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Role Of Heat Stable Fraction Of Alkaline Phosphatase , Lipid Peroxidation, And Uric Acid In The Early Detection Of Pre-Eclampsia

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Abstract: *In many areas of world, hypertensive disease in pregnancy is the single most common cause of maternal death. Pregnancy associated hypertension remains unsolved despite decades of intensive research and remains the most significant problem in obstetrics. Though pre-eclampsia has been recognized clinically, its etiology and pathophysiology remains enigmatic. Early detection of pre-eclampsia using pregnancy associated parameters is beneficial to reduce the number of morbidity and mortality. The role of placental alkaline phosphatase(heat stable fraction of alkaline phosphatase HSALP), uric acid and malondialdehyde (MDA), a marker of oxidative stress, were evaluated in early pregnancy in both primigravidae and multigravidae. The parameters were determined using standardized methods.The activity of placental alkaline posphatase was significantly decreased (P<0.001) in pre-eclampsia developed individuals when compared to individuals not developed pre-eclampsia.MDA levels were significantly (P<0.001) elevated in individuals developed pre-eclampsia in both primigravidae and multigravidae individuals. How ever the uric acid showed no significant decrease between pre-eclampsia developed individuals when compared to individuals not developed pre-eclampsia The results of the study suggested that placental ALP activity and MDA levels can be used as early markers for predicting pre-eclampsia later in pregnancy.***

Key words: *Hypertension; Multigravidae; Oxidative stress; Pre-eclampsia; Primigravidae;*

I. Introduction

In modern obstetrics, hypertensive disorders of pregnancy encompass a clinical spectrum of abnormalities ranging from minimal elevation in blood pressure to severe hypertension with multi organ dysfunction [1]. In many areas of world, hypertensive disease in pregnancy is a single most common cause of maternal death [2]. Major maternal hazards from the consequences of severe hypertension are grandmal seizures and damage to end organs [1]. How pregnancy incites or aggravates hypertension remains to be unsolved despite decades of intensive research and hypertensive disorders remain among the most significant unresolved problem in obstetrics [2].

Pre-eclampsia is a pregnancy specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation [2]. Proteinuria is an important sign of pre-eclampsia and the diagnosis is questionable in its absence [2]. Cardiac dysfunction, thrombocytopenia, hepatocellular necrosis and faulty placentation were detected in pre-eclampsia [2]. Based on this, investigators have attempted to identify early markers of faulty placentation, reduced placental perfusion, endothelial cell dysfunction, and activation of coagulation. Based on these an ideal screening test can be made out for the purpose of predicting pre-eclampsia. An effective predictive measure that would allow careful surveillance of mothers at high risk for the pre-eclampsia to reduce the risk of complications.

Placenta is clearly involved in the pathophysiologic mechanism of pre-eclampsia. Chorionic gonadotropin is the predominant placental hormone which has peak in the late first trimester and early second trimester when definite placental development occurs many markers of impending pre-eclampsia appears. Placental alkaline phosphatase is an isoenzyme of alkaline phoshatase which originates from syncytiotrophoblast. Serum level is positively correlated with enzyme content in placenta. Enzyme levels reflect the weight of fetoplacental unit.

Reactive oxygen species (ROS) have been implicated in the pathophysiology of a number of human ailments [3]. Free radicals promoted vascular malfunction has been reported to be a basic pathophysiology of pre-eclampsia [1]. Reactive oxygen species, particularly superoxide anions, evoke endothelial cells activation through many pathways. In the current study we evaluated the role of oxidative stress, placental alkaline phosphatase, and uric acid in early pregnancy in order to prove the predictive efficacy of these parameters in development of pre- eclampsia in late pregnancy.

II. Material And Methods

2.1 Selection Of Patients

The subjects were selected from outpatients attending the antenatal out patient of Obstetrics and Gynaecology department. The study was conducted with the permission of Institutional Human Ethics Committee. Subjects enrolled for the study were primigravidae and multigravidaeat 16-20 weeks of gestation with singleton pregnancy without any complication.Patients with complications like renal disease, heart disease, diabetes and epilepsy are excluded from the study.

Blood samples were collected by venous puncture using disposable syringes. Serum was separated by centrifugation at 3000 revolutions per minute for 15 minutes and stored in air tight containers at 4^0C until use. All tests were done within 3 days of collection of serum.

2.2 Determination Of Serum Parameters

Serum heat stable ALP activities were determined by the method of Curzen and Morris [6,10], after incubating serum at 65^0c for 30 min. The estimation was done with the help of a semi-automatic analyzer ERBA CHEM–5 (semiautomated biochemistry analyzer of Transasia) Malondialdehyde (MDA) was determined by reacting with thiobarbituric acid [7]. Uric acid was estimated according to uricase-peroxidase method [8]. All the patients were followed up till delivery whether they develop preeclampsia or not.

2.3 Statistical Analysis

Baseline parameters were compared using unpaired t test**.** Significant factors were chosen for prediction of preeclampsia by noting the mean values of parameters and significance was determined using unpaired t test .P value of <0.05 was considered significant. .Prediction modeling to predict pre-eclampsia was noted by using logistic regression technique

III. Results And Discussion

The frequency of pre-eclampsia development in the primigravidae and multigravidae individuals was given in table 1. Of the 52 primigravide women analysed, 10 developed pre-eclampsia and of the 48 multigravide women with past history of PIH or IUGdeveloped pre-eclampsia.

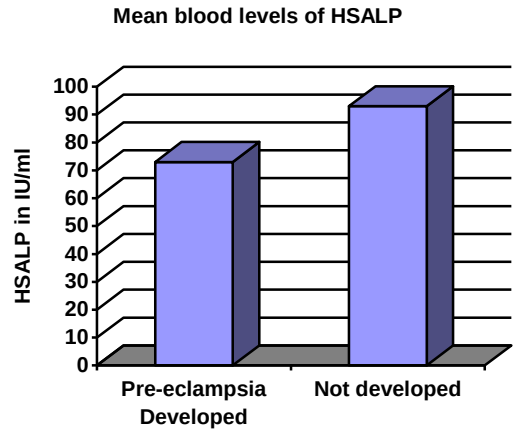
Table 1 : Pre-eclampsia developed or not

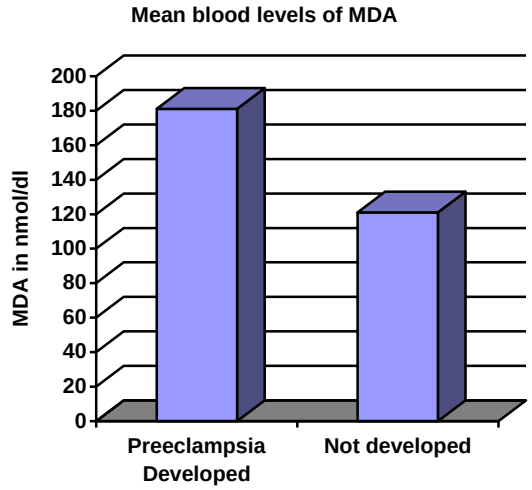
Pre-eclampsia developed or not	Frequency	Percentage
Developed n=19		
Primi	10	52.6
Multi	09	47.4
Not Developed n=81		
Primi	42	51.9
Multi	39	48.1

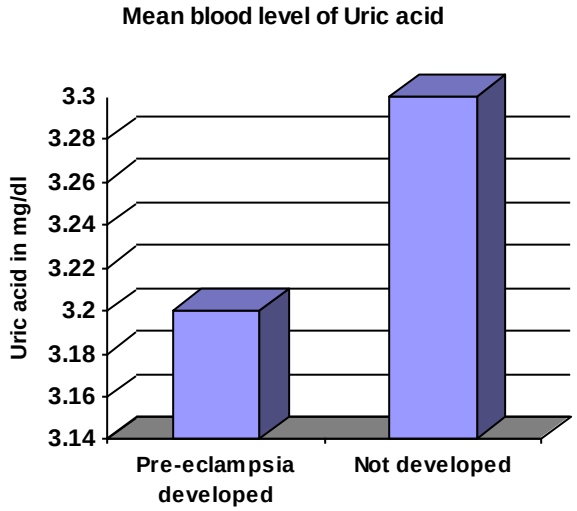
The levels of serum MDA, and uric acid and the activities of HSALP are given in table 2 All the serum parameters placental alkaline phosphatase and malondialdehyde analyzed in the serum of individuals who later developed pre-eclampsia showed statistically significant difference between these parameters than those who not developed pre-eclampsia. There was no statistically significant difference in uric acid levels between these two groups.

Testing the difference in the mean values using t test

	Those who developed pre-eclampsia	Those who not developed pre-eclampsia	t value & degree of freedom	P value
MDA	181.79 $\pm$ 39.75	121.61 $\pm$ 33.09	8.862 & 98	0.001*
HsALP	73.41 $\pm$ 23.39	93.85 $\pm$ 17.68	4.252 & 98	0.001*
Uric acid	3.267 $\pm$ 0.964	3.355 + 1.096	0.321 & 98	0.749 $^-$







Difference in mean values in those who developed pre-eclampsia and those who did not develop pre-eclampsia were found to be statistically significant for β hCG , MDA and HSALP . No significant difference was observed in uric acid values . Prediction modeling to predict pre-eclampsia using the significant factors using the logistic regression technique.

Omnibus tests of model coefficients

	Chi-square	df	Sig
Model	76.919	3	0.001

Model summary

-2 log likelihood	Cox & Snell R square
20.325	0.537

Variables in the equation

	B	SE	Sig	df	Odds ratio
β-hCG	000	000	0.002	1	1.000
MDA	0.078	0.030	0.009	1	1.081
HsALP	0.064	0.03	0.04	1	0.938

So there is 53.7% of the variability in the prevalence of pre-eclampsia can be explained by using the two parameters namely placental ALP and MDA together estimated during 16-20 weeks of gestation.

IV. Discussion

The present study was conducted to assess the predictive efficacy of various parameters in development of pre-eclampsia by noting the level of malondialdehyde (lipid peroxidation marker), placental alkaline phosphatase and uric acid early in pregnancy and noting development of pre-eclampsia later in pregnancy.

In this study MDA levels are significantly increased in those who developed pre-eclampsia where as levels were slightly increased in those those who do not developed pre-eclampsia. This is because pregnancy is a stressful condition with altered physiological and metabolic functions which are much more exaggerated in pre-eclampsia. Recent studies evaluating the pathological mechanism of eclampsia have suggested that free radicals could be likely promoters of vascular malfunction in pre-eclampsia excessive lipid peroxidation, reduced antioxidant activity, red blood cell dysfunction all collectively lead to excess generation of free radicals [4]. This in turn increases oxidative stress to foetus and causes damage to endothelium. In this process endothelium derived relaxing factor and prostacyclin synthase get inactivated [4]. The end results of these changes are smooth muscle contraction, platelet aggregation and vasospasm and finally pregnancy induced hypertension. The pre-eclamptic placental cells are found to have more capacity for reactive oxygen species generation due to the over expressed of xanthine oxidase activity [2]. The generated free radical initiated the self-perpetuating chain reaction, lipid peroxidation. The increased tumor necrosis factor (TNF-α) in pre-eclamptic placenta could also stimulate monocytes which, in turn produces excess reactive oxygen species generation leading to oxidative stress [3].

Heat stable fraction of alkaline phosphatase levels were significantly decreased in those who developed pre-eclampsia when compared to those who remained normotensive. Low levels seem to reflect low weight of fetoplacental unit [11]. Several studies had suggested the association between increased heat stable alkaline phosphatase levels and pre-eclampsia [13] Human placental alkaline phosphatase is an enzyme synthesized in the placenta during pregnancy and is also expressed by cancerous tissues in many patients. The enzyme originates from syncytiotrophoblast and the serum level is positively correlated with enzyme content in the placenta[29]. Various studies document the numerous roles played by this single enzyme which include DNA synthesis, transfer of IgG molecules from maternal to foetal circulation, tumour marker identification and monitoring of disease status .The function of phosphatase is unknown but it is reasonable to believe that this membrane associated enzyme participates in membrane transport mechanisms that might be of vital importance for placental metabolism and thus for the foetus[30].

Several studies suggest a possible association between increased heat stable ALP levels and pre-eclampsia [15,16,17,21] placental insufficiency and low birth weight [18,19,20,21] .

Uric acid levels at 16-20 weeks of gestation shows no significant change between those who developed pre-eclampsia when compared to those who did not develop pre-eclampsia. It is unlikely that uric acid levels will prove very useful in predicting development of pre-eclampsia in late pregnancy, the results of the present study have not proven to be useful in differentiating established gestational hypertension from pre- eclampsia [2]. Pre-eclampsia has been recognized clinically since the time of Hippocrates. The toxaemia theory which proposes that the compromised placenta produces substances leading to the maternal syndrome of pre-eclampsia, remains the favored hypothesis [12]. Trophoblast invasion is defective in pre-eclampsia and the uteroplacental circulation remains in a state of high resistance [14]. Persistent placental under perfusion is thought to stimulate the release of pre-eclamptic factors that on gaining access to the maternal circulation lead to vascular dysfunction.

V. Conclusion

The current study concluded that the parameters which indicate faulty placentation (placental alkaline phosphatase) and the parameters of oxidative stress (Malondialdehyde) can be used as early markers for predicting pre-eclampsia developing later in pregnancy.

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