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Original Research Paper

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2-METHOXY ESTRADIOL AND PRE-ECLAMPSIA-CORRELATION WITH THE SEVERITY OF THE DISEASE

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ABSTRACT

The study was conducted with the aim of comparing mid trimester serum levels of 2-Methoxy estradiol in patients with pre eclampsia and normotensive pregnant women and to study its association with the severity of pre eclampsia.

Materials And Methods- This was a prospective case-control study, conducted at the department of Kasturba Hospital, Delhi during time period of January 2019-December 2019. 200 ante natal women attending ANC clinic at Kasturba Hospital, at 14-20 weeks of gestation, ranging from 18-40 years of age, and fulfilling the inclusion and exclusion criteria, were chosen for the study. Blood samples were drawn at their first visit to ANC clinic and serum level of 2-ME was determined by using 2-methoxyestradiol ELISA kit. Patients were followed up in antenatal period, during delivery and post partum period till 6weeks. Women who developed hypertensive disorder of pregnancy during their ante natal period were taken as cases. The cases were evaluated further for development of pre eclampsia/severe pre eclampsia in accordance with the ACOG Taskforce on Hypertension in Pregnancy 2103. Women who remained normotensive during their ante natal period were taken as control.

Result- The mean value of mid trimester serum levels of 2-methoxy estradiol was found to be 1134.45±332.60ng/L in controls,723.95±38.54ng/L in gestational hypertension group,433.54±124.16ng/L in patients of pre eclampsia and 124.32±20.03ng/L in severe pre eclampsia group. Statistical analysis showed that mid trimester serum levels of 2-methoxy estradiol was significantly reduced in patients with severe pre eclampsia as compared to patient with pre eclampsia and gestational hypertension respectively($p<0.001$).ROC curve showed that at the cut off value of 781.17ng/L for 2-ME, the sensitivity and specificity of the test to establish association with severity of pre eclampsia was 100%.

Conclusion- Mid trimester serum levels of 2-ME was found to be significantly negatively correlated with the severity of hypertensive disorder of pregnancy.It was significantly reduced in patients with severe pre eclampsia when compared to patients with pre eclampsia and gestational hypertension respectively.

KEYWORDS : Pre eclampsia, 2-methoxy estradiol,ELISA

INTRODUCTION

Pre eclampsia is a multi-factorial pregnancy specific disorder that involve failure of various body systems.According to American college of Obstetricians and Gynaecologists Task force on Hypertension in pregnancy, Pre eclampsia is the leading cause of maternal and perinatal morbidity and mortality, with an estimated 50,000-60,000 pre eclampsia related deaths per year worldwide[1]. Despite the amount of resources invested in research and treatment of this disorder, it remains difficult to accurately predict and almost impossible to prevent.

The pathophysiology of preeclampsia has been extensively studied over the past decades. Recent research has been focused on the role of catechol-O-methyltransferase (COMT)and its product 2-methoxyestradiol (2ME) in pathophysiology of preeclampsia. 2ME is a naturally occurring metabolite of 17 beta estradiol generated via enzyme catechol-O-methyl transferase (COMT). Recent evidence has demonstrated that 2-ME under low-oxygen conditions is critical for the proper cytotrophoblast invasion, placental vascular development and regulation of oxygen tension during normal pregnancy; therefore, impaired 2-ME synthesis/release has been implicated in the etiopathogenesis of PE[2] .In support of this concept, Kanasaki et al[3] has also reported that COMT-deficient mice exhibit a PE-like phenotype and placental hypoxia in pregnancy and such PE-like features could be rescued by subcutaneous injection of 2-ME, further confirming the major roles of COMT/2-ME interactions in PE.

MATERIALS AND METHOD

The present study is a prospective case control study, conducted in the department of obstetrics and gynaecology, Kasturba Hospital, Delhi for period of 1 year from January 2019 to December 2019,after clearance from Institutional ethical committee.

Total sample size was 200. Formula used was:- For comparing mean of two groups

$$N >= 2\frac{(\text{standard deviation})^2}{(\text{mean difference})^2}*(Z\alpha + Z\beta)^2$$

Where Z_α is value of Z at two sided alpha error of 5% and Z_β is value of Z at power of 99% and mean difference is difference in mean values of two groups.

Inclusion Criteria

- Mid trimester (14-20weeks) pregnant women of age 18-40 years with singleton pregnancy who were sure of dates, with LMP or with dating scan.

Exclusion Criteria

- Past history of cardiac, renal, hepatic dysfunction.
- Chronic hypertension, Diabetes Mellitus, thyroid disorder or any chronic disorder.
- Multiple pregnancy, Molar pregnancy.
- Ultrasound proven fetal gross congenital anomalies.

Plan Of Work

After informed consent, random 200 ante natal women attending ANC clinic at Kasturba Hospital, at 14-20 weeks of gestation, ranging from 18-40 years of age, and fulfilling the inclusion and exclusion criteria, were chosen for the study.

Blood samples were drawn at their first visit to ANC clinic and serum level of 2-methoxy estradiol (2-ME) was determined by using 2-methoxyestradiol ELISA kit, which was based on competitive ELISA principle. The Standard curve range of 2-methoxyestrdiol ELISA kit was 10- 2400ng/L with the sensitivity of 6.03ng/L.

Patients were followed up in antenatal period, during delivery and post partum period till 6weeks.

Case group-Women who developed hypertensive disorder of pregnancy during their ante natal period were taken as cases. The cases were evaluated further for development of pre eclampsia/severe pre eclampsia in accordance with the ACOG Taskforce on Hypertension in Pregnancy 2103.

Pre Eclampsia-
It is defined as SBP≥140mmHg or DBP≥90mmHg on 2 occasions at least 4 hours apart in previously normotensive patient, after 20 weeks of gestation.And, any one of these:-

- Proteinuria ≥0.3gm in 24hour urine specimen
- Protein/creatinine(mg/dl)≥0.3
- Urine dipstick protein+1

Severe Pre eclampsia-
It is defined as SBP≥160mmHg or DBP≥110mmHg,on 2 occasions at least 4 hours apart.And, any one of the following:-

- Impaired hepatic function as indicated by double the concentration of liver enzymes (SGOT/SGPT ≥ 70 IU/ml), severe persistent epigastric pain that does not respond to pharmacotherapy
- Progressive renal insufficiency (serum creatinine >1.1mg/dl or doubling of serum creatinine concentration in the absence of any renal disease)
- New onset cerebral/visual disturbances
- Pulmonary edema
- Thrombocytopenia(<1lakh/microlitre)

Control Group-
Women who remained normotensive during their ante natal period were taken as control.

METHODOLOGY

After taking informed consent, all the women were subjected to the detailed history,general examination, systemic examination, obstetrical examination, and various investigations were sent.

BP Measurement-
Using an appropriate-sized cuff, BP was measured in the right arm sitting position in relaxed state with the arm kept at the level of the heart, using sphygmomanometer. Two readings were taken 4 hours apart.

Investigations-
Routine Antenatal Investigations-
Hemoglobin, ABO Rh typing, VDRL, blood sugar screening, viral markers, Liver function tests(AST, ALT, Alkaline Phosphatase) Serum proteins, Kidney function test(serum urea, creatinine, USG (obstetrical), and urine routine microscopy were carried out.

Special Investigation-
PT/APTT, fundoscopy, USG Doppler wherever necessary was done.

Sample Collection And Analysis-
After informed consent,5ml of venous blood was collected under all aseptic precautions in 14-20 weeks of gestation.2-ME ELISA kit was used to estimate the mid trimester serum levels of 2-methoxyestradiol in ante natal women.

Patients were followed monthly till 28 weeks, then every fortnightly till 36 weeks, and then weekly till delivery, and weekly till 6 weeks in the post partum period and managed according to hospital protocol.

Statistical Analysis-
The collected data was transformed into variables, coded and entered in Microsoft Excel and was analyzed and statistically evaluated using SPSS-PC-19 version.

- Quantitative data was expressed in mean±standard deviation or median with inter-quartile range and difference between two comparable groups were tested by student's t-test (unpaired) or Mann Whitney 'U' test while more than two groups were compared by ANNOVA test or Kruskal Wallis H test followed by posthoc test.
- Qualitative data were expressed in percentage and statistical differences between the proportions were tested by chi square test or Fisher's exact test.
- Multiple linear regression analysis was performed to the correlation of 2-ME with different parameters.
- ROC curve was prepared to predict association of 2-ME with pre-eclampsia and based on that sensitivity, specificity, PPV and NPV were calculated.

'P' value less than 0.05 was considered statistically significant.

RESULTS-

Out of 200 patients taken, 1 had spontaneous abortion and 2 of them had missed abortion before 20 weeks of gestation and hence only 197 could be followed further for outcome measurement. In the further follow up,68 patients developed hypertensive disorder of pregnancy and were considered as cases, while those who remained normotensive were the control group.

The Case And Controls Were Matched For Demographic And Clinical Variables.(table 1)

Characteristics		CASE(%)	CONTROLS (%)	P Value
Age(Years)	18-25 years	55.9	59.7	0.44
	26-30years	38.2	34.9	
	>30years	5.9	5.4	
Mean Age(Years)		24.75±3.77	24.32±3.75	
Parity	Primi	60.3	58.1	0.87
	Multi	39.7	41.9	
Average Systolic BP(mmHg)		152.21±9.13	116.23±6.79	<0.001
Average Diastolic BP(mmHg)		100.97±9.18	74.06±6.44	<0.001

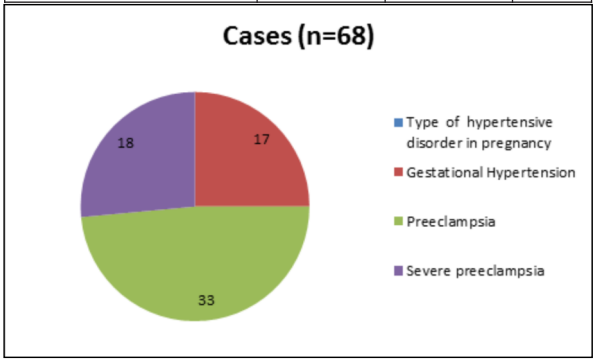

Figure1- Distribution Among Cases According To Type Of Hypertensive Disorder In Pregnancy.

Table 3:comparison Of Mid Trimester Levels Of 2-methoxy Estradiol Among Study Group.

	Group				P value
	Controls (n=129)	GTN (n=17)	PE (n=33)	Severe PE(n=18)	
2-Methoxy estradiol(2-ME) level (ng/L)	1134.45± 332.60	723.95± 38.54	433.54± 124.16	124.32± 20.03	<0.001

Statistical analysis showed that mid trimester levels of 2-methoxy estradiol was significantly reduced in patients with severe pre eclampsia as compared to patients with pre eclampsia and gestational hypertension respectively. (p<0.001).

Table 4: Association Of 2-ME With Biomarkers Of Severity Of Pre Eclampsia Among Cases.

Parameter	Correlation coefficient	p value	Regression equation
SBP	-0.74	<0.001	2ME = 3189.99-17.77 * SBP
DBP	-0.71	<0.001	2ME = 2718.46-21.94 * DBP
Platelet count	0.23	0.01	2ME = 593.44 + 154.35*PC
Hb (gm%)	0.07	0.23	2ME = 651.85+22.27*Hb
Blood Urea	-0.29	<0.01	2ME = 1313.69-22.75*BU
Creatinine (mg/dl)	0.19	0.03	2ME = 1445.17-752.08*Creatinine
SGOT	-0.48	<0.01	2ME = 1221.40-13.15*SGOT
SGPT	-0.49	<0.01	2ME =1321.81-10.27*SGPT

Plasma levels of 2-ME was significantly negatively correlated with SBP(r=-0.74,p<0.001) and DBP(r=-0.71,p<0.001). Regression analysis revealed that with one unit increase in SBP,2-ME decreased by 3172.22 units; and with one unit increase in DBP,2-ME decreased by 2696.52 units.

2-ME plasma levels were mildly significantly positively correlated with platlet count, blood urea and serum creatinine while moderately significantly negatively correlated with liver enzymes, SGOT and SGPT(r=-0.48,r=-0.49)respectively.

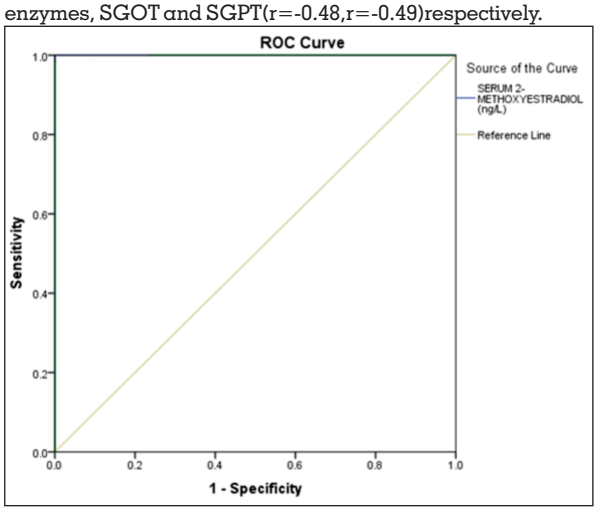

Figure 2- Receiving operating curve showing the sensitivity and specificity of 2-ME for pre-eclampsia

The ROC curve obtained in the study showed area under the curve was 1.0 for the plasma 2ME levels, at the cut off value of ≤ 781.17ng/L. At this cut off value of plasma 2-ME levels , the sensitivity of this test to establish association of 2-ME with severity of pre eclampsia was found to be 100%, specificity 100% , positive predictive value of 100% , negative predictive value of 100% and accuracy of 100%. Thus the sensitivity and specificity of 2-ME for diagnosing severity of pre eclampsia was found to be high.

DISCUSSION

Numerous studies have suggested that preeclampsia often developed at a certain age and parity groups. **Sanjay Gupta et al**[4] reported that pre eclampsia is a disease of the first-time pregnant woman as in his study ,81 % of the patients with preeclampsia were primigravida. The study further reported that 76.34 % of the patients were between 21 and 30 years of age thus rendering a very young population morbid and at risk of mortality. In this study, we did not find any significant difference in the risk of developing preeclampsia based on maternal age and parity.

In our study, we found a statistically significant negative correlation between mid trimester serum levels of 2ME with the severity of pre eclampsia..The mean value of mid trimester serum levels of 2-methoxy estradiol was found to be 1134.45±332.60ng/L in controls. Among cases, the mid trimester serum levels of 2-methoxy estradiol was 723.95±38.54ng/L in gestational hypertension group, while it was 433.54±124.16ng/L in patients with pre eclampsia. In severe pre eclampsia group, the mid trimester serum levels of 2-methoxy estradiol was found to be 124.32±20.03ng/L.

Since our results have been consistent with previous studies done over the years, it can be inferred that 2ME levels correlate well with the severity of preeclampsia, being lowest in those suffering from severe form of the disease and can be considered as a marker of the disease severity.

In our study mid trimester serum levels of 2-ME was significantly negatively correlated with systolic and diastolic blood pressure. Regression analysis revealed that with one unit increase in SBP,2-ME decreased by 3172.22 units; and with one unit increase in DBP,2-ME decreased by 2696.52 units. **Miriam pertegal et al**[5] conducted a similar study to evaluate the correlation of 2ME plasma levels with the clinical severity indices of pre eclampsia. The study reported that 2-ME plasma levels was significantly higher in controls(n=66) as compared to women with pre eclampsia(n=50; 2906.43 +200.69 pg/mL vs 1818.41 + 189.25 pg/mL; P < .001).Further the study also found that the 2ME serum levels was significantly and negatively correlated with systolic peak blood pressure, correlation coefficient of - 0.283, and p = 0.47.But 2-ME plasma levels did not correlate with DBP,p=0.66. **Alejandera perez et al**[6] also reported in their study that 2-ME levels in early pregnancy may be able to predict the subsequent development of pre eclampsia, since plasma 2-ME levels at 11-14 weeks of gestation were decreased in women who later developed pre eclampsia(PE:1.87±2pg/ml vs normotensivecontrols:61.73±27pg/ml,p<0.05).Further they hypothesized that the possible explanation for the molecular mechanism responsible for these reduced serum levels was-(1)decreased expression/activity of COMT enzyme.(2) decreased bioavailability of 17 beta estradiol likely due to alteration in aromatase pathway.

Jobe et al[7] described the role of estrogen and its metabolites in maternal circulation in pre eclampsia.They explained the probable role of 2-ME in the pathogenesis of pre eclampsia through the mechanism of uteroplacental circulatory vascular growth and inhibition of angiogenic factors and factors inducing tissue hypoxia. Role of increased expression of HIF-1alpha in suppression of angiogenic factors such as vascular endothelial growth factor(VEGF) and increase in expression of anti-angiogenic factors like soluble fms like tyrosine kinase(sFlt-1) ,causing hypoxia and vascular defects in placenta has been studied by **Kanasaki et al**[3] The effect of this over-expression of sFlt-1 due to decrease in 2-ME levels which is also associated with increase in HIF-1 alpha ,was also reported by **Miriam peretgal et al**[5] study. The study showed that 2-ME serum levels was significantly negatively associated with sFlt-1(p=0.006) and was significantly and positively associated with angiogenic factors(p=0.005).

CONCLUSION

Our study proved that Mid trimester serum levels of 2-ME was found to be significantly negatively correlated with the severity of hypertensive disorder of pregnancy. The mid trimester serum levels of 2-ME was significantly reduced in patients with severe pre eclampsia when compared to patients with pre eclampsia and gestational hypertension

respectively. We also provided clinical evidence that the levels of 2ME correlate negatively and significantly with the systolic and diastolic blood pressures of those suffering from preeclampsia. 2ME may not only be used as a prognostic and predictive marker but also a therapeutic agent for the patients of preeclampsia, but further studies and clinical trials involving larger population are needed to study the same.

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