

Study of serum markers alfa fetoprotein, β -hcg and microalbuminuria as a early predictors of pre-eclampsia

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ABSTRACT

Background: Preeclampsia is a serious blood pressure disorder developing after 20 weeks of pregnancy diagnosed by high blood pressure and often proteinuria or organ damage. Without management it can lead to seizures or fatal complication. It can affect both mother and fetus. The early identification of patients with an increased risk for preeclampsia is therefore one of the most important goals in obstetrics. Today, several markers may offer the potential to be used, most likely in a combinatory analysis, as predictors or diagnostic tools. With the advancement of science we aim to find out an optimum method to predict adverse maternal and fetal effects of pre-eclampsia. This will help us in deciding about the treatment modality for pre-eclampsia. **Aims and objectives:** The Aim of this study to determine the level of β hcg, Alpha Foetoprotein and microalbuminuria in serial samples of antenatal subjects from early pregnancy to full term pregnancy. **Material and Method:** This is a prospective observational study conducted on 96 women who were attending antenatal OPD and wards of Department of Obstetrics and Gynaecology, R. D. Gardi Medical College in first trimester. Detailed history was taken and routine investigation was done. Along with that, special investigations i.e. alfafeto-protein, β -hcg & microalbuminuria were done. All these subjects were followed in 2nd & 3rd trimester and all these investigations were taken serially. By analyzing these investigations we could predict the pre-eclampsia at its earlier stages and prevent the maternal & fetal complications. **Result:** We found significant association between high serum AFP in first, second & third trimester and subsequent development of PE [$p < .05$]. Mean values of AFP 61.7 ng/ml [$p < .001$] in first, 97.87 ng/ml [$p < .001$] in second and 163.57 ng/ml [$p < .01$] in third trimester. Our data also support a significant association between elevated first, second and third trimester microalbuminuria, β -hcg level and development of PE.

Keywords: Preeclampsia, β hcg, Alpha Foetoprotein, microalbuminuria, hypertension.

INTRODUCTION

Preeclampsia is a multi-system disorder of pregnancy, which is characterized by new onset hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mm Hg, respectively, on two occasions, at least 6 hours apart) and proteinuria (protein excretion of ≥ 300 mg in a 24 h urine collection, or a dipstick of $\geq 2+$), that develop after 20 weeks of gestation in previously normotensive women (1, 2). The precise origin of preeclampsia remains elusive, but it is believed to be likely multifactorial. A certainty is the central role

played by the placenta in its pathology (3, 4, 5, 6). Preeclampsia occurs in 2–5% of pregnancies in the incident, but it complicates up to 10% of pregnancies in the developing countries, where emergency care is often inadequate or lacking. Therefore we are in need of a widely applicable and affordable test that could permit presymptomatic diagnosis in order to identify and monitor the patients at risk and thus provide the best prenatal care for these women and their child. Studies of the differences between normal pregnancies and those complicated by PE may give important

information, useful for finding a possible predictor of PE. Identification of such a predictor would make intervention and close surveillance a possibility and, hopefully, reduce the negative consequences of the disorder.

Aims and objective-

- The Aim of this study to determine the level of β hcg, AlphaFoetoprotein and microalbuminuria in serial samples of antenatal subjects from early pregnancy to full term pregnancy and correlate the abnormal markers with onset of preeclampsia.

MATERIALS AND METHODS

STUDY DESIGN

This is a prospective observational study conducted on 96 women who were attending antenatal OPD and wards of Department of Obstetrics and Gynaecology, R. D. Gardi Medical College in first trimester. Detailed history of patients was taken including the past and family history regarding pre-eclampsia, DM, HTN. Weight, blood pressure & detailed general examination (general condition, pallor, edema, respiratory system, cardiovascular system examination) was done. Then routine investigations were done which includes Hb%, platelet count, TLC, DLC, urine albumin, urine sugar, and ultrasonography. Along with that, special investigations i.e. alfafeto protein, β -hcg & microalbuminuria were done. All these subjects were followed in 2nd & 3rd trimester and all these investigations were taken serially. Data analysed to note if there was any correlation of abnormal values of markers with development of pre-eclampsia.

5 ml blood was collected in plain bulb and random mid stream urine sample was collected in sterile container. The bulb and container were tagged and labeled and sent to microbiology lab for analysis.

RESULTS

Over a period of one and a half year 96 antenatal women (n=96) were recruited consecutively in study.

In this study we correlated the following parameters: age, weight, blood pressure, various symptoms of patients, Hb, platelet count. AFP, β -hcgµalbuminuria were carried out sequentially in all three trimesters from outdoor and indoor clinics. The results are as follows:

Table 1: Age distribution of patients

Age (yrs)	No. of patients (n=96)	Percentage (%)
<20	19	20
21-25	52	54
26-30	21	22
>30	4	4

Following phenomenon were observed:

- 1.Changes in β -hcg, Alpha Foetoprotein, Microalbuminuria indices with rise in blood pressure levels.
2. Fetal outcome changes observed with pre-eclampsia.
3. Change in maternal & fetal outcome observed with changes in various parameters.

The data was entered in Excel[®] (Microsoft Corporation Ltd.) and then transferred to Stata 10.0 for further analysis (Stata Corporation, College Station, TX, USA). Pearson chi square test was applied and & values compounded. By analyzing these investigations we could predict the pre-eclampsia at its earlier stages and prevent the maternal & fetal complications.

Selection of study subjects

Inclusion Criteria

There was no fixed criterion to enroll a woman in study. Patients selected who were coming in first trimester of pregnancy, patient with regular menstrual cycles and who were sure of their date of LMP, singleton pregnancy

Exclusion Criteria

Patient with 2nd or 3rd trimester, Pregnancy with congenital anomaly of foetus, Multiple Pregnancy, Hydatiform mole, Patients with any chronic medical disorder like preexisting hypertension, DM, Asthma

Ethical Aspect

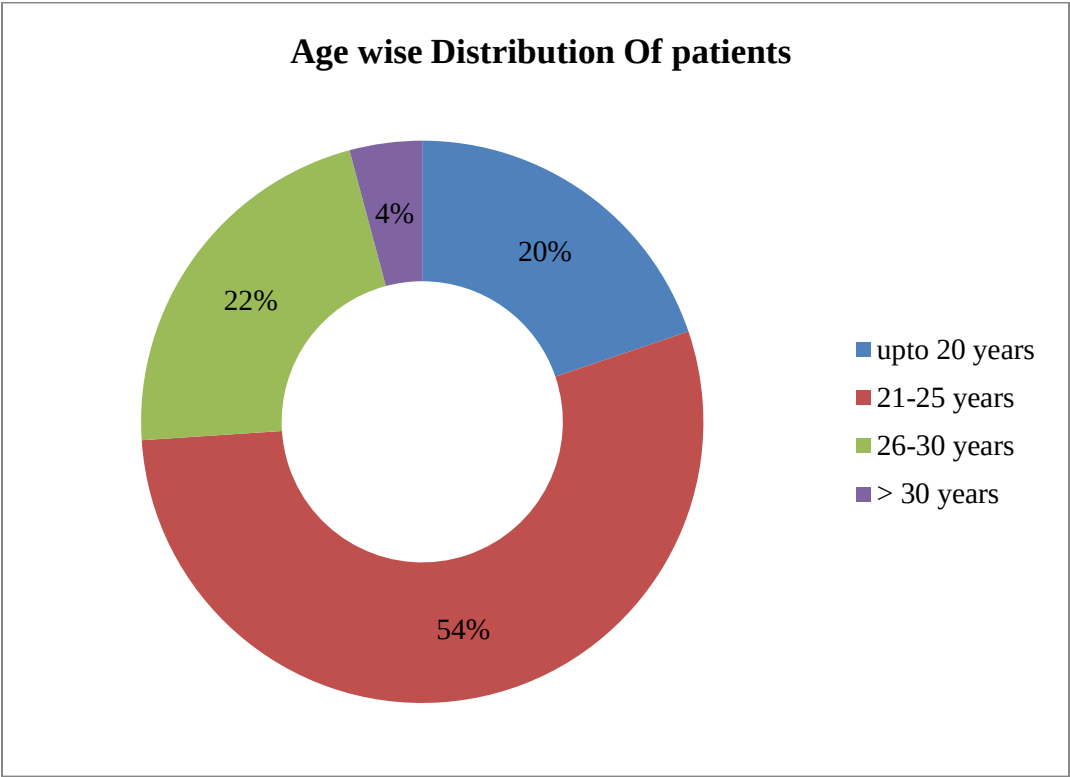
Before entering the trial patient was explained about the study and informed consent was taken. The institutional ethical committee cleared the proposal.

Lab Estimation of Markers

Serum Markers studied were:

1. Microalbuminuria - Turbidometric immunoassay
2. Beta hcg- Chemiluminescence Assay
3. Alpha Foetoprotein - Chemiluminescence Assay

Figure 1



The mean age of women was 23.70 years (95% confidence interval 22.98 to 24.42; with the age range of 18 to 35 years).

Mean Blood Pressure of Patients Trimester Wise:

Figure 2

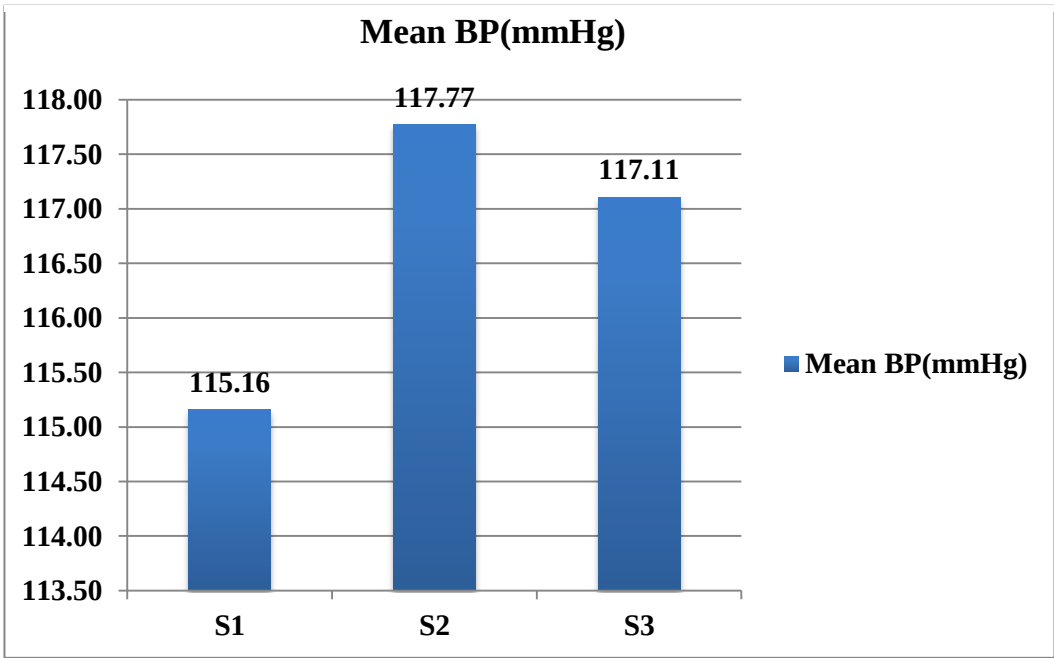
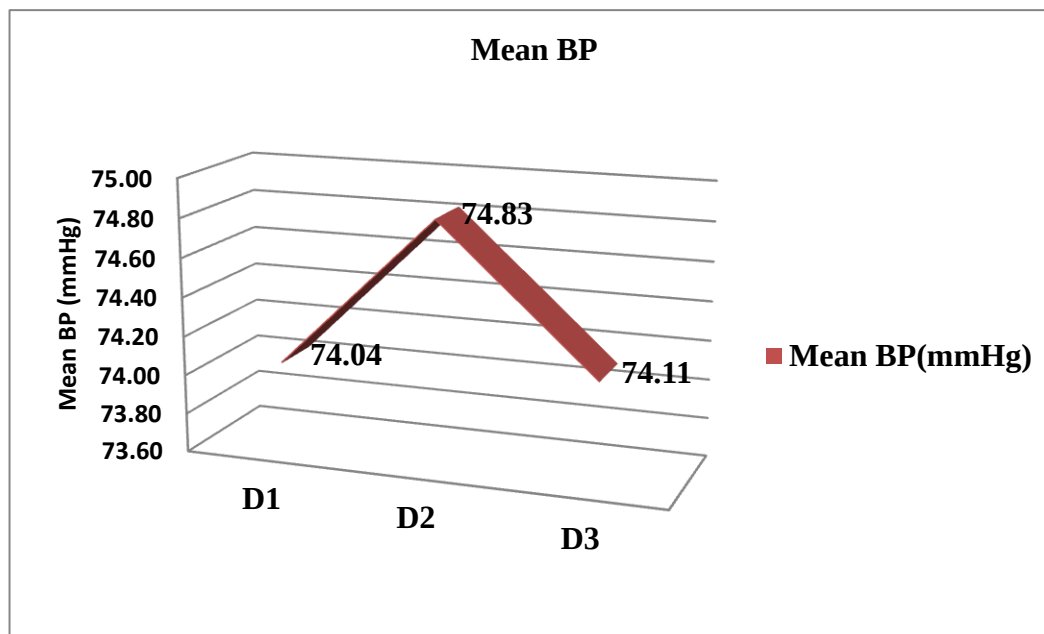


Figure 3



In 1st Trimester

Mean systolic blood pressure was 115.16 mm of Hg (95% confidence interval 113.31 to 117) with the range of 150 to 100 mm of Hg. Mean diastolic blood pressure was 74.04 mm of Hg (95% confidence interval 73.25 to 76.42) with the range of 100 to 60 mm of Hg .

In 2nd Trimester

Mean systolic blood pressure was 117.77 mm of Hg (95% confidence interval 115.72 to 119.82) with the

range of 160 to 100 mm of Hg. Mean diastolic blood pressure was 74.83 mm of Hg (95% confidence interval 73.25 to 76.42) with the range of 110 to 70 mm of Hg.

In 3rd Trimester

Mean systolic blood pressure was 117.11 mm of Hg (95% confidence interval 115.08 to 119.13) with the range of 150 to 100 mm of Hg. Mean diastolic blood pressure was 74.11 mm of Hg (95% confidence interval 72.63 to 75.58) with the range of 100 to 70 mm of Hg.

Distribution of patients according to mean Blood Pressure in PE &non PE

Table 2

	1 st Trimester		2 nd Trimester		3 rd Trimester	
	S1	D1	S2	D2	S3	D3
PE	127.44	82	140.22	91.56	140.57	90.29
Non PE	113.89	73.22	115.45	73.10	115.22	72.80
p-value	<.001	<.001	<.001	<.001	<.001	<.001

In 1st Trimester

Systolic blood pressure in patients who develop PE (n=9). The mean value was 127.44 mm Hg (95% CI = 117.32 to 137.56) compared with a mean of 113.89 mm Hg (95% CI= 112.26 to 115.51) (n=87). The difference of mean was 13.55 (p<.001) which is statistically significant. Diastolic blood pressure in patients who develop PE (n=9). The mean value was 82 mm Hg (95% CI = 73.82 to 90.17) compared with a mean of 73.22 mm Hg (95% CI= 72.17 to 74.27) (n=87). The difference of mean was 8.78 (p<.001) which is statistically significant.

In 2nd Trimester

Systolic blood pressure in patients who develop PE (n=9). The mean value was 140.22 mm Hg (95% CI = 130.27 to 150.16) compared with a mean of 115.45 mm Hg (95% CI= 114.11 to 116.79) (n=87). The difference of mean was 24.77 (p<.001) which is statistically significant. Diastolic blood pressure in patients who develop PE (n=9). The mean value was 91.56 mm Hg (95% CI = 82.30 to 100.80) compared with a mean of 73.10 mm Hg (95% CI= 72.11 to 74.10) (n=87). The difference of mean was 18.46 (p<.001) which is statistically significant.

In 3rd trimester

Systolic blood pressure in patients who develop PE (n=7). The mean value was 140.57 mm Hg (95% CI = 132.89 to 148.25) compared with a mean of 115.22 mm Hg (95% CI= 113.67 to 116.76) (n=87). The difference of mean was 25.35 (p<.001) which is statistically significant. Diastolic blood pressure in patients who develop PE (n=7). The mean value was 90.29 mm Hg (95% CI = 81.01 to 99.56) compared with a mean of 72.80 mm Hg (95% CI= 71.71 to 73.90) (n=87). The

difference of mean was 17.49 (p<.001) which is statistically significant.

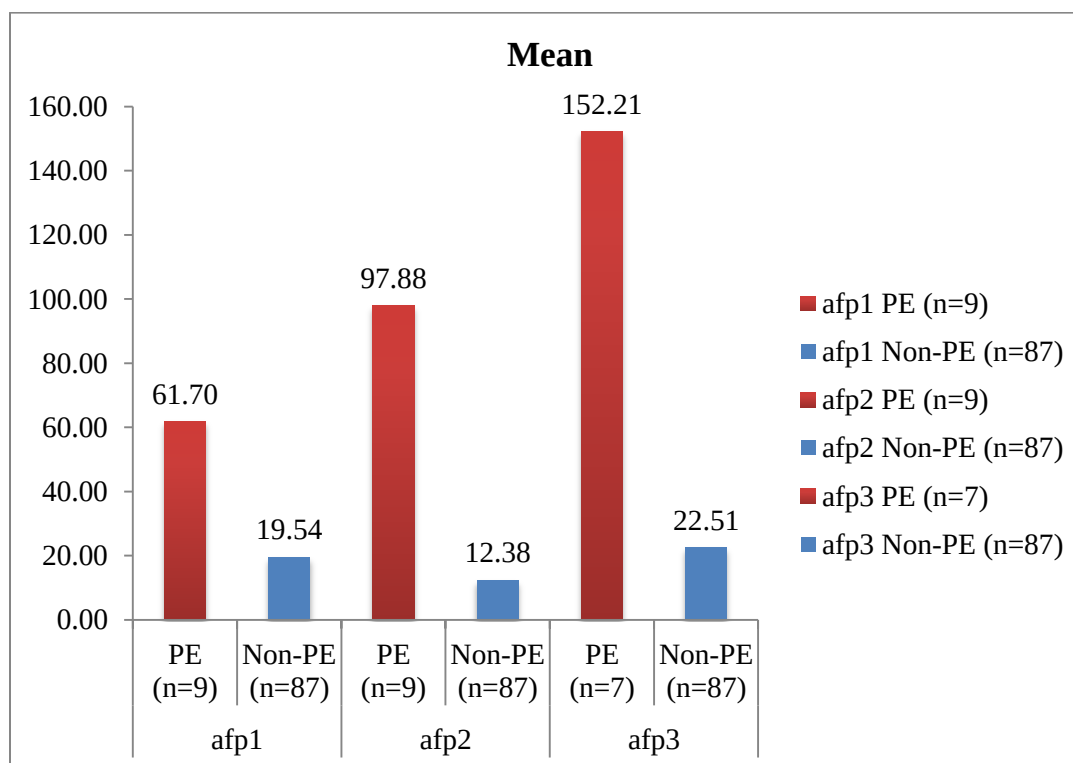
Platelet count in patients who develop PE (n=7). The mean value was 2.39 lacs/cumm (95% CI =1.74 to 3.02) compared with a mean of 2.28 lacs/cumm (95% CI= 2.20 to 2.37) (n=87). The difference of mean was 0.10 (p=0.53) which is statistically not significant.

Trimester wise no.& mean AFP Of patients in PE &non PE

Table 4

	1 st Trimester mean AFP	2 nd Trimester mean AFP	3 rd Trimester mean AFP
PE	61.70	97.88	152.21
Non PE	19.54	12.38	22.51
p-value	<.001	<0.001	<.01

Figure 4



In 1st Trimester

AFP in patients who develop PE (n=9). The mean value was 61.7 ng/ml (95% CI= 31.74 to 91.65) compared with a mean of 19.54 ng/ml (95% CI =12.19 to 26.90) (n=87). The difference of mean was 42.16 (p<0.001) which is statistically significant.

In 2nd Trimester

AFP in patients who develop PE (n=9). The mean value was 97.88 ng/ml (95% CI= 46.42 to 149.32) compared with a mean of 12.38 ng/ml (95% CI = 7.44 to 17.31) (n=87). The difference of mean was 85.5 (p<.001) which is statistically significant.

with a mean of 12.38 ng/ml (95% CI =7.44 to 17.31) (n=87). The difference of mean was 85.5 (p<.001) which is statistically significant.

In 3rd Trimester

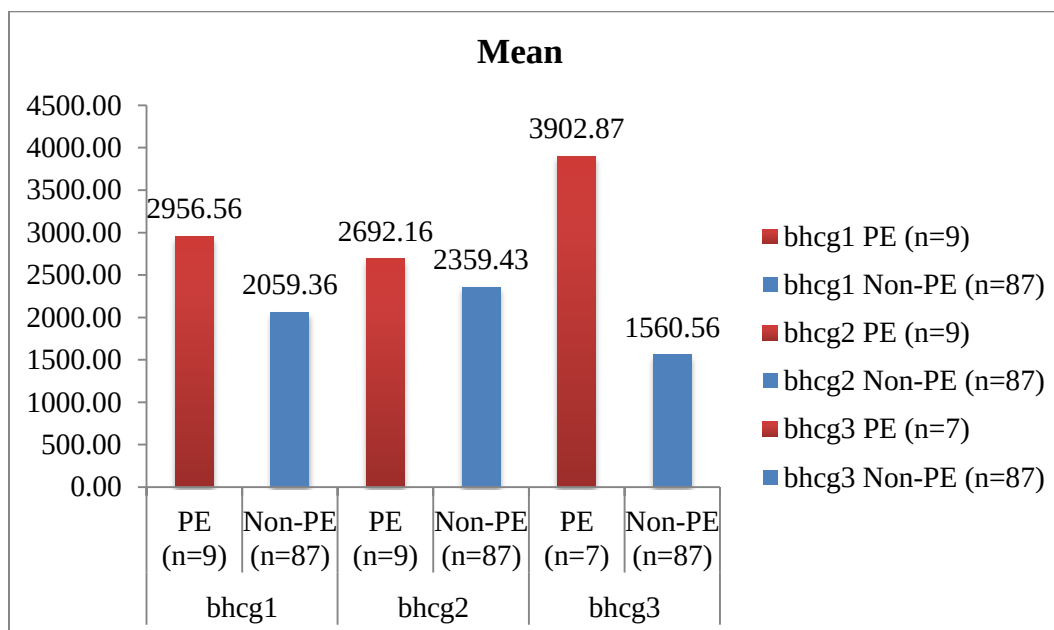
AFP in patients who develop PE (n=7). The mean value was 152.21 ng/ml (95% CI= -49.30 to 353.72) compared with a mean of 22.51 ng/ml (95% CI = 2.92 to 42.11) (n=87). The difference of mean was 129.70 (p<.01) which is statistically significant.

Trimester wise no.& mean β -hcgOf patients in PE &non PE

Table -5

	1 st Trimester mean β -hcg	2 nd Trimester mean β -hcg	3 rd Trimester mean β -hcg
PE	2956.56	2692.16	3902.87
Non PE	2059.36	2359.43	1560.56
p-value	<.05	.508	<.001

Figure 5



In 1st Trimester

β -hcg level in patients who develop PE (n=9). The mean value was 2956.56 mIU/ml (95% CI = 1082.79 to 4830.31) compared with a mean of 2059.36 mIU/ml (95% CI= 1827.72 to 2290.99) (n=87). The difference of mean was 897.2 (p<0.05) which is statistically significant.

In 2nd Trimester

β -hcg level in patients who develop PE (n=9). The mean value was 2692.16 mIU/ml (95% CI = 758.11 to 4626.19) compared with a mean of 2359.43 mIU/ml

(95% CI= 2085.84 to 2633.02) (n=87). The difference of mean was 332.7 (p=0.508) which is statistically not significant.

In 3rd Trimester

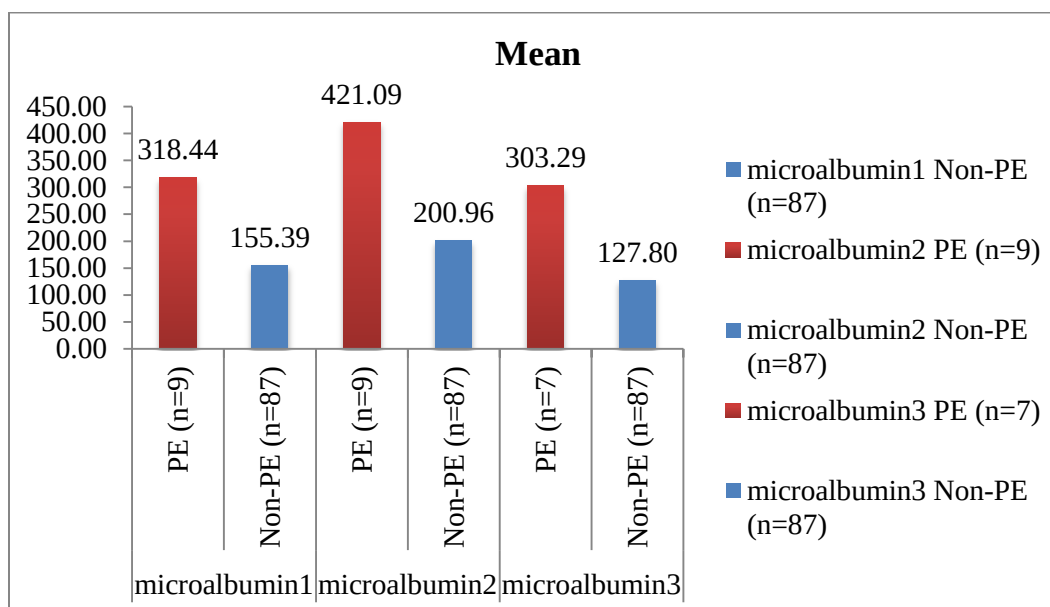
β -hcg level in patients who develop PE (n=7). The mean value was 3902.87 mIU/ml (95% CI = 888.41 to 6917.32) compared with a mean of 1560.56 mIU/ml (95% CI= 1310.93 to 1810.20) (n=87). The difference of mean was 2342.31 (p<.001) which is statistically significant.

Trimester wise no.& mean microalbuminuria of patients in PE &non PE

Table 6

	1 st Trimester mean MA	2 nd Trimester mean MA	3 rd Trimester mean MA
PE	318.44	421.09	303.29
Non PE	155.39	200.96	127.80
p-value	<.02	<.02	<.001

Figure 6



In 1st Trimester

Microalbuminuria in patients who develop PE (n=9). The mean value was 318.44 mg/L (95% CI = 197.21 to 439.67) compared with a mean of 155.39 mg/L (95% CI= 112.97 to 197.82) (n=87). The difference of mean was 163.05 mg/L (p<.02) which is statistically significant

Similarly in 2nd Trimester

Microalbuminuria in patients who develop PE (n=9). The mean value was 421.09 mg/L (95% CI = -2.66 to 844.84) compared with a mean of 200.96 mg/L (95% CI= 159.66 to 242.26) (n=87). The difference of mean was 220.13 mg/L (p<.02) which is statistically significant

Similarly in 3rd Trimester

Microalbuminuria in patients who develop PE (n=7). The mean value was 303.29 mg/L (95% CI= 128.37 to 478.19) compared with a mean of 127.80 mg/L (95% CI = 106.30 to 149.30) (n=87). The difference of mean was 175.48 mg/L (p<.001) which is statistically significant

Thus we found that systolic and diastolic blood pressure, AFP and microalbuminuria in all three trimesters, are significantly different in the two groups. Also β -hcg is significantly different in first and 3rd trimester. Thus AFP, β -hcg and microalbuminuria can be taken into consideration to predict occurrence of pre-eclampsia.

DISCUSSION

Pre eclampsia is one of the major causes of maternal and perinatal morbidity and mortality worldwide accounting for 2-8% mortality. PE increases perinatal mortality five-

fold, with most deaths caused by iatrogenic prematurity [7]. Preterm birth in itself is responsible for the majority of neonatal deaths and nearly one half of all cases of congenital neurologic disability [8].

This study is an effort to identify a woman at risk for developing PE before the disease manifests by doing consecutive estimations of AFP, β -hcg in blood and Microalbuminuria in the three trimesters.

We have taken systolic and diastolic blood pressure in all patients and found statistically significant difference in all three trimesters in both the groups (PE & non PE) (p<.001) [Table 3].

In this cohort study we followed these patients through the 3 trimesters and found that presence or absence of various prodromal symptoms. In our study mean AFP of patients in first trimester in PE patients is 61.70 ng/ml & non PE patients is 19.54 ng/ml (p < 0.001). In second trimester mean AFP in PE patients is 97.88 ng/ml & non PE patients is 12.38 ng/ml (p < .001) and in third trimester in PE patients is 152.21 ng/ml & non PE patients is 22.51 ng/ml (p < 0.01) which are statistically significant. [Table 4]

Mean β -hcg of patients in 1st trimester in PE patients is 2956.56 mIU/ml & non PE patients is 2059.36 mIU/ml (p < 0.05). In 2nd trimester mean β -hcg in PE patients is 2692.16 mIU/ml & non PE patients is 2359.43 mIU/ml (p < .508) and in 3rd trimester in PE patients is 3902.87 mIU/ml & non PE patients is 1560.56 mIU/ml (p < 0.001) which are statistically significant in first and third trimester. [Table 5].

Luckas et al, Benn et al, Ashour et al [9-11] indicated that an unexplained elevation of serum hcg significantly correlated with the occurrence of PE. Steier et al [12] Ashour [11], Lambert et al [13], DaOur study shows

mean values of β -hcg in PE is 2956.55 [$p < .05$] in first, 2692.15 [$p = .508$] in second and 3886.68 [$p < .001$] in third trimester. [Table 5]

By contrast Pouta et al [14] and Aquilina et al [15] demonstrated no relation between levels of serum hcg and development of PE. Stamilo et al, [16] also found no association between severe PE and elevated hcg levels. Vidson EJ et al [17][188] also found significant association of serum hcg with the development of PE.

Alpha-FetoProtein

We found significant association between high serum AFP in first, second & third trimester and subsequent development of PE [$p < .05$]. Mean values of AFP 61.7 ng/ml [$p < .001$] in first, 97.87 ng/ml [$p < .001$] in second and 163.57 ng/ml [$p < .01$] in third trimester [Table 4]

In many studies unusually high AFP values have been associated with PE.

Raty et al also found the AFP values in the PE group differed significantly from all other groups. Aquilina et al [15] observed positive correlation between AFP levels and occurrence of preeclampsia. Nearly similar were the observations of Duric et al [18]. Onderoglu LS, Grobman WA [19] studies suggested that AFP was predictive of severe pre-eclampsia.

By contrast Stamilio et al [16] reported that AFP was not a useful marker for pre-eclampsia.

Microalbuminuria

There is an association between microalbuminuria and subsequent development of PE. Our data also support a significant association between elevated first, second and third trimester microalbuminuria and development of PE.

In our study mean values of microalbuminuria 318.44 mg/L [$p < .02$] in first, 421.08 mg/L [$p < .02$] in second and 237.166 mg/L [$p < .001$] in third trimester. [Table 6]

Jaiswar SP et al [20] found the sensitivity of predicting PE by measuring microalbumin in early pregnancy varied between 50% to 68% and the specificity varied between 58 to 97%, PPV varied between 26 to 61% and the NPV varied between 87-94%. Salako et al [21] has reported that a single estimation of microalbuminuria at booking had a high sensitivity [88.9%] but a low PPV [22%]. However, Chhabra [22] have found that estimation of microalbuminuria around 18 weeks of gestation seemed useful, especially in primigravida.

Singh R et al [23] after studying 144 patients concluded microalbuminuria in mid-pregnancy may be a significant predictor of development of subsequent pre-eclampsia.

Overall this study documents strong correlation of AFP, β hcg & microalbuminuria in serial assays to development of PE in later trimester.

Strengths of the Study

This study has several strengths. It used a prospective observational design and followed 96 antenatal women from the first trimester through the third trimester. Serial measurements of AFP, β -hCG, and microalbuminuria were performed, allowing changes in these markers to be assessed over pregnancy. The study also evaluated the association of these biochemical markers with blood pressure and subsequent development of preeclampsia. The trimester-wise assessment provides useful information regarding the potential role of these markers in the early identification of women at risk of preeclampsia.

Limitations of the Study

The study has some limitations. The sample size was relatively small, with only 96 participants, and the number of women who developed preeclampsia was limited. The study was conducted at a single center, which may limit the generalizability of the findings. Diagnostic performance measures such as sensitivity, specificity, positive predictive value, and negative predictive value were not comprehensively evaluated. Furthermore, potential confounding factors were not assessed using multivariable statistical analysis. The findings, particularly for β -hCG, were not consistent across all three trimesters.

Future Scope

Future studies should include larger sample sizes and multicenter prospective designs to validate these findings. Further research should establish trimester-specific cutoff values for AFP, β -hCG, and microalbuminuria and evaluate their sensitivity and specificity for predicting preeclampsia. Combining these markers with maternal clinical risk factors and other biochemical or ultrasonographic parameters may improve predictive accuracy. Advanced statistical approaches, including multivariable regression and ROC curve analysis, should also be considered to develop reliable predictive models for early identification of women at risk of preeclampsia.

CONCLUSION

There is a significant association of symptoms viz. headache, nausea, vomiting, epigastric pain, blurring of vision from 2nd trimester with PE later in pregnancy. Serial estimation of AFP & microalbuminuria in first, second & third trimester have significant association with the development of PE later in pregnancy. Also values of β hcg in first & third trimester have significant association with the development of PE later in pregnancy. The major finding of this research work is significant co-relation of microalbuminuria to PE.

Declaration

Ethics Approval and Consent to Participate

The study was conducted after approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants before enrollment in the study.

Consent for Publication

Not applicable.

Funding

The authors received no specific funding for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

All authors contributed to the conception and design of the study, data collection, analysis and interpretation of data, preparation of the manuscript, and approved the final version of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to appropriate ethical and privacy considerations.

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