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Role of Alphafeto Protein, Beta Human Chorionic Gonadotropin and Unconjugated Estriol as Predictor of Preeclampsia.

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ABSTRACT:

Background: Pre-eclampsia remains a major cause of prenatal morbidity and mortality worldwide. Cause of pre-eclampsia is still ill defined and there is no appropriate test for predicting occurrence of the disorder.This study aimed to assess association between pre-eclampsia and serum levels of alphafeto protein (AFP), β-human chorionic gonadotropin (β-hCG) , and unconjugated estriol (uE_3 or free estriol). **Method:** The study carried out on 500 pregnant women admitted to R.N.T. Medical college ,Udaipur.Subjects were divided into 3 groups normotensive pregnancies,mild preeclampsia and severe preeclampcia .The level of β-hCG ,AFP and unconjugated estriol were measured using Enzyme-linked lmmunosorbent Assay (ELISA) method and results were analyzed statistically using SPSS softwere. **Results:** The Mean ± SD levels of serum urea, creatinine, uric acid, AFP and β-HCG were found to be significantly increased and unconjugated estriol was found significantly decreased in mild & severe ($P<0.001$) pre-eclamptic women ,as compared to normotensive controls.**Conclusion :** The population-specific median values for the three biomarkers (AFP, β- HCG, uE3) may be used as reference values during prenatal screening in pregnant women.

Keywords: AFP,β-HCG, uE_3, pre-eclampsia

INTRODUCTION: Pre-eclampsia is a multisystem disorder of unknown etiology with hypertension, proteinuria and/or edema which predisposes to potentially lethal complications such as eclampsia, abruption- placentae, acute renal failure, cerebral hemorrhage and circulatory collapse. Approximately 7 to 10% of all pregnancies are complicated by hypertensive disease, 70% of which are gestational hypertension – pre-eclampsia related and 30% are due to chronic hypertension (sibai, 2010). Pre-eclamsia is defined as a systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg on 2 occasions at least 4 hrs apart after 20 weeks gestation in women with a previously normal blood pressure or ≥ 160 mm Hg systolic or ≥ 110 mm Hg diastolic, confirmed with in a short interval (minutes) to facilitate timely antihypertensive therapy and proteinuria ≥ 300 mg / 24 hrs or a protein / creatinine ratio ≥ 0.3 mg / dl or a dipstick reading of ≥ 1+. In the absence of proteinuria, pre-eclamsia is diagnosed as new – onset hypertension with the new onset of any the following

: thrombocytopenia, renal insufficiency, or cerebral or visual systoms. (ACOG, 2013) Alfa – fetoprotein is a glycoprotein produced by the fetal liver and gastrointestinal tract. Its level is raised due to functional alteration of trophoblastic cells, leading to increased leakeage, as trophoblastic dysfunction is the primary problem in pre-eclamsia (Dayal M, 2011). It has been suggested that maternal serum alpha – fetoprotein (MSAFP) screening, apart from identifying fetuses with open neural tube defects and chromosomal abnormalities, could also identify pregnancies at high risk of adverse outcomes (seppala M, 1973). The human chorionic gonadotropin (hCG) is a glycoprotein composed of two non – covalently linked subunits, α and β, and is produced by syncytiotrophoblast cells of the placenta. Maternal serum hCG peaks at 8 – 10 wk of gestation and then declines to reach a plateau at 18 – 20 wk of gestation. The free β - subunit can derive from three sources, namely, direct trophoblast cell production, dissociation of hCG into free α and free β – subunits, and by macrophage or neutrophil enzymes nicking the hCG molecule (cole LA et al 1993). The free β – hCG circulating in maternal serum corresponds to only about 0.3 – 4% of the total hCG (spencer K 1991).The normal palcenta differentiates during pregnancy with the cytotrophoblast dominant in early gestation and the syncytotrophoblast dominant in late pregnancy. Placental vascular damage leading to decreased oxygen supply might result in increased hCG production by hyperplasic cytotrophoblastic cells (Majumdar S et al 2005). Although the placenta is the source of estriol, this hormone may reflect fetal steroid genesis. The fetal adrenal glands produce dehydroepiandrosterone sulfate (DHEA-S), which is hydroxylated by the fetal liver into 16—hydroxy-DHEA-S. The latter is transported to the placenta where it undergoes desulfation by steroid sulfatase and is finally aromatized to estriol **(Newby D et al 2000)**. In the early phase of pregnancy, fetal adrenal DHEA-S production is independent of fetal ACTH, but in the second trimester ACTH is required for adrenal function. Henceforth, 90% of the estriol production originates from DHEA-S synthesized by the fetal adrenal glands. In fact, unlike total estriol, unconjugated estriol is produced almost entirely by the fetal-placental unit and therefore is a more sensitive indicator of fetal health. The aim of this present study was to find out the role of AFP , β – hCG and unconjugated estriol in pathogenesis of pre-eclamsia and its association with severity of pre-eclamsia.

MATERIAL AND METHODS: The present study was conducted at the Department of obstetrics and gynecology, R.N.T. Medical College, Udaipur, after taking approval from ethical committe . The prospective randomized study was conduction on 500 pregnant women of gestational age between 12-24 weeks with singleton pregnancy. Patients with chronic hypertension, twin pregnancy, molar pregnancy, chromosomally abnormal fetus, diabetes, chronic renal diseases, autoimmune

disorders,throbophelias, familyhistory of diabetes mellitus, cardiovascular diseases were excluded from the study. A part from routine hematological investigations, estimation of AFP , β – hCG and unconjugated estriol_levels in maternal serum were done by ELISA technique. Blood samples were collected with all aseptic precautions. Preeclampsia was considered as defined by American college of Obstetrics and Gynecologists (ACOG, 2013) the systolic blood pressure $\geq$ 140 mm Hg or $\geq$ 90 mm Hg diastolic on two occasions at least 4 hrs apart after 20 wks gestation in women with a preciously normal blood pressure. Severe preeclampsia is defined by the systolic blood pressure $\geq$ 160 mm Hg or diastolic $\geq$ 110 mm Hg on 2 occasions 4 hours or more apart while the patient is bed rest. Statistical analyses were performed with SPSS software. The differences of pregnancy out comes among the control, mild pre–eclampsia, and severe pre–eclampsia groups were carried out with ANOVA, student's t-test. * p-value <0.05 is significant.

RESULTS:

Table 1: Demographic characteristics of normal pregnancy and pre- eclampsia cases :

Sr. No.	Parameters	Normal Pregnancy (n=250)	Mild Pre-eclampsia (n=200)	Severe Pre-eclampsia (n=50)	P- Value
1.	Means gestational age (weeks)	20.2 ± 2.25	22.42 ± 3.25	21.3 ± 2.9	p> 0.05
2.	Mean maternal age (years)	20.58 ± 2.3	23.2 ± 3.1	21.8 ± 2.9	p> 0.050
3.	Mean systolic blood pressure (mm Hg)	114.25 ± 7.42	156.24± 7.90	183.86 ± 8.24	p< 0.001
4.	Mean diastolic blood pressure (mm Hg)	76.61 ± 8.67	99.51 ± 4.87	113.06 ± 5.11	p< 0.001

Table 2: Laboratory data of normal pregnancy, Mild and severe pre-eclampsia :

Sr. No.	Parameters	Normal Pregnancy (n=250)	Mild Pre - eclampsia (n=200)	Severe Pre-eclampsia (n=50)	P- Value
1.	Urea (mg/dl)	15.50 ± 2.59	24.52 ± 3.99	35.46 ± 4.94	p< 0.001
2.	Creatinine (mg/dl)	0.74 ± 0.14	0.83 ± 0.07	1.46 ± 0.27	p< 0.001
3.	Uric acid (mg/dl)	4.85 ± 1.31	5.83 ± 1.00	7.60 ± 0.77	p< 0.001
4.	AFP (ng / ml)	52.50± 15.52	116.41± 7.92	151.04 ± 7.2	p< 0.001
5.	Beta HCG (mIU / ml)	8091.44 ± 1493.68	15850.26 ± 789.53	19791.70 ± 987.02	p< 0.001
6.	uE_3(ng / ml)	10.78 ± 1.43	7.02 ± 1.87	5.42 ± 1.81	p< 0.001

Mild pre-eclampsia cases of those who showed ≥ 140mmHg systolic or ≥ 90mmHg diastolic one 2 occasions at least 4 hrs apart after 20wks gestation in women with a previously normal blood pressure . Severe pre-eclampsia cases of those who showed ≥ 160 mmHg systolic or ≥ 110mmHg diastolic ,on 2 occasions 4 hours or more apart while the patients is an bed rest(ACOG,2013). Out of 250 pre eclampsia patients ,200 were mild pre-eclampsia and 50 were severe preeclampsia. Table:1 illustrates the Mean ± SD levels of Systolic and diastolic blood pressure were significantly increased in mild and severe (P<0.001) pre-eclampsia women ,when compared with normotensive . Table-2 shows, the Mean ± SD levels of serum urea, creatinine, uric acid, AFP and β-HCG were found to be significantly increased and unconjugated estriol was found significantly decreased in mild & severe (P<0.001) pre-eclamptic women ,as compared to normotensive controls.

DISCUSSION : In preeclampsia the rise of blood pressure is due to vasoconstriction and impaired angiogenesis leading to hypoxia and hyperplasia of trophoblastic cells which causes hypersecretion of placental hormone ultimately leading to high level of circulating β– hCG. In this study, we found that serum β– hCG levels were significantly elevated in severe preclampsia, compared with the centrols. This finding indicates that an abnormal secretory function exists in patients with severe preeclampsia. In preeclampsia, placental pathologic examination reveals focal cellular necrosis in the syncytiotrophoblast and increased mitotic activity with cellular proliferation in the cytotrophoblast (Jones CJP 1980). In addition, the proliferating cytotrophoblast in severe pre–eclampsia is rapidly transformed into syncytio- trophoblast with in 72 hours (Hoshina, 1982). The normal placenta differentiates during pregnancy with the cytotrophoblast dominate in late pregnancy (Enders AC 1965). It is well known that the cytotrophoblast is an undifferentiated stem cell and the syncytiotrophoblast is a differentiated trophoblast transformed from the cytotrophoblast (Kliman HJ, 1987). In 1934, Smith et al talked about increasing hCG levels in severe preeclampsia for the first time. Luckas M (1998), Benn PA (1996) & Ashour AM (1997) indicate that an unexplained elevation of serum hCG significantly correlated with the occurrence of preeclampsia. By contract pouta et al and Aguilina et al demonstrated no relation between levels of serum hCG and severity of pre – eclampsia .Stamilio et al also found no association between severe preeclampsia and elevated second trimester hCG levels. Alpha – feto protein (AFP) is produced in the fetal liver and yalksac, and secreted into the fetal circulation and amniotic fluid, passed into the maternal circulation via the placenta and its concentration is 100 fold increase in the first trimester of pregnancy compared with non pregnant women. In our study, unexplained high levels of MSAFP have been associated with pre – eclampsia. Our findings are consistent with the study by Tikkanen et al (2007),

Waller et al (1996) and Willaims et al (1992) about the correlation of pre – eclampsia and MSAFP, while Khoo's study (1978) showed, in preeclampsia women; significantly lower mean AFP values were obtained. Raly et al also found the AFP values in the severe pre – eclampsia group differed significantly from all other groups. Brazerol et al (1999) reported that the explanation for the association between elevated maternal serum alphafeto protein and adverse pregnancy outcome is not clear, but is probably a marker of placental dysfunction, including partial placental abruption, feto maternal bleeding and abnormal implantation. Although the placenta is the source of estriol, this hormone may reflect fetal steroidogenesis. The fetal adrenal glands produce dehydroepiandrosterone sulfate [DHEA-S], which is hydroxylated by the fetal liver into 16—hydroxy-DHEA-S. The latter is transported to the placenta where it undergoes desulfation by steroid sulfatase and is finally aromatized to estriol. In the early phase of pregnancy, fetal adrenal DHEA-S production is independent of fetal ACTH, but in the second trimester ACTH is required for adrenal function. Henceforth, 90% of the estriol production originates from DHEA-S synthesized by the fetal adrenal glands. In fact, unlike total estriol, unconjugated estriol is produced almost entirely by the fetal placental unit and therefore is a more sensitive indicator of fetal health. Unconjugated estriol is a variable independent of maternal age and therefore it can be used alone or in combination with maternal age for the determination of the relative risk of Down's syndrome or Edward's syndrome.In a routine screening programme, maternal serum unconjugated estriol has poor predictive power if used as a single marker, but its inclusion contributes to improving the predictive value of age and alpha feto-protein.

CONCLUSION: The new markers (AFP,β-hCG, uE3) provide an opportunity to study the early natural history of disease and possibly to conduct treatment trails. The present study confirmed the elevated levels of AFP, β-hCG and low level of unconjugated estriol are associated with pre-eclampsia.

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