased production, as reported by one author.[31]

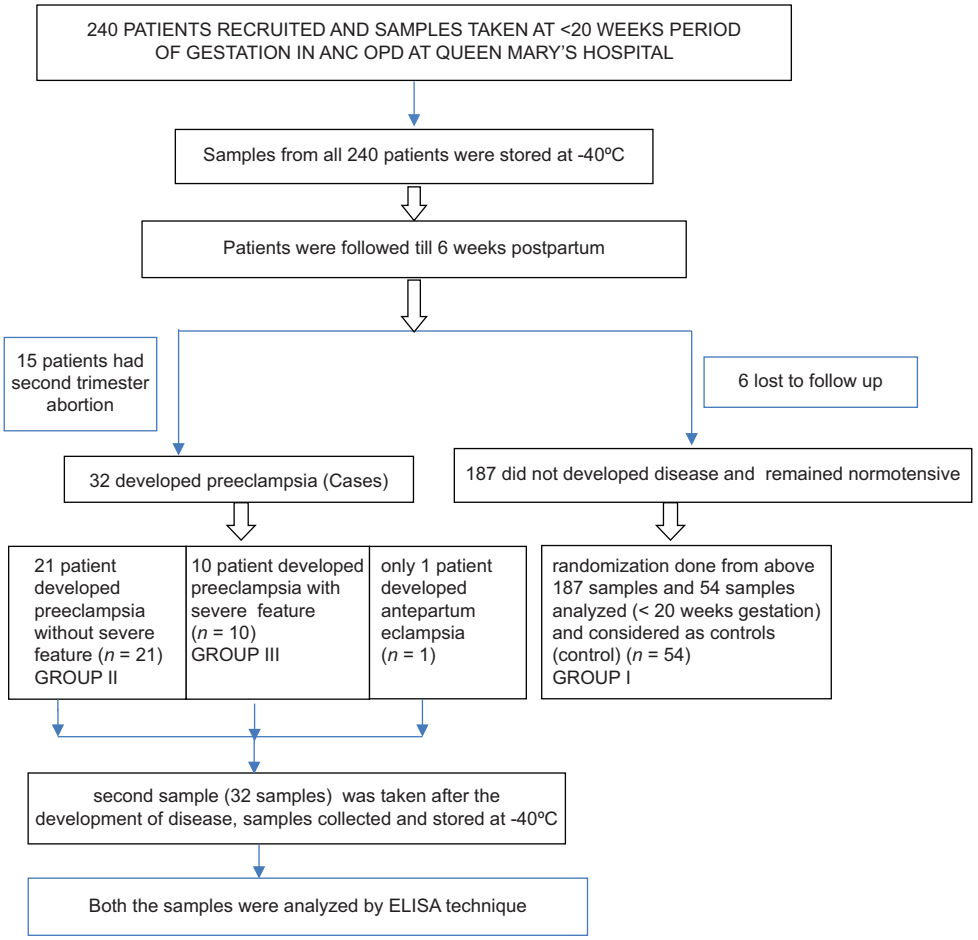
In the present study, there was one antepartum eclampsia, which was excluded during analysis. Primiparous patient with a BMI of 25, from an urban area, belonging to the lower-middle socioeconomic level S. GlyFn level was 126.15 ng/mL at the time of enrollment (<20 weeks of gestation), and S. GlyFn level was lower at 87.75 ng/mL after the development of disease.

After receiver operator curve (ROC) analysis, S. GlyFn (<20 weeks of gestation) at a cutoff value of 92.80 ng/mL discriminated the cases of preeclampsia from healthy controls with 100.00% sensitivity and 48.10% specificity with 52.54% PPV and 100% NPV at AUC = 0.614 ($P = 0.080$).

Table 4: Serum glycosylated fibronectin at the time of enrollment and after the development of disease among cases (*n*=31)

	Groups, mean±SD			*P*
	Preeclampsia with severe features (Group III) (*n*=10)	Preeclampsia without severe features (Group II) (*n*=21)	Total (*n*=31)	
Serum GlyFn levels (ng/mL) at <20 weeks of gestation	144.28±24.53	119.64±25.94	127.59±27.68	0.018
Serum GlyFn levels (ng/mL) after the development of PE (ng/mL)	87.84±24.25	73.09±14.62	77.85±19.19	0.043

Applied unpaired *t*-test for significance. SD=Standard deviation, PE=Preeclampsia, GlyFn=Glycosylated fibronectin



Flow Diagram 1: Participant flow through the study

Glycosylated fibronectin at a cutoff value of 126.70 ng/mL significantly ($P = 0.034$) discriminated the cases of preeclampsia with severe features from healthy controls. At an AUC of 0.712, S. GlyFn had 90.00% sensitivity, 63.00% specificity, 31.03% PPV, and 97.14% NPV.

Glycosylated fibronectin cutoff value of 92.80 ng/mL discriminates cases of preeclampsia without severe features from healthy controls, AUC = 0.568 ($P = 0.364$), with 100.00% sensitivity, 48.10% specificity, 42.86% PPV, and 100% NPV.

The present study was a nested case–control study in which, at a cutoff value of S. GlyFn of 98.8 ng/ml, sensitivity was 100%, specificity was 48.10%, PPV was 52.54%, and NPV was 100%.

In another study, which was also a nested case–control study, at a cutoff value of S. GlyFn of 176.4 ng/ml, sensitivity was 97%, specificity was 93%, PPV was 47%, and NPV was 94%.[16]

In one of the studies that was a prospective observational study, at a cutoff value of S. GlyFn of 315 ng/ml sensitivity was 91% and specificity was 86%.

In another prospective observational study, at a cutoff value of S. GlyFn of 263 ng/ml sensitivity was 98.5% and specificity was 92.8%. Other authors also observed an increase in fibronectin in the first trimester of pregnancy, which would eventually lead to preeclampsia in the course of pregnancy.[32-34]

In the current study, we found significantly higher levels of S. GlyFn concentration in women with preeclampsia

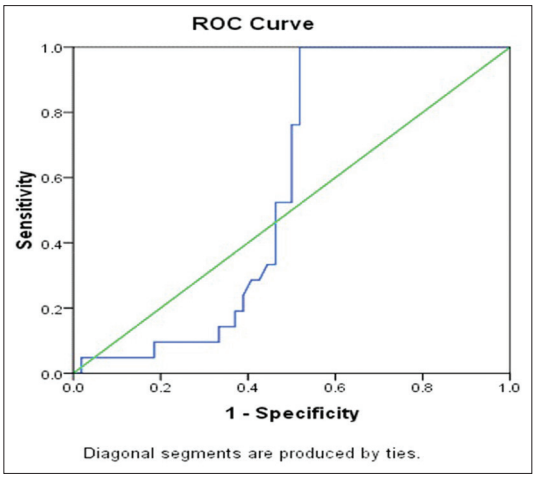


Figure 3: Diagnostic accuracy of serum glycosylated fibronectin to discriminate the cases of preeclampsia without severe features from normotensive control at cutoff value of 92.80 ng/ml, AUC = 0.568 (P = 0.364) had 100.00% sensitivity, 48.10% specificity

as compared to controls at <20 weeks of gestational age (P = 0.059). Furthermore, in preeclampsia with severe features, the mean level of S. GlyFn was significantly higher as compared to preeclampsia without severe features (P = 0.018). This finding was consistent with various other studies.[30,31,35-37]

S. GlyFn can be used as a biomarker for the prediction of preeclampsia. Fibronectin is synthesized in the endothelial cells, and its increased level indicates damage to the endothelium.

Early recognition of preeclampsia will prevent complications and help reduce morbidity and mortality; therefore, the availability of a simple predictive biomarker of preeclampsia would allow preventive measures to treat preeclampsia.[38]

The result of our study provides a potential background for future studies to establish the role of S. GlyFn in the prediction of preeclampsia. S. GlyFn is not only a predictor of preeclampsia; it is also helpful in the prognostication of a disease. S. GlyFn in preeclampsia had good sensitivity, so it is a good screening marker. S. GlyFn has good diagnostic accuracy.

Although the numbers of recruited women were good, the number of cases who developed preeclampsia on follow-up was lower. For a better outcome, a larger sample size is required. Due to the different types of methods (such as the point-of-care test, radial immune diffusion assay, and ELISA) used in detecting the level of S. GlyFn in various other studies, a standard cutoff value could not be exactly assessed.

Our study shows S. GlyFn has the potential to serve as a good screening tool (due to its high sensitivity) in the first or early second trimester in normotensive, healthy pregnant women to predict preeclampsia in the later part of the pregnancy. Its high NPV rules out the disease.

Conclusion

S. GlyFn levels elevated in preeclampsia as compared to normotensive healthy controls. Because of this early rise in pregnancy (between 14 and 20 weeks of gestation) and its high sensitivity S. GlyFn can be considered good biomarker before 20 weeks of gestation for prediction of preeclampsia. Furthermore, S. GlyFn level was found to be elevated in preeclampsia with severe features as compared to preeclampsia without severe features, and this explains its role as a prognostic marker in preeclampsia. S. GlyFn at a cutoff value of 92.80 ng/mL discriminates between PE and control with 100.00% sensitivity, 100% NPV, at a cutoff value of 126.70 ng/mL it discriminates preeclampsia with severe features from controls with 90.00% sensitivity and 97.14% NPV.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. Clin J Am Soc Nephrol 2007;2:543-9.
2. Sajith M, Nimbargi V, Modi A, Sumariya R, Pawar A. Incidence of pregnancy induced hypertension and prescription pattern of antihypertensive drugs in pregnancy. Int J Pharm Sci Res 2014;23:4.
3. Mikat B, Gellhaus A, Wagner N, Birdir C, Kimmig R, Köninger A. Early detection of maternal risk for preeclampsia. ISRN Obstet Gynecol 2012;2012:172808.
4. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American college of obstetricians and gynecologists' task force on hypertension in pregnancy. Obstet Gynecol 2013;122:1122-31.
5. American College of Obstetricians and Gynecologists. Committee on practice bulletins – Obstetrics. ACOG practice bulletin number 222: Gestational hypertension and preeclampsia. Obstet Gynecol 2020;135:e237-60.
6. Griffin M, Shennan AH. Clinical applications of biomarkers in preeclampsia. Biomark Med 2014;8:459-70.
7. Angeli F, Angeli E, Reboldi G, Verdecchia P. Hypertensive disorders during pregnancy: Clinical applicability of risk prediction models. J Hypertens 2011;29:2320-3.
8. Myatt L, Clifton RG, Roberts JM, Spong CY, Hauth JC, Varner MW, *et al.* First-trimester prediction of preeclampsia in nulliparous women at low risk. Obstet Gynecol 2012;119:1234-42.
9. White ES, Baralle FE, Muro AF. New insights into form and function of fibronectin splice variants. J Pathol 2008;216:1-14.
10. Ylätupa S, Haglund C, Mertaniemi P, Vahtera E, Partanen P. Cellular fibronectin in serum and plasma: A potential new tumour marker? Br J Cancer 1995;71:578-82.
11. Voskuyl AE, Emeis JJ, Hazes JM, van Hogezand RA, Biemond I, Breedveld FC. Levels of circulating cellular fibronectin are increased in patients with rheumatoid vasculitis. Clin Exp Rheumatol 1998;16:429-34.
12. Kanters SD, Banga JD, Algra A, Frijns RC, Beutler JJ, Fijnheer R. Plasma levels of cellular fibronectin in diabetes. Diabetes Care 2001;24:323-7.
13. Magee LA, Sharma S, Nathan HL, Adetoro OO, Bellad MB, Goudar S, *et al.* The incidence of pregnancy hypertension in India, Pakistan, Mozambique, and Nigeria: A prospective population-level analysis. PLoS Med 2019;16:e1002783.
14. Berhe AK, Kassa GM, Fekadu GA, Muche AA. Prevalence of

hypertensive disorders of pregnancy in Ethiopia: A systemic review and meta-analysis. BMC Pregnancy Childbirth 2018;18:34.
15. Payne BA, Hanson C, Sharma S, Magee LA, von Dadelszen P. Epidemiology of the hypertensive disorders of pregnancy. In: Magee LA, von Dadelszen P, Stones W, Mathai M, editors. The FIGO textbook of pregnancy hypertension. London: Global Library of Women's Medicine; 2016.
16. Melese MF, Badi MB, Aynalem GL. Perinatal outcomes of severe preeclampsia/eclampsia and associated factors among mothers admitted in Amhara region referral hospitals, North West Ethiopia, 2018. BMC Res Notes 2019;12:147.
17. Luo B, Ma X. Risk factors for preeclampsia: A case-control study. Hypertens Pregnancy 2013;32:432-8.
18. Das S, Das R, Bajracharya R, Baral G, Jabegu B, Odland JØ, *et al.* Incidence and risk factors of pre-eclampsia in the Paropakar Maternity and women's hospital, Nepal: A retrospective study. Int J Environ Res Public Health 2019;16:3571.
19. Majhi MM, Bhatnagar N. Updated B.G Prasad's classification for the year 2021: Consideration for new base year 2016. J Family Med Prim Care 2021;10:4318-9.
20. Sarma HK, Talukdar B. Eclampsia: A clinical prospective study in a referral hospital. J Obstet Gynaecol Barpeta 2014;1:57-61.
21. Silva LM, Coolman M, Steegers EA, Jaddoe VW, Moll HA, Hofman A, *et al.* Low socioeconomic status is a risk factor for preeclampsia: The generation R study. J Hypertens 2008;26:1200-8.
22. Motedayen M, Rafiei M, Rezaei Tavirani M, Sayehmiri K, Dousti M. The relationship between body mass index and preeclampsia: A systematic review and meta-analysis. Int J Reprod Biomed 2019;17:463-72.
23. Wataganara T, Boriboonhirunsarn D, Titapant V, Kanokpongsakdi S, Sunsaneevithayakul P, Vantanasiri C. Maternal body mass index at term does not predict the severity of preeclampsia. J Med Assoc Thai 2008;91:1166-71.
24. Khosravi S, Dabiran S, Lotfi M, Asnavandy M. Study of the prevalence of hypertension and complications of hypertensive disorders in pregnancy. Open J Prev Med 2014;4:860.
25. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: Systematic review and meta-analysis of large cohort studies. BMJ 2016;353:i1753.
26. Grum T, Seifu A, Abay M, Angesom T, Tsegay L. Determinants of pre-eclampsia/eclampsia among women attending delivery services in selected public hospitals of Addis Ababa, Ethiopia: A case control study. BMC Pregnancy Childbirth 2017;17:307.
27. Bdolah Y, Elchalal U, Natanson-Yaron S, Yechiam H, Bdolah-Abram T, Greenfield C, *et al.* Relationship between nulliparity and preeclampsia may be explained by altered circulating soluble fms-like tyrosine kinase 1. Hypertens Pregnancy 2014;33:250-9.
28. Luo ZC, An N, Xu HR, Larante A, Audibert F, Fraser WD. The effects and mechanisms of primiparity on the risk of pre-eclampsia: A systematic review. Paediatr Perinat Epidemiol 2007;21 Suppl 1:36-45.
29. Dekker GA, Robillard PY, Hulsey TC. Immune maladaptation in the etiology of preeclampsia: A review of corroborative epidemiologic studies. Obstet Gynecol Surv 1998;53:377-82.
30. Nagalla SR, Janaki V, Vijayalakshmi AR, Chayadevi K, Pratibha D, Rao PV, *et al.* Glycosylated fibronectin point-of-care test for diagnosis of pre-eclampsia in a low-resource setting: A prospective Southeast Asian population study. BJOG 2020;127:1687-94.
31. Brubaker DB, Ross MG, Marinoff D. The function of elevated plasma fibronectin in preeclampsia. Am J Obstet Gynecol 1992;166:526-31.
32. Ballegeer V, Spitz B, Kieckens L, Moreau H, Van Assche A, Collen D. Predictive value of increased plasma levels of fibronectin in gestational hypertension. Am J Obstet Gynecol 1989;161:432-6.
33. Friedman SA, de Groot CJ, Taylor RN, Golditch BD, Roberts JM. Plasma cellular fibronectin as a measure of endothelial involvement in preeclampsia and intrauterine growth retardation. Am J Obstet Gynecol 1994;170:838-41.
34. Eriksen HO, Hansen PK, Brocks V, Jensen BA. Plasma fibronectin concentration in normal pregnancy and pre-eclampsia. Acta Obstet Gynecol Scand 1987;66:25-8.
35. Huhn EA, Hoffmann I, Martinez De Tejada B, Lange S, Sage KM, Roberts CT, *et al.* Maternal serum glycosylated fibronectin as a short-term predictor of preeclampsia: A prospective cohort study. BMC Pregnancy Childbirth 2020;20:128.
36. Rasanen J, Quinn MJ, Laurie A, Bean E, Roberts CT Jr., Nagalla SR, *et al.* Maternal serum glycosylated fibronectin as a point-of-care biomarker for assessment of preeclampsia. Am J Obstet Gynecol 2015;212:82.e1-9.
37. Chen CK, Lee CN, Shyu MK, Hwa HL, Chen CD, Sheu BC, *et al.* Fibronectin levels in normal pregnancy and preeclampsia. J Formos Med Assoc 1994;93:921-4.
38. Backes CH, Markham K, Moorehead P, Cordero L, Nankervis CA, Giannone PJ. Maternal preeclampsia and neonatal outcomes. J Pregnancy 2011;2011:214365.