

Full Length Research Paper

Comparative evaluation of serum soluble endoglin level in preeclampsia and normotensive pregnant women

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This study was conducted to evaluate the serum soluble endoglin (CD105) levels in preeclamptic and normotensive pregnant women after 22 weeks of pregnancy. One hundred pregnant women from 22 weeks of gestation till term were divided into 2 groups in this case control study. Group I included 50 women with features of preeclampsia while Group II had 50 gestationally matched normotensive controls. Soluble endoglin levels were measured by Enzyme-linked immunosorbent assay (ELISA) and results analysed. The endoglin levels in the study varied between 2.37 to 77.77 mg/ml. The levels were significantly higher in Group I and were inversely proportional to the gestational age. The maternal and neonatal outcomes were better in Group II of the study. Serum soluble endoglin could be of possible use as a predictive clinical test for preeclampsia risk assessment.

Key words: Soluble endoglin, preeclampsia.

INTRODUCTION

Hypertensive disorders of pregnancy form a major cause of maternal mortality, morbidity, perinatal deaths, preterm births and intrauterine growth retardation. Although it is of immense importance to identify women at risk and to confirm the diagnosis in suspected cases, there is no accurate biomarker for the same. Chemical agents like interleukin 2-receptor, insulin like growth factor-1, insulin like growth factor binding protein-1, placenta growth factor, hepatocyte growth factor, inhibin A, activin A and human chorionic gonadotrophin have been tried in efforts to find the most suitable presymptomatic marker for preeclampsia. Recently, there have been occasional reports of elevation of two antiangiogenic peptides (soluble film like tyrosine kinase -1 (sFlt-1) and soluble endoglin (sEng) in women with established preeclampsia (Levine et al., 2004, 2006). Soluble endoglin may have a potential

role in the pathophysiology of preeclampsia. Hence, this study was planned to evaluate the serum soluble endoglin levels in cases of preeclampsia and normotensive pregnant women after 22 weeks of pregnancy.

Aim

The aim was to compare the serum soluble endoglin levels in preeclamptic women and normotensive pregnant women after 22 weeks of pregnancy.

MATERIALS AND METHODS

One hundred pregnant women with singleton pregnancy from 22 weeks of gestation till term were enrolled into this longitudinal case control study at the time of their antenatal visit to the outpatient department of Obstetrics and Gynaecology and were divided into two groups. Group I included 50 women with signs and symptoms of preeclampsia while Group II consisted of an equal number of normotensive women who served as controls. Only those women who did not develop preeclampsia till delivery were included in

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Group II of the study. Preeclampsia was defined as a systolic blood pressure greater than or equal to 140 mmHg and/or diastolic blood pressure greater than or equal to 90 mmHg and the presence of + or more of proteinuria after 20 weeks of gestation. The blood pressure was measured in lateral recumbent position in the left arm with the woman's legs resting on a flat surface, by a mercury sphygmomanometer kept at the level of the heart. The first sound (Korotkoff 1) heard was taken as the systolic blood pressure while the diastolic blood pressure was taken at complete disappearance of sounds (Korotkoff 5). In the event of absence of Korotkoff 5, muffling (Korotkoff 4) was accepted. Preeclampsia was graded as mild if the systolic blood pressure was between 140 to 159 mmHg and the diastolic pressure was between 90 to 109 mmHg and as severe if the systolic and diastolic blood pressure were more than 160 and 110 mmHg, respectively. Early onset preeclampsia was defined as onset of preeclampsia at less than 34 weeks of gestation while late onset preeclampsia was diagnosed if the onset was at or after 34 weeks of pregnancy.

Women with multiple pregnancy, collagen vascular disorders, chronic hypertension, renal or thyroid disease and tobacco abuse were excluded from the study.

After a detailed history, a general physical, systemic and obstetric examination, all women underwent haematologic, ultrasonographic abdominal evaluation and urinary estimation of proteins and sugar. Proteinuria was measured by dipstick test and was graded as + (30 mg/dl), ++ (100 mg/dl), +++ (300 mg/dl) and ++++ (>2000 mg/dl). A complete haemogram, blood grouping, uric acid, urea, creatinine, alkaline phosphatase, SGOT and SGPT estimations were the other haematologic investigations carried out.

The serum soluble endoglin level was measured in all patients after an informed consent at recruitment by ELISA technique using commercial kits (R&D systems). It is a 4.5 h solid phase ELISA test which employs the quantitative sandwich enzyme immunoassay technique. Blood was collected in women of Group I after the diagnosis of preeclampsia was made. The normal value of serum soluble endoglin varies from 2.54 to 7.06 ng/ml and values >7.06 ng/ml were regarded as elevated. All the women were followed till delivery. Secondary outcome measures were mode of delivery, birth weight and perinatal morbidity and mortality. The data was analysed statistically using Student's t-test (unpaired) and chi-square tests.

RESULTS

The patients of both the groups in the study were comparable in terms of age and place of residence. The mean age of patients was 23.7±3.8 years and 24.5±3.4 years in Group I and II, respectively ($p = 0.288$). The two groups were comparable in terms of parity, the mean parity being 0.82±1.89 in Group I and 0.74±0.964 in Group II ($p = 0.743$).

The gestational age in Group I ranged from 23 to 40 weeks with median value of 34 weeks (mean 33.3±4.3 weeks) while in Group II, it ranged between 23 to 42 weeks with median of 31 weeks (mean 32.0±5.0 weeks), the difference being insignificant ($p = 0.176$).

All patients of Group I were hypertensive at enrolment with mean systolic pressure of 154.4±15.4 mmHg and median pressure of 150 mmHg. In contrast, all women in

Group II were normotensive with mean systolic pressure of 115.8±6.5 mmHg and a median value of 111 mmHg. This difference in the two groups was highly significant ($p < 0.0001$). The diastolic blood pressure ranged between 90 and 140 mmHg in Group I (median = 100 mmHg, mean 103.92±9.96 mmHg) while in Group II, the range was 70 and 94 mmHg (median 80 mmHg), mean 80.24±5.73 mmHg ($p = 0.001$).

All women of Group I had proteinuria at enrolment, five (10%) women excreted upto 100 mg/dl and 45 (90%) had more than 100 mg/dl proteins in urine. In Group II, five (10%) women had trace of proteins in urine while 4 (8%) had between 30 and 100 mg/dl proteins which disappeared on follow up visit ($p < 0.001$).

Forty six (92%) women of Group I had pedal oedema while headache was reported by 39 (78%), epigastric pain by 18 (36%) and blurring of vision by 13 (26%) women. In Group II, 7 (14%) had headache, 2 (4%) had pedal oedema and 2 (4%) had history of preeclampsia in a previous pregnancy.

The normal range of blood urea was taken as 15 to 40 mg%. It was elevated in 4 (8%) cases of Group I only. The mean blood urea was 29.78±20.79 mg% and 21.04±3.10 mg% in Groups I and II respectively ($p = 0.004$).

The mean serum uric acid as 4.04±1.04 in Group I and 3.50±0.84 mg/dl in Group II ($p = 0.006$). The upper limit of normal serum creatinine in pregnancy was taken as 0.8 mg/dl and was found elevated in 4 (8%) cases of Group I and none of Group II. The mean value was 1.02±0.29 mg% in Group I and 0.85±0.207 mg% in Group II ($p < 0.002$).

Table 1 depicts the serum soluble endoglin level in the study at enrolment. The difference of values of this protein in the 2 groups of the study was very highly significant ($p < 0.001$). Table 2 shows the distribution of serum soluble endoglin levels according to period of gestation. Between 22 and 28 weeks gestation, the mean levels in Group I were raised in all 6 patients of Group I (mean 23.79±8.34 ng/ml) in comparison to 13 patients of Group II (mean 5.05±3.70 ng/ml) ($p < 0.001$). Between 28 and 34 weeks of pregnancy, all 20 patients of Group I had raised levels (mean 16.91±9.78 ng/ml) while all 19 patients of Group II had levels within normal range (mean 5.44±1.78) ($p = 0.001$). From 34 weeks till term pregnancy, there were 24 patients in Group I and 18 in Group II. The mean serum endoglin level was 16.43±17.58 ng/ml in Group I and 9.41±17.13 ng/ml in Group II ($p = 0.195$).

The mean values of blood urea, serum uric acid, creatinine and soluble endoglin levels in Group I of the study were 29.78±20.79, 4.04±1.04, and 1.02±0.298 mg%, and 17.49±14.2 ng/ml, respectively. The corresponding values in Group II were 21.04±3.21, 3.50±0.844, and 0.85±0.207 mg% and 6.77±10.50 ng/ml and the

Table 1. Distribution of serum soluble endoglin levels in the study.

Serum soluble endoglin (ng/ml)	Group I (n=50)		Group II (n=50)	
	No. of patients	Percentage	No. of patients	Percentage
<2.5	2	4	7	14
2.54-7.06	5	10	34	68
>7.06-17	24	48	8	16
>17-27	12	24	-	-
>27-37	2	4	-	-
>37-47	2	4	-	-
>47-57	1	2	-	-
>57-67	1	2	-	-
>67-77	-	-	-	-
>77	1	2	1	2
Total	50	100	50	100
Median (range)	14.47 (1.02-77.77)		5.48 (1.02-77.11)	
Mean±SD	17.49±14.2		6.77±10.50	

Table 2. Serum soluble endoglin levels according to period of gestation.

Period of gestation (weeks)	Mean soluble endoglin level (ng/ml)					
	Group I (n=50)			Group II (n=50)		
	No. of patients	Median (range)	Mean soluble endoglin (ng/ml)	No. of patients	Median (range)	Mean soluble Endoglin (ng/ml)
22-24	1	-	26.72	3	3.49 (2.37-3.68)	3.18
>24-26	4	21.19 (7.43-37.99)	20.95	4	4.31 (2.80-15.37)	6.69
>26-28	1	-	38.77	6	4.80 (1.81-8.86)	4.88
>28-30	4	28.49(10.58-19.15)	16.74	5	5.67 (4.53-7.52)	5.67
>30-32	9	16.84 (7.52-49.6)	19.64	11	5.67(1.02-9.15)	5.28
>32-34	7	8.96 (6.55-19.74)	11.47	3	6.51 (3.68-6.75)	5.65
>34-36	8	13.38 (5.54-29.03)	14.00	4	6.03 (1.45-10.31)	5.95
>36-38	10	10.09 (4.95-59.4)	16.11	6	6.56(3.0-11.3)	7.015
>38-40	6	10.01 (1.02-77.77)	23.24	6	3.90 (2.24-5.98)	4.14
>40-42	-	-	-	2	39.365 (1.62-77.11)	39.36

differences in the 2 groups were significant. Table 3 depicts the serum endoglin levels in women with early and late onset preeclampsia.

The mean systolic blood pressure at enrolment was 154.4±15.4 mmHg in Group I and 115.8±6.5 mmHg in Group II, while the mean diastolic blood pressure was 103.9±9.9 and 80.2±5.7 mmHg in Groups I and II, respectively. The mean systolic blood pressure at delivery in Group I was 153.4±14.4 mmHg while in Group II, it was 114.8±7.7 mmHg ($p<0.001$). The diastolic blood pressure at delivery in Groups I and II was 100.8±8.0 and 77.9±6.0mmHg respectively ($p<0.001$).

The gestational age at delivery ranged from 28 to 40 weeks in Group I and from 37 to 42 weeks in Group II,

the mean age being 35±2.7 weeks in Group I and 39±1.0 weeks in Group II. Thirty two (64%) women of Group I and none from Group II delivered before 37 weeks ($p<0.001$). Thirty four (68%) women of Group I and 42 (84%) of Group II had vaginal delivery and the remaining were delivered abdominally.

Of the 30 preterm deliveries in Group I, 23 delivered vaginally and 7 by cesarean section. The mean weight was 2.14±0.66 and 2.97±0.33 kg in Groups I and II, respectively ($p < 0.001$).

Seven patients of Group I had eclampsia, 6 had ascites, 3 each had acute renal failure and HELLP syndrome while 2 mothers died. The only maternal complication observed in the neonates, 35 from Groups I and 5 from Group II

Table 3. Serum soluble endoglin levels in early onset and late onset preeclampsia.

Reference	Serum soluble endoglin level in early onset preeclampsia (ng/ml)	Serum soluble endoglin level in late onset preeclampsia (ng/ml)
Kim et al (2008)	105.4±37.9	66.3±36
Stepan et al (2008)	67.5±4.0 vs. control 6.5±2.0	31.7±2.0 vs. control 6.5±2.0
Present study (2007-09)	18.63±9.75 vs. control 5.33±2.7	16.43±17.58 vs. control 9.11±16.70

Group II was antepartum hemorrhage in 2 cases. Among required transfer to neonatal intensive care unit (NICU). Twenty three of these were premature and 3 of them expired within a week due to sequelae of prematurity. All the babies of Group II who were shifted to NICU were discharged satisfactorily within 7 days. The mean hospital stay of babies of Groups I and II was 11.2±5.58 and 0.36±1.27 days, respectively ($p = 0.001$).

DISCUSSION

Endoglin, a soluble antiangiogenic protein, binds TGF-beta, thereby preventing activation of eNOS and downstream vascular vasodilatation (Robinson and Johnson, 2007). Inhibition of normal vascular physiology as induced by this protein leads to increased vascular permeability and to induced hypertension, both classic findings in preeclampsia. The present study has attempted to evaluate the role of serum soluble endoglin in preeclampsia in comparison to normotensive pregnant women. The baseline patient characteristics and the demographic profile in the 2 groups of the study were comparable.

Slahuddin et al. (2007) reported a significantly higher mean soluble endoglin level in preeclampsia (69.2±42.5 ng/ml) than in normotensive pregnant women (15.5±6.9 ng/ml; $p=0.01$). Staff et al. (2007) found a 4.4 fold higher median maternal serum endoglin concentration than in control group as compared to 2.8 fold value in preeclamptic women in present study. Placental ischemia leads possibly to release of placental factors and an imbalance of angiogenic factors, causing endothelial dysfunction in preeclampsia and an altered expression of antiangiogenic peptides (like soluble endoglin) from it.

The present study indicates 86% women with preeclampsia to be having raised endoglin levels. Hirashima et al. (2008) in their study, reported raised endoglin in 88% of preeclamptic women. A significant elevation of this protein in early onset and severe preeclampsia than in late onset or mild disease could suggest that various antiangiogenic factors could be involved in the pathogenesis of preeclampsia with synergistic though different roles (Kim et al., 2009).

In our study, the mean serum endoglin levels in the

preeclampsia group were higher than in normotensive group. Within the preeclampsia group, the levels were higher in women with early onset preeclampsia than those with late onset disease. Levine et al. (2006), reported higher mean soluble endoglin levels in preterm preeclampsia as compared to matched controls (46.4 vs. 9.8 mg/ml; $p<0.001$). Similar elevated values, though with lesser difference, were reported by them among 16 cases of term preeclampsia in comparison to matched controls (31.0 vs. 13.3 ng/ml; $p<0.001$).

Serial measurements of serum endoglin may help in early detection of preeclampsia and in prediction of its severity. Smith et al reported 100% sensitivity and 72.7% specificity for predicting early onset preeclampsia in second trimester pregnancies by combining endoglin measurements with doppler sonography of uterine vessels (Smith et al., 2009).

From our study, it is evident that serum endoglin rises during normal as well as preeclamptic pregnancy and that the rise in preeclampsia is much higher. As suggested by Baumann et al. (2008), the rise in endoglin levels may occur as early as the first trimester in pregnancies which later develop preeclampsia. Hence, used alone or in combination with other predictive parameters like measurement of uterine perfusion, the measurement of soluble serum endoglin has the potential for use as a predictive clinical test for preeclampsia risk assessment.

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