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*RESEARCH ARTICLE*

# MATERNAL OXIDANT-ANTIOXIDANT HOMEOSTASIS DURING NORMOTENSIVE AND PRE-ECLAMPTIC PREGNANCY.

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## *Abstract*

**Objectives:** To evaluate changes in oxidant milieu in normotensive pregnant women (No PE) and those who developed pre-eclampsia (PE) and to define a relation between these changes and PE development.

Patients & Methods: All pregnant women gave three blood samples at 12th week (S1) gestational age, start of 3rd trimester (S2) and 48-hr postpartum (S3) for spectrophotometeric estimation of serum levels of malondialdehyde (MDA) and uric acid (UA), and activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase activity. Control group included a similar number of non-pregnant women. Blood pressures were estimated at 1st visit and monthly for diagnosis of PE.

**Results:** 65 pregnant women developed PE; 54 had mild and 11 had severe PE; while 19 women developed early and 46 late PE. Sample S1 levels showed non-significant differences between all study participants. S2 serum MDA levels were significantly higher in pregnant women than their S1 and control levels and in PE versus No PE women. S2 serum UA levels were significantly higher in PE women than their S1, control and corresponding levels in No PE women. S2 serum SOD and GPx activity was significantly lower in PE women than their S1, control and corresponding measures in No PE women. Serum GR and catalase activities were significantly lower in S2 samples of pregnant women than their S1 and control activities with significantly lower activity in No PE women. S3 serum levels of MDA and UA were decreased and activities of antioxidant enzymes were adjusted in S3 sample than in S2 sample, but with significant difference between PE and No PE women. Statistical analysis defined serum MDA at ≥13.2±0.128 µmole/L and GPx activity at ≥450 U/L as risk cutoff point for PE development.

**Conclusion:** Pregnancy is stressful condition associated with activation of oxidative stress (OS) and overproduction of oxidative products and disturbed activity of antioxidant enzymes. Exaggerated OS compromises placental functions and induces development of hypertensive manifestations. Elevated serum MDA and GPx enzyme

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activity could predict the progression for development of PE especially the late-onset type.



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## Introduction:-

Pregnancy is a unique physiological process that involves intricate interplay of inflammatory and anti-inflammatory milieu, hormonal changes, and cellular and molecular events at the maternal-fetal interface [1]. Pre-eclampsia (PE) is a pregnancy-specific disorder [2] characterized by new onset of maternal hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman [3]. PE is a multisystem disorder involves altered homeostasis of oxidants-antioxidants, inflammatory process and endothelial dysfunction [4]

During the physiological process of oxidation of organic materials by molecular oxygen to produce energy, a number of reactive oxygen species (ROS) are formed [5]. Normally, ROS are neutralized by a broad spectrum of naturally occurring protective antioxidants, such as superoxide dismutase (SOD), so as to prevent oxidative stress (OS) [6] that implies an imbalance between ROS production and cellular and tissue antioxidant defense system [7]. OS leads to damage all cellular components and modifies their physiological functions [8].

Oxidative stress is a common feature of normal pregnancy, but persistent, overwhelming OS leads to consumption and decline of antioxidants, affecting placental antioxidant capacity with subsequent damage of placental tissue components [9]; thus inducing a form of accelerated placental ageing [10]. In addition, ROS could stimulate platelet adhesion and aggregation leading to intravascular coagulopathy with subsequent placental infarction and impairment of the uteroplacental blood flow [11]. OS also plays an important immuno-regulatory role during pregnancy [12]. Moreover, the balance between the protective and destructive mechanisms of placental autophagy and apoptosis are often influenced by OS, and the proper interactions between them play an important role in placental homeostasis [13]. OS, immune disturbances and OS-induced imbalance between autophagy and apoptosis seem to be linked with adverse pregnancy outcomes such as spontaneous abortion, PE and intrauterine growth restriction [6].

**Objectives:-**

This study aimed to evaluate the changes in oxidant milieu in normotensive pregnant women and those who developed PE so as to define a relation between these changes and development of PE.

**Design:-**

Prospective comparative clinical multi-center study

**Setting:-**

Benha and Tanta University Hospitals

**Patients & Methods:-**

After approval of the study protocol by the Local Ethical Committee, all primigravida, attended the outpatient clinic for assurance of diagnosis of being pregnant, were eligible for evaluation for inclusion and exclusion criteria. Primigravida fulfilled the inclusion criteria, signed a written fully informed consent for study inclusion and underwent complete clinical and gynecological examination and GA was calculated since the 1st day of the last menstrual period and confirmed by crown–rump length measurement on US examination.

Exclusion criteria included multiple pregnancy, fetal abnormalities, pre-conception diabetes, essential hypertension, renal, hepatic or cardiac diseases. Smokers, alcoholics, exposed to free radicals at work places or livening communities or had diseases inducing oxidant stresses were excluded form the study.

**Preliminary and follow-up protocol and grouping:-**

1. Preliminary examination conducted at 1st visit included estimation of systolic and diastolic blood pressures (SBP & DBP) to assure for being normotensive for all study attendants.
2. Throughout the antenatal visits, all women underwent estimation of SBP and DBP and those developed hypertensive manifestations suggestive of PE were grouped as PE group. PE was diagnosed by the development of gestational hypertension after the 12th week GA in women who were normotensive at time of 1st antenatal visit with SBP ≥140 mmHg and/or DBP ≥90 mmHg on at least two occasions, 4 hours apart, and proteinuria

(one dipstick measurement ≥2+ on a voided random urine sample) [14, 15]. PE women were categorized as Early PE if they were diagnosed around the 20th week GA and as Late PE if diagnosed later to the 20th week GA. Also, PE was categorized according to its severity as Severe PE if SBP was >160 mmHg, DBP was >110 mmHg, and proteinuria was >5 g/in a 24-h period, but if not it was considered as mild PE [16].

3. Women who were maintained as normotensive till delivery was grouped as No PE group.
4. The study also included cross-matched number of non-pregnant women as control group, for laboratory parameters and were collected from those attending the family planning unit, fulfilled the inclusion and exclusion criteria for the study and accepted to give a blood sample for estimation of study parameters.

**Blood sampling:-**

1. For all pregnant women, three blood samples were obtained, at 12th (S1) and start of the 3rd trimester (S2) and 48-hr postpartum (S3). For control women, only one blood sample was obtained.
2. All study participants gave 5 ml blood sample that was withdrawn under complete aseptic conditions, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in sterile Eppindorff tube and stores at -80°C till be assayed.

**Laboratory investigations:-**

All study parameters were estimated using spectrophotometer for estimation of serum levels of:

1. Malondialdehyde (MDA) which reacts with thiobarbituric acid to form a pink colored complex; serum MDA level was estimated by measuring absorbance at 535 nm [17].
2. Uric acid is converted by uricase to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidases, oxidizes 3,5-dichloro-2 hydroxy benzene sulphonic acid and 4 amino phenazone to form red violet quinine imine; serum uric acid level was determined by measuring absorbance at 505 nm [18].
3. Superoxide dismutase (SOD) activity was measured based on the inhibition of the formation of phenazenemethosulphate-Nitro blue tetrazolium formazon complex. The color formed at the end of the reaction can be extracted into butanol and measured at 560 nm [19].
4. Glutathione peroxidase (GPx) catalyzes the oxidation of reduced glutathione by hydrogen peroxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione is converted to the reduced form with simultaneous oxidation of NADPH measured as decrease in absorbance at 340 nm [20].
5. Catalase activity was determined depending on the fact that catalase rapidly breaks down hydrogen peroxide leading to decrease in absorbance, so the difference in the absorbance at 240 nm per minute is considered as a measure of catalase activity [21].

**Statistical analysis:-**

Obtained data were presented as mean±SD, numbers and percentages. Results were analyzed using paired t-test and Chi-square test ($X^2$ test). Possible relationships were investigated using Spearman's linear regression. Sensitivity & specificity of estimated parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

## Results:-

Throughout the study duration, 81 women developed PE, 16 were excluded for not fulfilling the study inclusion criteria and 65 women were enrolled as PE group; 54 women developed mild and 11 women developed severe PE; while 19 women developed early and 46 developed late PE. Another 65 pregnant women of cross-matched age and completed their pregnancy free of PE were collected as No PE group. There was non-significant (p>0.05) difference between both groups as regards enrolment data determined at 12th week GA as shown in table 1 with non-significant difference versus the control group (Table 1). At time of development of PE, both SBP and DBP were significantly higher in women of PE group compared to women of No PE and control group (Fig. 1).

**Table 1:-**Baseline data determined at the 12th week GA

| Data | | Control (n=65) | No PE (n=65) | PE (n=65) | P value |
|---|---|---|---|---|---|
| Age (years) | | 26.3±4.8 | 26.2±6.1 | 26.1±5.4 | 0.978 |
| BMI data | Weight (kg) | 73.3±6.5 | 75.8±6.8 | 75.3±7.6 | 0.098 |
| | Height (cm) | 170±2 | 169.4±2.5 | 169.8±2.4 | 0.338 |
| | BMI ($kg/m^2$) | 25.4±2.5 | 26.5±2.4 | 26.1±2.8 | 0.059 |
| Blood pressure measures (mmHg) | Systolic | 118.2±3.8 | 118±4.6 | 118.4±3.7 | 0.863 |
| | Diastolic | 82.5±4.3 | 83.6±3.1 | 82.8±3.9 | 0.227 |
| Proteinuria‡ | No | 59 (90.8%) | 60 (92.3%) | 58 (89.2%) | 0.832 |
| | Present (+) | 6 (9.2%) | 5 (7.7%) | 7 (10.8%) | |

Data are presented as mean±SD & numbers; percentages are in parenthesis; PE: Pre-eclampsia; BMI: Body mass

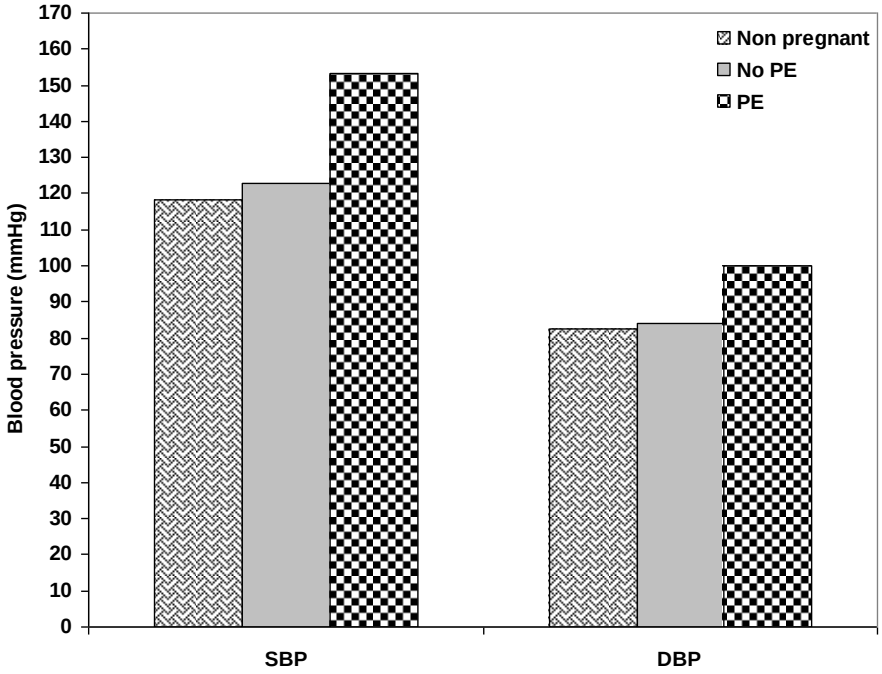


**Fig. (1): Mean SBP and DBP at time of development of PE in the three groups**

In S1 sample (at the 12th wk GA), there were non-significant differences between pregnant and control (non-pregnant) women and between normotensive (No PE) and PE pregnant women as regards serum levels of oxidation products; MDA and UA and estimated activities of antioxidant enzymes.

In S2 sample (at the start of the 3rd trimester), serum levels of MDA increased significantly in pregnant women compared to their baseline and control levels and in PE versus No PE women. Mean S2 serum UA levels were significantly higher in PE women compared to S1 and control levels and to S2 levels in No PE women, with non-significantly higher S2 levels in No PE compared their S1 and control levels. In No PE women, mean S2 activity of serum SOD was significantly higher compared to S1 and control activity measures and to S2 activity measures in PE women with non-significantly higher activity measures in PE women compared to their S1 and to control activity measures. Estimated activity of GPx enzyme in S2 sample was significantly lower in PE women than their S1 and control activity levels and S2 measure in No PE women, while was significantly higher in S2 than S1 sample of No PE women. On contrary, GR and catalase activities were significantly lower in S2 samples of pregnant women compared to their activity in S1 and that of controls with significantly lower activity in S2 sample of No PE compared to PE women

Interestingly, serum levels of MDA and UA decreased in S3 samples in pregnant women compared to their corresponding levels in S2 despite being still higher than levels in S1 sample and control samples. Similarly, activities of antioxidant enzymes adjusted their levels in S3 sample than in S2 sample. Despite these improvements, in estimated measures of studied parameters, the difference was still significant between PE and No PE women (Table 2).

**Table 2:-**Mean levels of oxidation products and mean activity levels of studied antioxidant enzymes estimated in studied women

| | | Control (n=65) | No PE (n=65) | PE (n=65) | P value |
|---|---|---|---|---|---|
| MDA (µmole/L) | S1 | 4.3±1.54 | 4.2±1.7 | 4.4±1.59 | 0.783 |
| | S2 | | 7.57±4.33*† | 14.22±5.32*† | <0.00001 |
| | S3 | | 5.66±2.4*†‡ | 11.79±2.86*†‡ | <0.00001 |
| Uric acid (mg/ml) | S1 | 3.49±1.03 | 3.55±0.89 | 3.54±0.91 | 0.929 |
| | S2 | | 4±1.87 | 6.64±2.52*† | <0.00001 |
| | S3 | | 3.77±1.56 | 5.72±1.78*†‡ | <0.00001 |
| SOD (U/L) | S1 | 7.36±3.67 | 7.21±2.99 | 7.13±2.85 | 0.918 |
| | S2 | | 11.45±2.21*† | 8.59±4.85 | 0.00003 |
| | S3 | | 9.6±3.25*† | 7.91±1.8 | 0.00035 |
| GPx (U/L) | S1 | 551.3±178.2 | 554.4±178.1 | 523.8±205 | 0.592 |
| | S2 | | 605.2±113.6*† | 483.3±27.2*† | <0.00001 |
| | S3 | | 590.8±141.6 | 671±195.6*†‡ | 0.008 |
| GR (U/L) | S1 | 33.9±12.88 | 32±15.4 | 30.2±15 | 0.673 |
| | S2 | | 13.33±5.63*† | 22.78±13.92*† | <0.00001 |
| | S3 | | 29.55±17‡ | 24.3±14.4*† | 0.098 |
| Catalase (U/L) | S1 | 125.8±58.9 | 127.2±66.8 | 130.3±63.4 | 0.498 |
| | S2 | | 51.86±20.3*† | 83.5±28.7*† | <0.00001 |
| | S3 | | 101.5±51.3*† | 117.5±57.4*† | <0.00001 |

Data are presented as mean±SD; PE: Pre-eclampsia; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GR: glutathione reductase; S1: sample 1 obtained at the $12^{th}$ wk GA, S2: *: indicates significance of difference versus control; †: indicates significance of difference versus $12^{th}$ wk levels; ‡: indicates significance of difference versus control; P value indicates significance of between PE and No PE groups

Spearman's correlation analysis showed that presence of pregnancy and development of PE was significantly associated with high serum levels of MDA and uric acid. Presence of pregnancy showed positive significant association with serum SOD activity, while was negatively associated with activity of serum GR and catalase. Development of PE showed positive significant correlation with activity serum GR and catalase, while showed negative significant association with activity of SOD and GPx (Table 3).

**Table 3:-**Spearman's correlation between presence of pregnancy and development of PE and estimated oxidation products and activity of antioxidant enzyme in studied women

| | Pregnancy | | Development of PE | |
|---|---|---|---|---|
| Serum parameter | Rho | p | Rho | p |
| MDA (µmole/L) | 0.539 | <0.001 | 0.538 | <0.001 |
| Uric acid (mg/ml) | 0.369 | <0.001 | 0.523 | <0.001 |
| SOD (U/L) | 0.307 | <0.001 | -0.249 | 0.004 |
| GPx (U/L) | -0.025 | >0.05 | -0.761 | <0.001 |
| GR (U/L) | -0.529 | <0.001 | 0.398 | <0.001 |
| Catalase (U/L) | -0.552 | <0.001 | 0.514 | <0.001 |

Rho: Spearman's correlation coefficient, PE: Pre-eclampsia; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; GR: glutathione reductase

Kaplan-Meier regression analysis defined serum MDA at ≥13.2±0.128 µmole/L (95% CI: 12.95-13.45) as a risk cutoff point for possibility of development of PE with increasing risk with increased levels (Fig. 2) and defined

serum level of Gpx activity at ≥450 U/L for a possibility of PE development and at ≥593±10.2 U/L (95% CI: 572-613) as a risk cutoff point for sure development of PE (Fig. 3).

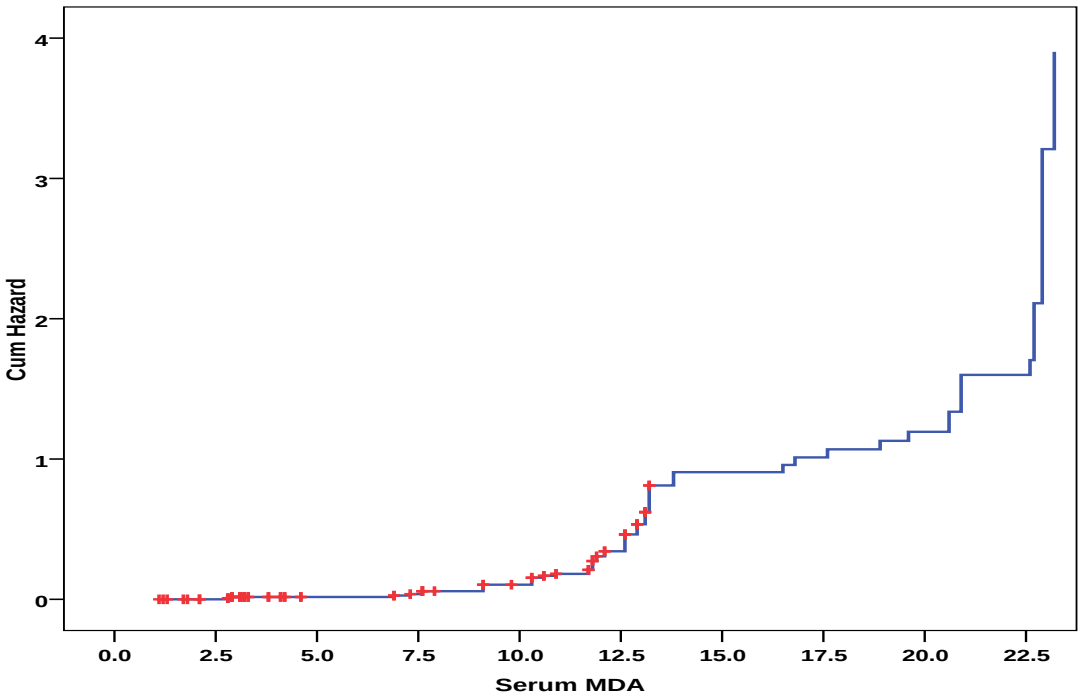


**Fig. 2:**-Kaplan-Meier regression analysis of S2 sample serum MDA defined ≥13.2 µmole/L as a risk cutoff point for possibility of PE development

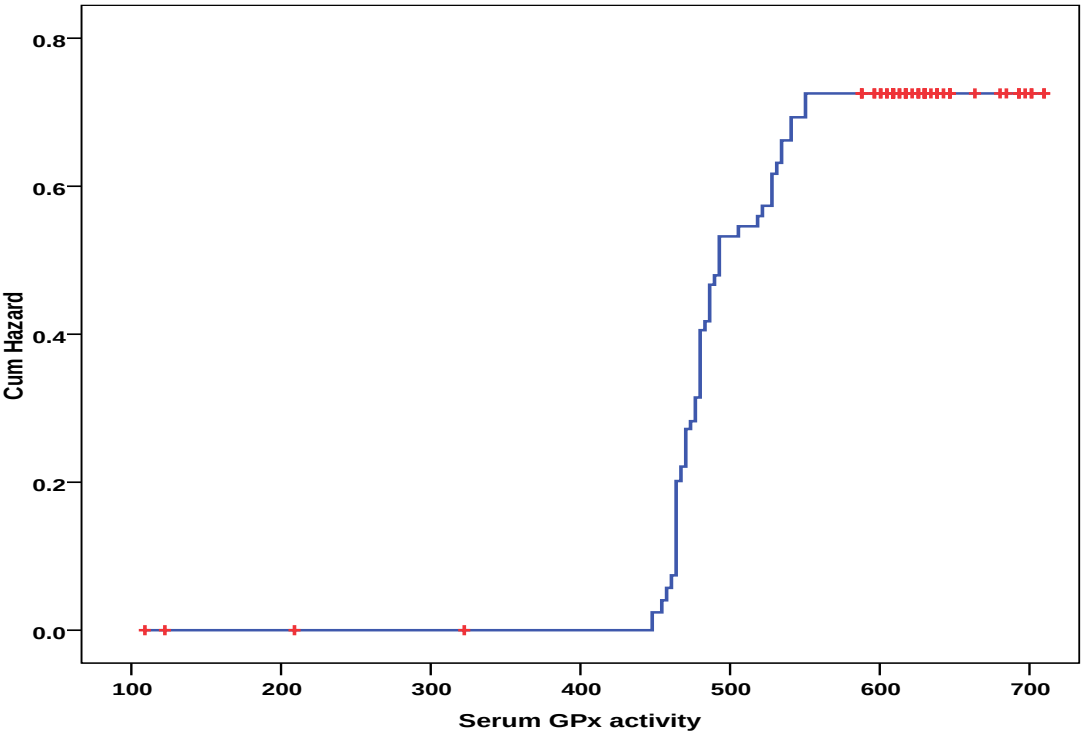


**Fig. 3:**-Kaplan-Meier regression analysis of S2 sample serum Gpx activity at ≥450 U/L for a possibility of PE development and at ≥593±10.2 as a risk cutoff point for sure development of PE

## Discussion:-

The current study detected significantly altered homeostasis of oxidants-antioxidants in pregnant women manifested as significantly higher serum levels of oxidative products; MDA and UA with increased serum activity of oxidant and decreased activity of antioxidant enzymes estimated at the start of the 3rd trimester (S2 sample) compared to control women, a finding indicating a deleterious effect of pregnancy on oxidants-antioxidants homeostasis and compared to levels estimated at the 12th wk GA (S1 sample); a 2nd finding indicating progressive impact of pregnancy on oxidants-antioxidants homeostasis.

Moreover, the reported alterations were more manifest in PE women compared to No PE women, a 3rd finding indicating a relationship between disturbed oxidants-antioxidants homeostasis and placental function with subsequent release of vasopressor mediators that induce elevation of blood pressure and affection of peripheral vascular resistance.

In line with these findings, **Namdev et al.** [22] found oxidative stress was increased among PE women compared to controls as evidenced by increased mean MDA levels and decreased mean total antioxidant status. **Bakacak et al.** [23] reported significantly higher MDA levels in PE women than in hypertensive and normotensive pregnancy and were significantly higher in hypertensive than normotensive pregnancy and **Bernardi et al.** [24] detected significantly lower plasma nitric oxide levels and SOD activity with significantly higher endothelin-1 levels and arginase activity in PE women than controls.

Also, **Elmas et al.** [25] found blood pressure and serum levels of UA, xanthine oxidase activity (XOA) and allantoin levels were higher in PE patients when compared with pregnant controls and **Shastri et al.** [26] reported significantly higher lipid peroxides and lower paraoxonase-1 activity during 1st trimester in pregnant women with high hemoglobin than women with low/normal hemoglobin concentration. The obtained data go in hand with **Pourghassem Gargari et al.** [27] who detected significantly higher serum levels of MDA and significantly lower TAC in women with PE compared to controls.

Thereafter, **Bambrana et al.** [4] reported that in PE women XOA, MDA and UA levels were significantly increased, while total antioxidant status (TAS) and levels of SOD and glutathione reductase (GR) were decreased in PE versus normotensive pregnant women. Recently, **Barneo-Caragol et al.** [28] found serum UA levels were significantly different between non-pregnant and normotensive pregnant women in the 1st, 2nd and 3rd trimesters and increases gradually as the pregnancy progresses.

Interestingly, 46 pregnant women developed late onset PE, this finding on one side and increased serum levels of oxidation products and altered serum activity of oxidant and antioxidant enzymes on the other hand assured the progressive deleterious effect of pregnancy on oxidants-antioxidants homeostasis which surely played a significant role in development of PE. In line with these findings, **Atiba et al.** [29] found plasma MDA level and glutathione peroxidase (GPx) activity were significantly higher and SDO activity was significantly lower in the 3rd trimester of normotensive and PE pregnant women compared to their corresponding levels in the 2nd trimester.

The reported changes were found to be reversed in S3 sample (48-hr postpartum), a finding indicating a placental role for the reported disturbed oxidants-antioxidants homeostasis. Similarly, **Bambrana et al.,** [4] reported reversal of the changes in oxidants-antioxidants homeostasis evinced within 48 h after delivery in PE.

In support of these data, statistical analysis defined a positive significant correlation between pregnancy per si and development of PE and serum levels of oxidation products and decreased activity of antioxidant enzymes that point to either disturbed biosynthesis or simple consumption secondary to increased production of oxygen free radicals that lead to decreased serum activity of SOD and GPx with concomitant increased GR activity. These correlations coincided with **Elmas et al.** [25] who found serum levels of UA, XOA and allantoin levels showed high correlations with blood pressure in cases of PE.

Multiple studies tried to propose explanations for pregnancy-induced disturbed oxidants-antioxidants homeostasis and their role in development of PE; OS could activate maternal endothelial cells as a precursor to PE [30], or cause, conflate, or be the consequence of the altered inflammatory response in PE pregnancies [31]. As another explanation, ROS can cause apoptosis of the syncytiotrophoblast during the placentation process and impair the normal arteriolar remodeling [32]. Thereafter, **Bakacak et al.** [23] detected high serum Cu/Zn ratios in PE than in hypertensive and

normotensive women and documented that these increased ratios may reflect vascular complications of PE, and the ensuing increases in lipid peroxidation may play important pathogenic roles in PE. **Elmas et al.** [25] attributed high serum UA, XOA and allantoin levels to placental cell death because of abnormal trophoblastic activity observed in PE.

Statistical regression analyses defined high serum MDA and serum GPx activity as significant predictors for severe PE. In line with this finding, considering persistently elevated or progressively elevating liver enzymes as a manifestation of severe PE [33], **Atiba et al.** [25, 34] reported positive correlation between serum MDA levels and serum levels of both AST and ALT and concluded that elevated liver enzymes seen in PE women may be due to free radical injury to the liver.

## Conclusion:-

Pregnancy is a stressful condition associated with activation of oxidative stress and overproduction of oxidative products and disturbed activity of antioxidant enzymes. Exaggerated oxidative stress compromises the placental functions and induces development of hypertensive manifestations. Elevated serum MDA and GPx enzyme activity could predict the progression for development of PE especially the late-onset type. However, wider-scale studies are mandatory to establish the proposed cutoff points for prediction of development of PE.

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