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A Study on Maternal Serum Glycosylated Fibronectin as a Predictor of Pre-Eclampsia in Antenatal Women between 20-36 Weeks of Gestational Age

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Abstract: ***Background and aim: Preeclampsia remains a major problem of modern obstetrics with insufficiently elucidated etiology. Early detection would diminish maternal and fetal mortality and morbidity. Preeclampsia is a multi system disorder and develops typically after 20 weeks of gestation with proteinuria in a previously normotensive and non proteinuric women. Incidence of hypertensive disorders contributes to 9% of maternal mortality in Asia and12% in India. Preeclampsia is associated with increased maternal glycosylated fibronectin and several studies have reported that during the clinical phase of PE the maternal serum maternal fibronectin concentration is increased. Aim: The aim of this study is to determine the maternal serum values of glycosylated fibronectin in antenatal women between 20-36weeks of gestational age. In order to evaluate their relevance in the predictor of preeclampsia. This study is to investigate the prognostic value of abnormal serum glycosylated fibronectin as predictor of preeclampsia. Methods: It is a prospective randomized observational study in Pregnant woman in gestational age between 20-36 weeks of gestational age coming to regular Antenatal OPD in the Department of Obstetrics and gynecology, government Rajaji Hospital attached to Madurai Medical College, Madurai for the period is 8months. The study is conducted on 150 cases. The study subjects will be followed up till delivery for each visit pt advised to check BP, urine albumin and watch for development of preeclampsia. Results: In our study, most of the patients were between the age group of 20 to 35years. There is development of preeclampsia in a women with increased levels of maternal serum glycosylated fibronectin. Conclusion: In our study there is significant independent contribution from maternal serum glycosylated fibronectin which was found to be increased in screening of PE women compared to normotensive pregnant women. Early prediction of Preeclampsia could potentially improve the outcome by close surveillance of the patient and would be the basis of the prophylactic medications, starting from the first trimester to improve placental invasion,, uteroplacental circulation and so decreasing the prevalence of the disease.***

Keywords: preeclampsia, maternal glycosylated fibronectin, predictor, ultrasonography.

1. Introduction

Preeclampsia (PE) is associated with 10–15% of all maternal deaths during pregnancy and childbirth, making it the second-leading cause of maternal mortality, resulting in an estimated 76, 000 maternal deaths annually1. PE also accounts for 25% of stillbirths and 25% of neonatal deaths2. Over 99% of this maternal and fetal/ neonatal mortality attributed to PE occurs in low-and middle-income countries, particularly Africa and the Indian subcontinent3.

Specifically, the "traditional" diagnostic criteria of new-onset hypertension > 140/90 mmHg and proteinuria > 300 mg/24 h after 20 weeks of gestation were revised, and proteinuria is no longer required as long as another maternal organ dysfunction (i. e., renal insufficiency, liver involvement, neurological and hematological complications) is present. The International Society for Hypertension in Pregnancy (ISSHP), the Australasian Society for the Study of Hypertension in Pregnancy, and the Society of Obstetricians and Gynaecologists of Canada added uteroplacental dysfunction or intrauterine growth restriction (IUGR) to the diagnostic criteria for PE6. Eclampsia and the syndrome of Hemolysis, Elevated Liver enzymes, and Low Platelets (HELLP) can also occur in the absence of hypertension or proteinuria8. These "non-traditional" constellations of symptoms contribute to the difficulty in obtaining an accurate diagnosis of PE solely based on clinical criteria. This is particularly problematic in women with pre-existing proteinuria and pre-existing or gestational hypertension, in whom an accurate diagnosis of PE is critical.

More objective measures to help clinicians make a final and accurate diagnosis would greatly improve clinical care and, in many cases, could be lifesaving. An important alternative to diagnoses based on observable clinical presentation is determining the levels of predictive biomarkers that can be measured in body fluids such as blood, urine, or saliva. Several circulating factors are associated with PE, including 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 copeptin9.

Preeclampsia is a complex disorder in pregnancy. Classified as a hypertensive disorder in pregnancy, the routine clinical presentation is more often ambiguous or atypical than overt.

The criteria conventionally adopted in disease classification are the presence of Hypertension with sBP ≥ 140 mm Hg

and/or dBP ≥ 90 mm Hg from GA 20 weeks, together with the presence of proteinuria. This classification applies when the disease is overt and challenging when the clinical presentation is ambiguous. Recent guidelines such as NICE 2019 or ACOG have modified the diagnosis/detection to the presence of new onset hypertension of 140 / 90, along with any proteinuria, maternal organ damage, uteroplacental damage, in recognition of the complexity of the disease. Rapid clinical deterioration is associated with the sudden and unanticipated sharp increase in Hypertension and in severe forms, the subjects can develop eclampsia, have liver or kidney injury, develop coagulopathies and can lead to both maternal and fetal mortality. Preterm birth and small for gestational age babies are all outcomes of rapid disease progression and due to emergency interventions.

The hypoxic placenta releases soluble factors into the maternal circulation, inducing systemic endothelial dysfunction. This gives rise to the clinical features of hypertension, proteinuria, oedema and coagulation abnormalities10. The circulating factors secreted by the placenta responsible for the widespread endothelial dysfunction in pre-eclampsia have been difficult to elucidate despite extensive efforts. Maynard et al. and others discovered the placenta of pregnant women with pre-eclampsia produced increased levels of soluble FMS-like tyrosine kinase one receptor (sFlt-1) 11. sFlt-1 acts by binding to the receptor binding domains of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), another member of the VEGF family that is made predominantly by the placanta11. VEGF is known to have potent angiogenic properties and also promotes vasodilation13. VEGF exerts its biological effects through Flt-1, a membrane-bound receptor tyrosine kinase. sFlt-1 is the soluble secreted receptor variant that lacks the transmembrane and cytoplasmic domains and antagonises VEGF and PlGF14. Increased levels of sFlt-1 in the maternal circulation will result in reduced levels of free VEGF and free PlGF, creating an antiangiogenic state responsible for hypertension and proteinuria of pre-eclampsia11.

Biochemical markers help improve diagnostic accuracy and reliability, especially in ambiguous/atypical presentations. A recently introduced biochemical marker in India is Glycosylated Fibronectin. Published studies of GlyFN indicate good test sensitivity and specificity. High levels of GlyFN are associated with HELLP, SGA babies, preterm birth, and a set of adverse maternal and fetal outcomes. The published studies have considered cohorts of normotensive mothers, clinically diagnosed preeclampsia and high-risk pregnancies and in each of these presence or absence of disease has been well established.

Our study aims to establish the correlation of high GlyFN levels with disease progression between GA 32-37 weeks, where intervention and delivery will be initiated using existing clinical management protocols. This will be an observational study, therefore GlyFN results will not be used for management decisions. Data from this study will be helpful in planning a suitable protocol for management of ambiguous / atypical clinical presentations of Preeclampsia.

2. Materials and Methods

Source of data: This study was conducted at AN patient attending OP, IP at GRH Madurai

Study design: open label, comparative, prospective study, **Study period:** 8 months.

Sample design: simple random sampling, **Sample size:** 150.

Inclusion Criteria:

a) All pregnant mothers between GA 32 to 34 weeks irrespective of hypertensive status and risk factors present so includes normotensive women, chronic / gestational hypertension, preeclamptic and eclamptic women.
b) Includes singleton and twin pregnancies

Exclusion Criteria:

a) Pregnancies with fetal anomalies are not included in the study group
b) Whilst twin pregnancies are included, triplets and quadruplets are excluded from study
c) Subjects with chronic renal disease, chronic heart ailments are excluded

Data Collection

Glycosylated Fibronectin can be measured using a reader and test strip.5 ul of finger prick blood of the pregnant mother is the sample used for the test. The sample is placed into a buffer and 120 ul of this is placed on a test strip. The test strip is inserted into the reader which measures the concentration of GlyFN and reports it as a value range in 10 minutes. The values reported by the reader are Normal (< 250 ug / dL), Abnormal (251-350 ug / dL), Positive (351-600 ug / dL) and High Positive (> 600 ug / dL).

A Normal test result indicates absence of Preeclampsia with a high negative predictive value. An abnormal test result indicates mild or developing Preeclampsia. This requires a repeat test after 2 weeks. A positive test result confirms the presence of Preeclampsia therefore close surveillance and monitoring of the pregnancy is essential. A high positive test result indicates presence of severe preeclampsia and to prepare for imminent birth of the baby.

All pregnancies between GA 32 to 37 weeks will be tested with GlyFN, once. The number of pregnancies that receive GlyFN Positive results (Abnormal, Positive & High Positive) will be tracked and monitored closely for deterioration in clinical conditions warranting admission and intervention. The time to delivery from testing, any adverse maternal / fetal outcomes will be documented and equated with test results. All interventional decisions will be based on clinical assessment, hypertensive status, lab and ultrasound reports following existing protocols. The number of pregnancies that receive GlyFN Normal results and progress towards term deliveries will be tracked and monitored as well.

Ethical Committee Approval: The present project was approved by the ethical committee.

Statistical Analysis:
The data obtained were entered into Excel 2010 version and analyzed using Epi-info version 7.1. For descriptive statistics, the data were analyzed as follows: Categorical data were analyzed using percentages, and the continuous data were analyzed using mean and standard deviation. Chi-square test and 'regression analyses for inferential statistics. A probability value of <0.05 is considered significant.

3. Results

A total of 150 subjects were included in the final analysis

Table 1: Descriptive analysis of mothers age (in years) in study population (N=150)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Mothers age (In Years)	24.72 ± 4.47	24.00	17.00	40.00	24.00	25.44

Among the study population, them mean Mothers age (In Years) was 24.72 ± 4.47.

Table 2: Descriptive analysis of obestric history in the study population (N=150)

Obstetric History	Frequency	Percentages
Primi	76	50.67%
Multi	74	49.33%

Among the study population, 50.67% of them were Primi and 49.33% of them were multi.

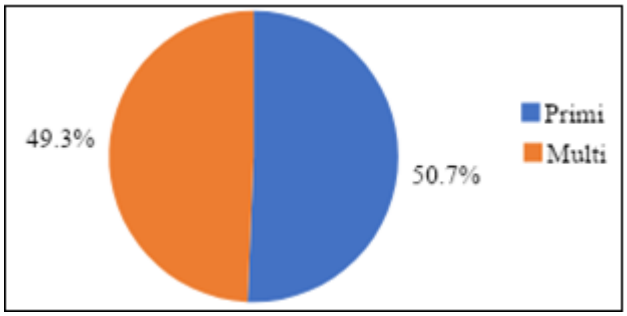

Figure 1: Pie chart of obestric history in the study population (N=150)

Table 3: Descriptive analysis of gestational age (in weeks) in study population (N=150)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Gestational Age (In Weeks)	33.4 ± 2.72	33.71	21.00	37.29	32.96	33.84

Among the study population, the mean Gestational Age (In Weeks) was 33.4 ± 2.72.

Table 4: Descriptive analysis of blood pressure in study population (N=150)

Blood pressure	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Systolic Bp (in mmhg)	111.03 ± 12.46	110.00	87.00	150.00	109.02	113.04
Diastolic Bp (in mmhg)	72.48 ± 9.17	70.00	50.00	104.00	71.00	73.96

Among the study population, the mean Systolic Bp (in mmhg) was 111.03 ± 12.46, the mean Diastolic Bp (in mmhg) was 72.48 ± 9.17.

Table 5: Descriptive analysis of proteinuria (dipstick / 24 hours urinary protein) in the study population (N=150)

Proteinuria (Dipstick / 24 Hours Urinary Protein)	Frequency	Percentages
1+	5	3.33%
2+	3	1.78%
4+	2	1.33%
TRACE	13	8.67%
NIL	127	84.67%

Among the study population, 8.67% of them proteinuria were TRACE, 3.33% of them proteinuria were 1+, 1.78% of them proteinuria were 2+, 1.33% of them proteinuria were 4+.

Table 6: Descriptive analysis of amniotic fluid in study population (N=150)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Amniotic Fluid	12.17 ± 3.31	12.00	5.00	25.00	11.63	12.70

Among the study population, the mean Amniotic Fluid was 12.17 ± 3.31.

Table 7: Descriptive analysis of weight in kg in study population (N=150)

Parameter	Mean ± SD	Median	Minimum	Maximum	95% C. I	
					Lower	Upper
Weight (in kg)	2.04 ± 0.36	1.95	1.30	3.20	1.98	2.10

Among the study population, the mean Weight (in kg) was 2.04 ± 0.36.

Table 8: Descriptive analysis of UA doppler in the study population (N=150)

UA Doppler	Frequency	Percentages
Abnormal	1	0.67%
Hydro aminos	2	1.33%
IUGR	1	0.67%
Low line placenta	1	0.67%
Normal	127	84.67%
Oligoamines	11	7.33%
Poli hydramnios	7	4.67%

Among the study population with UA doppler, 7.33% of them had Oligoamines, 4.67% of them had Polyhydramnios, 1.33% of them had of them had Hydroaminos, 0.67% of them found IUGR.

Table 9: Descriptive analysis of lab parameter in study population (N=150)

Lab parameter	Mean ± SD	Median	Minimum	Maximum
Haemoglobin G / Dl	10.11 ± 1.47	10.05	1.20	13.40
Platelets (X 10^3 / Ul)	2.59 ± 1.99	2.40	1.00	26.00

Among the study population, the mean Haemoglobin was 10.11 ± 1.47, the mean platelet was 2.59 ± 1.99.

Table 10: Descriptive analysis of bun / urea mg / dl, creatinine mg / dl in study population

Parameter	Mean ± SD	Median	Minimum	Maximum
Bun / Urea (in mg / dl) (N=34)	18.02 ± 5.93	16.0	11.0	44.0
Creatinine (in mg / dl) (N=150)	0.8 ± 0.22	0.8	0.5	2.9

Among the study population, the mean BUN/UREA was 18.02 ± 5.93, the mean creatinine was 0.8 ± 0.22.

Table 11: Descriptive analysis of clinical sign in the study population (N=150)

Clinical sign	Frequency	Percentages
Abdominal Pain	54	36.00%
Pedal Edema	27	18.00%
Extra weight gain	10	6.67%
Recurrent Head Aches	18	12.00%
Thyroid	1	0.67%

Among the study population with clinical sign, 36.00% of them had Abdominal Pain, 18.00% of them had Pedal Oedema, 6.67% of them had Extra weight gain, 12.00% of them had Recurrent Head Aches, 0.67% of them had Thyroid.

Table 12: Descriptive analysis of clinical diagnosis in the study population (N=150)

Clinical Diagnosis	Frequency	Percentages
Anemia	15	10.00%
GDM	9	6.00%
Hypothyroidism	7	4.67%
Oligoamines	7	4.67%
Bp Elevated	6	4.00%
Polydramnoios	4	2.67%
CHT	3	2.00%
Lowing Placenta	3	2.00%
Fibroids	2	1.33%
High risk	2	1.33%
Severe MS	2	1.33%
Thyroid	2	1.33%
Others	16	10.67%

Among the study population with clinical diagnosis, 10.00% of them had Anemia, 6.00% of them had GDM, 4.67% of them had Hypothyroidism, 4.67% of them Oligoamines, 4.00% of them had Bp Elevated, 2.67% of them had Polydramnoios, 2.00% of them had CHT, 2.00% of them had Lowing Placenta.

Table 13: Descriptive analysis of lumella test result in the study population (N=150)

Lumella test result	Frequency	Percentages
Normal	47	31.33%
Abnormal	70	46.67%
Positive	26	17.33%
High positive	7	4.67%

Among the study population with Lumella test result, 31.33% of them were normal, 46.67% of them were abnormal, 17.33% of them were positive, 4.67% of them were high positive.

4. Discussion

An ongoing effort has been made to predict the development of pre-eclampsia by performing tests during the early stages of pregnancy. maternal serum biomarkers have gained attention as a potentially more consistent parameter for determining disease risk. a significant proportion of pre-eclampsia cases present late in pregnancy in developing countries, resulting in increased complications. in our research we have evaluated the glycosylated fibronectin as a predictor of pre-eclampsia among Antenatal women between 20-36 weeks of gestational age.

Characteristics of our study participants:
The mean mother's age (In years) in the study population was 24.72 ± 4.47. Primi made up 50.67% of the study population, while multi made up 49.33%. The mean Gestational Age (In Weeks) of the study population was 33.4 ± 2.72 and the mean Weight (in kgs) was 2.04 ± 0.36

Recent research has focused on the investigation of pregnancies with signs and symptoms suggestive of PE, with the goal of detecting the development of PE within the next 1–4 weeks. The PROGNOSIS (Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia) study found that a sFlt-1/PlGF ratio of 38 had a good NPV of 99.3 percent for ruling out PE or HELLP within 1 week and a PPV of 36.7 percent for ruling in PE within 4 weeks.14 Another prospective multicentre study found that the AUC for PlGF < 5th percentile prediction of PE within 2 weeks was 0.87.15

Hypertension is a pregnancy-related multisystem disorder, whose pathophysiology is unknown. Preeclampsia is also referred as a disease of placenta since it is triggered by placental insufficiency. It is one of the most frequently encountered pregnancy-related medical complications next to gestational diabetes mellitus. Preeclampsia is one of the

leading causes of maternal mortality and morbidity, neonatal and fetal mortality, and preterm birth. It is estimated that in developing countries, preeclampsia accounts for 15% of maternal deaths every year. In our study the mean Systolic BP (in mm Hg) was 111.03 ± 12.46, the mean Diastolic BP (in mm Hg) was 72.48 ± 9.17 which quite low than other studies conducted in India.14, 15

Estimating the amniotic fluid volume (AFV) has become an important part of foetal evaluation. Although the accuracy and predictive value of the sonographic techniques currently available in clinical practise are limited, careful amniotic fluid index (AFI) estimation provides a fair assessment of foetal well-being. AFI at its extremes has been associated to a poor foetal outcome. The unadjusted total median concentration in the Sudhanshu Shekhar 15, was 248 found to be highest for MP (20.92 ng/mL), followed by PP (19.22 ng/mL), then EP (1.97 ng/mL) 249 and BP (1.11ng/mL) in the maternal blood plasma and similar trend was found in amniotic fluid 250 where the unadjusted total median concentration was found to be 18.92 ng/mL, 18.82 ng/mL, 251 1.89 ng/mL and 1.37 ng/mL for MP, PP, EP and BP respectively. In our current study the mean Amniotic Fluid was 12.17± 3.31.

The presence of proteins in the urine signals the onset of a hypertensive complication, either proteinuric gestational hypertension or superimposed preeclampsia over preexisting renal disease. Proteinuria is best quantified by measuring its daily renal excretion. Nonpregnant women can excrete up to 150mg per day, whereas pregnant women can excrete up to 300 mg per day.12 which is similar to our study. In our study 8.67% of them proteinuria was TRACE, 3.33% of them proteinuria was 1+, 1.78% of them proteinuria was 2+, 1.33% of them proteinuria was 4+.

5. Conclusion

Our findings show that in pregnant women with hypertension, the glycosylated fibronectin POC test has a high predictive value for pre-eclampsia occurring within 10 days of testing. It will be a useful cost-effective tool in low-resource settings for appropriate triage and timely referral to higher centres, and it can be used in tertiary centres to triage PE patients to inpatient vs. outpatient management. Larger studies are needed in the future to determine the sensitivity and specificity that can reduce the frequency of biochemical and sonographic monitoring in hypertension cases during pregnancy.

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