

A Study of Different Biochemical and Sonological Parameters as Predictors of Preeclampsia

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ABSTRACT

BACKGROUND

Preeclampsia-eclampsia is a very common cause of maternal & perinatal mortality & morbidity. Predictors of preeclampsia in apparently healthy pregnant patients by some biochemical & sonological parameter, either individually or in combination may be helpful.

METHODS

This is an OPD based prospective study at medical college, Kolkata, dept. of G & O from 1st January 2013 to 30th June 2014. Singleton normotensive patients at 12-14 wks. gestation were included in the study. Patients with chronic hypertension, multifetal pregnancy, heart disease, renal disease & diabetes mellitus were excluded. Serum parameters AFP, bHCG, inhibin A, urinary parameters like urinary calcium-creatinine ratio (spot sample), sonological parameters like uterine artery doppler study, RI, PI, notching were measured.

RESULTS

15 out of a total of 200 patients (7.5%) developed preeclampsia. Booking BMI & Uristix protein had significant association with preeclampsia. Among the biochemical & sonological markers maternal serum bHCG, uterine artery doppler RI (22-24 wks.) & notching (22-24 wks.) had the best sensitivity, specificity & predictive value. The combination of high maternal serum bHCG combined with persistent notching or high RI at 22-24 wks. uterine artery doppler had the highest specificity & predictive value for prevalence of development of preeclampsia.

CONCLUSIONS

Thus, those women with high β -hCG (>2.499 MOM) and either high RI (>0.625) or persistent notching in 22-24 weeks uterine artery Doppler scan should be followed up more intensively as they are at significantly high risk of developing preeclampsia.

KEY WORDS

Preeclampsia, Prediction, Serum Biochemical Marker, Beta HCG, Doppler Velocimetry

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BACKGROUND

Sometimes, pregnancy and childbirth are complicated by some unique disorders like preeclampsia & eclampsia that lead to significant morbidity and even mortality world wide^{1,2} in certain situations. Preeclampsia/Eclampsia causes approx. 1/4th of all maternal deaths in India (MMR-178 in 2010-12).³ Preeclampsia is defined as new onset hypertension $\geq 140/90$ mm Hg after 20 weeks of pregnancy with proteinuria ≥ 300 mg/24 hours or $\geq 1+$ dipstick (NHBPEP),⁴ (ISSHP).⁵ This disorder complicates 2-8% of all pregnancies.^{6,7,8} Early diagnosis and intensive management of at risk patients may improve both maternal and foetal outcome.

Recent understanding of aetiology and pathogenesis of preeclampsia has identified a two-stage model; poor placentation and oxidative stress & inflammation.⁹ Dysfunction of trophoblastic cells in preeclampsia leads to increased level of AFP, hCG & Inhibin A in maternal serum. Failure of trophoblastic invasion into the maternal spiral arteries, can be investigated by uterine artery doppler velocimetry at around 20 wks. of gestation. Persistence of increased resistance to uterine arterial flow indicates increased risk of preeclampsia.¹⁰ Decreased excretion of calcium in urine of asymptomatic patients will eventually help to develop preeclampsia.^{11,12} The calcium-creatinine ratio in urine is reduced in these women. A simple spot urine calcium-creatinine ratio in 2nd trimester may detect such patients.^{13,14}

We wanted to analyse the potential clinical use of these biochemical parameters (serum β -hCG, AFP, Inhibin-A, urine calcium-creatinine ratio) and sonological parameters (uterine artery doppler) individually and also in combination for the purpose of prediction of preeclampsia.

METHODS

The institutional ethics committee of Medical College, Kolkata has approved the study on 9.2.2013 after checking the required protocol. While undertaking the study voluntary informed consent was taken from each patient in writing (Bengali/ Hindi /English) & full confidentiality of the patients had been retained.

This was an OPD based prospective study. Sample technique was purposive sampling, a type of non-probability sampling, performed at Medical College Hospital, Kolkata, Department of Obstetrics and Gynaecology from 1st June 2013 to 30th June 2014. Singleton normotensive women attending antenatal clinic (OPD) at 12-14 weeks of gestation are included in the study. Antenatal women with chronic hypertension, multifoetal pregnancy, heart disease, renal disease & diabetes mellitus are excluded from the study. Data were collected from all eligible candidates with informed consent. Two hundred (200) patients had been investigated during the study process and were followed up till delivery. Sample size determination in this study was based on the cost, time & convenience of collecting the data & the need for it to offer sufficient statistical power. Detailed history was taken and examination of pregnant mothers at 12-14 weeks of gestation were done.

Uterine artery doppler study was done at 12-14 weeks & repeated at 22-24 wks. of gestation. Maternal serum markers i.e. β -hCG, AFP, Inhibin-A¹⁵ were done between 12 and 16 weeks of gestation by ELISA technique. Urine examination was done for calcium creatinine ratio (calcium by o-cresolphthalein complex reaction¹⁶ and creatinine by Jaffes method)¹⁷ at 20-24 weeks of gestation. We selected the spot test over 24-hour sample for the sake of feasibility.

How many of patients developed preeclampsia were seen and sensitivity, specificity and predictive values of each biochemical & sonological parameters were calculated in isolation and in combination-

- The statistical software SPSS version 20 & Microsoft excel 2013 has been used for the analysis.
- An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.
- Categorical variables are expressed as number & percentage of patie.
- Presence and absence of Preeclampsia is compared across different categorical variables using Pearson's Chi Square test for Independence of Attributes.
- Continuous variables are expressed as Mean \pm Standard Deviation and compared across the 2 groups (developed and not developed preeclampsia) using unpaired t test.
- ROC (receiver operating characteristic) curve analysis is done to establish the cut offs for MOM of selected biochemical & sonological parameters. Cut offs were determined by finding the specific value of each parameter for which the sum of sensitivity and specificity is highest.

RESULTS

Table 1 shows the incidence of preeclampsia in the study population (n=200) is 15, which is 7.5% Chart 1 & Table 2 shows the cut off values of the different biochemical and sonological parameters as determined from the analysis of the ROC curves & also the comparison of different parameters with development of preeclampsia. Among the 39 subjects whose MOM AFP was high i.e. above cut off, 10 developed preeclampsia, 5 subjects among 161 developed preeclampsia yet their MOM AFP level was normal. Among the 40 subjects whose MOM β -hCG was high i.e. above cut off, 12 developed preeclampsia, 3 subjects among 160 developed preeclampsia yet their MOM β -hCG level was normal.

Among the 51 subjects whose MOM inhibin-A was high i.e. above cut off, 11 developed preeclampsia, 4 subjects among 149 developed preeclampsia yet their MOM inhibin-A level was normal. Among the 60 subjects whose doppler RI (12-14 wks.) was high i.e. above cut off, 12 developed preeclampsia, 3 subjects among 140 developed preeclampsia yet their doppler RI (12-14 wks.) level was normal. Among the 129 subjects whose doppler PI (12-14 wks.) was high i.e. above cut off, 12 developed preeclampsia, 3 subjects among 71 developed preeclampsia yet their doppler PI (12-14 wks.) level was normal. Among the 48 subjects whose doppler RI (22-24 wks.) was high i.e. above cut off, 12 developed preeclampsia, 3 subjects among 152 developed preeclampsia yet their doppler RI (22-24 wks.) level was normal.

Among the 72 subjects whose doppler PI (22-24 wks.) was high i.e. above cut off, 11 developed preeclampsia, 4 subjects among 128 developed preeclampsia yet their doppler PI (22-24 wks.) level was normal. Among the 50 subjects whose CCR (20-24 wks.) was high i.e. above cut off, 10 developed preeclampsia, 5 subjects among 150 developed preeclampsia yet their CCR (20-24 wks.) level was normal. Among the 50 subjects who had notching in doppler (22-24 wks.), 12 developed preeclampsia. 3 subjects among the 150 did not have notching in doppler (22-24 wks.) yet they did develop preeclampsia. Table 3 shows the sensitivity, specificity and predictive value of each parameter used for prediction of preeclampsia. Tests with best sensitivity, specificity and predictive value are MS β-hCG, doppler RI (22-24 weeks) and doppler notching (22-24 weeks). Highest PPV and specificity MS hCG 30.0% and 84.86%, lowest PPV and specificity PI (12-14 wks.) 9.3% and 36.76%.

These 3 parameters are taken together for analysis to find the combined sensitivity, specificity and predictive values for various combinations of them. criteria used-

- Any 1 of the 3, positive then positive ('or' criteria)
That is when either of hCG or RI (22-24 weeks) or notching (22-24 weeks) is positive.
- Any 2 of the 3, positive then positive ('and' criteria)
That is when any two of hCG, RI (22-24 weeks), notching (22-24 weeks) is positive together.
- All 3 of 3 positive then positive ('and' criteria)
That is when all of hCG and RI (22-24 weeks) and notching (22-24 weeks) are positive together.

Preeclampsia	Frequency	Percent
Not Developed	185	92.5
Developed	15	7.5
Total	200	100.0

Table 1. Incidence of Preeclampsia in the Study Population

Sl. No.	Parameter	Cut off Value	AUC of the ROC Curve	Not Developed PE (N=185)		Developed PE (N=15)	
				High	Normal	High	Normal
				1	MOM MS AFP	2.158	0.753
2	MOM MS βHCG	2.499	0.857	28 (70%)	157 (98.13%)	12 (30%)	03 (1.87%)
3	MOM MS Inhibin A	2.454	0.784	40 (78.43%)	145 (97.32%)	11 (21.57%)	4 (2.68%)
4	Doppler RI (12-14 wks.)	1.605	0.805	48 (80%)	137 (97.86%)	12 (20%)	3 (2.14%)
5	Doppler PI (12-14 wks.)	1.775	0.545	117 (90.7%)	68 (95.67%)	12 (9.3%)	3 (4.33%)
6	Doppler RI (22-24 wks.)	0.625	0.625	36 (75%)	149 (98.03%)	12 (25%)	3 (1.97%)
7	Doppler PI (22-24 wks.)	1.93	0.659	61 (84.72%)	124 (96.88%)	14 (15.28%)	1 (3.12%)
8	CCR (20-24 wks.)	0.11	0.258	40 (80%)	145 (96.67%)	10 (20%)	5 (3.33%)
9	Notching in doppler (22-24 wks.)			Yes: 38 (76%)	No: 147 (98%)	Yes: 12 (24%)	No: 3 (2%)

Table 2. Different Parameters with Development of Preeclampsia

Table 4 shows the comparison of different tests; when any 1 of 3 tests, either 2 of the 3 tests and all 3 tests are abnormal. Among the 76 subjects who had either of the 3 tests abnormal 14 developed preeclampsia. Mong 124 of those who had all tests normal developed preeclampsia.

Among the 17 subjects who had (hCG + RI) & (hCG + notching) tests abnormal, 10 developed preeclampsia. 5 among 183 of those who had normal tests developed preeclampsia. Among the 45 subjects who had (RI + notching) tests abnormal 12 developed preeclampsia. 3 among 155 of those who had normal tests developed preeclampsia. Among the 17 subjects who had all the tests abnormal 10 developed preeclampsia. Table 5 shows the sensitivity, specificity and predictive value of the combined tests. Among them, when either of the 3 tests were positive sensitivity of prediction of preeclampsia improved significantly (93.33%) but at the cost of specificity and PPV (66.49% and 18.42%). Combination of hCG with RI and hCG with notching yielded specificity 96.22%, PPV 58.82%; so did combination of all three tests. For combination of RI and notching specificity was 82.16% and PPV 26.67%.

Parameter	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
MOM MS AFP (ng/mL) (12-16 weeks)	10	156	29	5	66.67	84.32	25.64	96.89
MOM MS β-hCG (IU/mL) (12-16 weeks)	12	157	28	3	80.00	84.86	30.00	98.13
MOM MS Inhibin-A (pg/mL) (12-16 weeks)	11	145	40	4	73.33	78.38	21.57	97.32
RI uterine artery Doppler (12-14 weeks)	12	137	48	3	80.00	74.05	20.00	97.86
PI uterine artery Doppler (12-14 weeks)	12	68	117	3	80.00	36.76	9.30	95.77
RI uterine artery Doppler (22-24 weeks)	12	149	36	3	80.00	80.54	25.00	98.03
PI uterine artery Doppler (22-24 weeks)	11	124	61	4	73.33	67.03	15.28	96.88
Urine calcium-creatinine ratio (20-24 weeks)	10	145	40	5	66.67	78.38	20.00	96.67
Notching (22-24 weeks)	12	147	38	3	80.00	79.46	24.00	98.00

Table 3. Sensitivity, Specificity and Predictive Values of Individual Parameters in Prediction of Development of Preeclampsia

Sl. No.	Parameter	Developed PE					
		No			Yes		
		Normal	Ab-normal	Total	Normal	Ab-normal	Total
1.	hCG/RI/notching (1 of 3 abnormal)	123 (99.19%)	62 (81.58%)	185	1 (0.81%)	14 (18.42%)	15
2.	hCG+ RI	178 (97.27%)	7 (41.18%)	185	5 (2.73%)	10 (58.82%)	15
3.	hCG + notching	178 (97.27%)	7 (41.18%)	185	5 (2.73%)	10 (58.82%)	15
4.	RI + notching	152 (98.06%)	33 (73.33%)	185	3 (1.94%)	12 (26.67%)	15
5.	hCG + RI + notching (all 3 abnormal)	178 (97.27%)	7 (41.18%)	185	5 (2.73%)	10 (58.82%)	15

Table 4. Comparison of Different Tests; When Any 1 of 3 Tests, either 2 of the 3 Tests and all 3 Tests are Abnormal

Scoring System	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
hCG/RI/notching	14 (18.42%)	123 (99.1%)	62 (81.58%)	1 (0.81%)	93.33	66.49	18.42	99.19
hCG + RI	10 (58.82%)	178 (97.27%)	7 (41.18%)	5 (2.73%)	66.67	96.22	58.82	97.27
hCG + notching	10 (58.82%)	178 (97.27%)	7 (41.18%)	5 (2.73%)	66.67	96.22	58.82	97.27
RI + notching	12 (26.67%)	152 (98.06%)	33 (73.33%)	3 (1.94%)	80.00	82.16	26.67	98.06
hCG + RI + notching	10 (58.82%)	178 (97.27%)	7 (41.18%)	5 (2.73%)	66.67	96.22	58.82	97.27

Table 5. Sensitivity, Specificity and Predictive Value of the Combinations

Table 6 shows the comparison of mean of the continuous variables of the tests for prediction of preeclampsia. All the comparisons were significant (p<0.05) with development of preeclampsia except PI of uterine artery Doppler (12-14 weeks) (2.05 vs 1.98, p=0.546). Thus, uterine artery doppler

RI, notching at 22-24 weeks and serum β -hCG are the tests that best predicted development of preeclampsia individually. Combination of β -hCG with any of the doppler parameters is more specific and has better predictive value than each test alone. Addition of all three tests does not increase the specificity.

	Developed Preeclampsia		p	Significance
	No	Yes		
	Mean \pm S.D.	Mean \pm S.D.		
MOM AFP (ng/mL) (12-16 weeks)	1.48 \pm 0.61	2.05 \pm 0.63	0.001	Significant
MOM β -hCG (IU/mL) (12-16 weeks)	1.59 \pm 0.69	2.73 \pm 0.8	<0.001	Significant
MOM Inhibin-A(pg/mL) (12-16 weeks)	1.7 \pm 0.7	2.48 \pm 0.68	<0.001	Significant
RI uterine artery Doppler (12-14 weeks)	0.56 \pm 0.06	0.63 \pm 0.05	<0.001	Significant
PI uterine artery Doppler (12-14 weeks)	1.98 \pm 0.4	2.05 \pm 0.35	0.546	Not Significant
RI uterine artery Doppler (22-24 weeks)	0.54 \pm 0.07	0.62 \pm 0.08	<0.001	Significant
PI uterine artery Doppler (22-24 weeks)	1.83 \pm 0.39	2.07 \pm 0.54	0.029	Significant
Urine calcium-creatinine ratio (20-24 weeks)	0.14 \pm 0.03	0.11 \pm 0.03	<0.001	Significant

Table 6. Comparison of Mean of the Continuous Variables of the Different Tests

DISCUSSION

There is no screening test for preeclampsia (WHO meta-analysis by Conde-Agudelo and associates in 2004¹⁸). Basic theme of various predictive tests including the present one for preeclampsia is to identify a specific pathogenic process that can be detected sufficiently early in asymptomatic stage that will prevent the development of preeclampsia syndrome. Analysis of the results showed- 15 of the total 200 patients developed preeclampsia. Thus, incidence was 7.5%. Consistent with previous studies, that stated incidence of 2-8%.^{6,7,8}

Serum parameters done between 12-16 weeks of pregnancy yielded the following data: MS AFP was found raised above the cut off (cut off 2.158 MOM) in 39 subjects; of them 10 subjects developed preeclampsia. MS β -hCG was found raised above cut off (cut off 2.499 MOM) in 40 subjects; of them 12 subjects developed preeclampsia. MS inhibin-A was found raised above cut off (cut off 2.454 MOM) in 51 cases among them 11 subjects developed preeclampsia. Comparison of mean of MOM of these parameters showed significant association with development of preeclampsia (2.05 vs 1.48 for AFP p=0.001, 2.73 vs 1.59 for β -hCG p<0.001, 2.48 vs 1.7 for inhibin-A p<0.001).

Study by Aquilina et al¹⁹ used β -hCG and inhibin-A (cut off >2.0 MOM) and found sensitivity of Inhibin-A to be greater than hCG (48.6% vs 37.4%) fixing specificity at 90% (p<0.05) for preeclampsia prediction. Yaron et al²⁰ found significant association with preeclampsia and other adverse pregnancy outcomes when β -hCG and AFP were >2.5 MOM (p<0.001). Our study found significant association with all the serum parameters namely β -hCG, AFP and inhibin-A and preeclampsia, consistent with previous studies. Uterine artery Doppler was performed twice in our study, first at 12-14 weeks and again at 22-24 weeks for RI, PI and notching (in 22-24 weeks scan). RI of uterine artery (12-14 weeks) was raised above cut off value of 0.605 in 60 cases, out of them 12

subjects developed preeclampsia whereas at 22-24 wks. it was raised above cut off value of 1.775 in 129 cases, out of them 12 developed preeclampsia. PI of uterine artery (12-14 weeks) was raised above cut off value of 1.775 in 129 cases, out of them 12 developed preeclampsia whereas at 22-24 wks. it was raised above cut off value of 1.775 in 129 cases, out of them 12 developed preeclampsia.

Notching in 22-24 weeks was present in 40 subjects out of them 12 subjects developed preeclampsia. Comparison of mean of these variables showed significant association with all the parameters with development of preeclampsia, except PI at 12-14 weeks (0.63 vs 0.56 for RI 12-14 weeks, p<0.001; 0.62 vs 0.54 for RI 22-24 weeks, p<0.001 and 2.07 vs 1.83 for PI 22-24 weeks, p=0.029). First and second trimester uterine artery Doppler have been tested by several authors for significance in preeclampsia prediction. Papageorgiou et al^{21,22} found persistence of high impedance pattern in uterine artery in 2nd trimester to significantly increase likelihood of preeclampsia (LR of 6 vs 5) These reports are consistent with our present study since we also found 2nd trimester Doppler indices to be more sensitive and specific for preeclampsia prediction. Urine calcium-creatinine ratio was done by a spot test between 20-24 weeks. CCR was below the cut off value of 0.11 in 50 cases, out of them 10 subjects developed preeclampsia

Comparison of mean showed significant relation between CCR and development of preeclampsia (0.11 vs 0.14, p<0.001). Studies by Ozcan et al²³ (using cut off 0.066), Saudan et al²⁴, Izumi et al¹⁸ (both using cut off 0.10) all found significant association of CCR in 1st or 2nd trimester with development of preeclampsia. Sensitivity and specificity of these studies ranged between 70-80% and 75-85% respectively, which is consistent with our current study. There was one (0.5%) case of severe preeclampsia that had all the parameters above the cut-off levels. There were 2 (1%) patients who delivered at 34 weeks and 8 (4%) patients who delivered at 36 weeks. All others were followed for more than 37 weeks of pregnancy. No patients had eclamptic convulsions. Thus, individually the tests that best predicted preeclampsia was MS β -hCG, uterine artery Doppler RI at 22-24 weeks and diastolic notching in uterine artery Doppler at 22-24 weeks. The combination of β -hCG (12-16 weeks) with RI/notching at 22-24 weeks appears to be the best panel for combination of tests for prediction of preeclampsia. A combination of all three tests does not yield higher predictive value than any of the two described above. However, a larger multicentric study is needed to establish their positive correlation and implement the same for predicting preeclampsia in practice.

CONCLUSIONS

Maternal serum β -hCG and uterine artery Doppler RI/notching (22-24 weeks) is the best combination of predictive tests for preeclampsia. Individually ms β -hCG (12-16 weeks), uterine artery Doppler RI (22-24 weeks) and uterine artery Doppler notching (22-24 weeks) are the tests having best sensitivity, specificity and predictive values for prediction of preeclampsia. All the other serum parameters (AFP, inhibin A), urine parameters (CCR) and sonological parameters (except Doppler PI 12-14 weeks) are significantly

associated with development of preeclampsia. Thus, those women with high β -hCG (>2.499 MOM) and either high RI (>0.625) or persistent notching in 22-24 weeks uterine artery Doppler scan should be followed up more intensively as they are at significantly high risk of developing preeclampsia.

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