Research Article

Correlation between Serum Inflammatory and Oxidative Stress Markers with Blood Pressure in Preeclampsia

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Abstract
Objectives: To evaluate the serum pro-inflammatory cytokines (Tumor necrosis alpha (TNF α)), oxidative stress marker(Malondialdehyde(MDA)) and serum antioxidant biomarker (Superoxide dismutase(SOD)) and study their correlations with blood pressure (BP) in preeclampsia (PE). **Methods:** Fifty pregnant women (18 - 35 years old) were divided into two groups each with 25 pregnant women: preeclampsia group and control group. **Results:** Blood pressure, mean serum TNF α, and MDAwere significantly higher, while, the mean serum value of SOD was significantly lower in the pregnant women with preeclampsia than in the control pregnant women. Also, there was a significant positive correlation between TNF α, MDA and BP. **Conclusion:** Elevated both serum TNF α, and MDA and decreased in SOD are the possible mechanisms involved in the pathogenesis of PE. Early detection of PE allow for planning of appropriate monitoring of PE and for prevention of complications.
Keywords: Preeclampsia, ROS,TNF α, Antioxidants, Oxidative stress.

Introduction
However, oxygen is critical for life of many living organisms; it may also be so harmful. Oxidants formed during oxygen metabolism is called reactive oxygen species (ROS) and include free radicals such as superoxide (O2·-) and hydroxyl radical (·OH) and non-radical hydrogen peroxide (H_2O_2). While ROS helps the immune system to fight pathogens, oxidative damage may cause DNA strand and base damage, alsoalteration of physiological cell signaling [1].

It is important to maintain redox hemostasis; so human body has developed enzymatic and nonenzymatic defense mechanisms to overcome the oxidative state. The major nonenzymatic antioxidants are glutathione, uric acid, and melatonin. Moreover, superoxide dismutase (SOD), catalase, glutathione peroxidase, and peroxiredoxin are considered the most important enzymatic antioxidants[2].

The interplay between ROS and antioxidant defense mechanisms is important in female reproductive physiology. ROS have been required for ovarian steroid biosynthesis, oocyte maturation, fertilization, implantation, formation of blastocysts, and luteal maintenance[3].

During healthy gestation, ROS is critical for normal placentation. ROS increases the uteroplacental blood-flow during trophoblast cells invasion of the myometrium and when trophoblast replaces endothelial and smooth muscle cells in uterine spiral arterioles[4].

Interestingly, a relatively low-oxygen is needed for early placental development to prevent ROS from harming the early embryo, and then the placenta will show a three-fold rise in oxygen concentration[5]. ROS should be maintained under physiological control to regulate trophoblast proliferation, invasion and to enhance angiogenesis, that is required for a normal pregnancy[6].

Conversely, any imbalance between pro-oxidants and antioxidants may lead tomany reproductive and pregnancy disorders as endometriosis, polycystic ovarian syndrome, infertilityand may cause adverse pregnancy outcomes[7].

Oxidative stress is the key role of both placental and endothelial dysfunction. The oxidative stress stimulates the release of cytokines, angiogenic factors, and apoptotic debris that

elicits an inflammatory response. Notably, in both pre-eclampsia and fetal growth restriction (FGR), the highest oxidative stress markers levels are predominant[8].

Preeclampsia (PE) is a serious gestational complication characterized by a new onset of high blood pressure following 20 weeks of gestation along with indications of harm to other organs such as the kidneys, liver, brain and coagulation system. Globally, PE affects 2-8% of pregnancies[9]. In a study conducted to estimate the prevalence of hypertensive diseases of pregnancy in Egypt (4.2%) had pregnancy induced hypertension, (3.8%) had PE and eclampsia was (0.3%)[10].

The pathological mechanism remains enigmatic also there are many risk factors, such as nulliparity, past preeclampsia, some genetic factors, maternal age, maternal obesity, metabolic disease as diabetes mellitus, and twin pregnancy all contribute to preeclampsia.

Also, underlying chronic kidney disease, defect in immune system, original hypertension, and macroproteinuriamay be considered as an addition risk factors for PE[11].

In order to compensate the blood flow deficiency, the mother develops hypertension to increase the blood flow, by the end of the second or third trimester of gestation. Interestingly, delivery of the placenta will ameliorate the disease which indicates the essential role of the placenta in the pathogenesis of PE.

The Pathogenesis of PE can be divided into two stages. The first stage is characterized by alterations of placental perfusion that may results from abnormalities of implantation and vascular remodeling[12]. While, the second stage can be called the maternal syndrome as there are reduced placental perfusion, vasoconstriction, and sometimes plasma volume decreases which lead to the systematically reduced perfusion. Also, PE is may show activation of the coagulation cascade[13].

In the first trimester, PE may be asymptomatic just is characterized by placental ischemia causing spiral artery remodeling defects and subsequent antiangiogenic factors release. This markedly increasesthe oxidative stress and causes systemic endothelial dysfunction leading to development of clinical feature of PE later in pregnancy[14].

Early detection of PE remains a challenge in the clinic. Research has attempted to disclose the number of biomolecules with diagnostic and prognostic values as potential candidates for PE. There is a serious need to identify accurate biomarkers that help in understanding the exact pathogenesis and early detection of PE.

The aim of the present study was to evaluate the correlation between proinflammatory cytokines TNF-α; MDA and SOD with the increase of systolic blood pressure (SBP); diastolic blood pressure (DBP) in (Preeclampsia group), and comparison with normal levels of blood pressure (control group).

Patients and methods

The subjects for this study were from the Obstetrics and Gynaecology Department of Beni-Suef University Hospital, Egypt. After enrolling in the study, a detailed obstetric history and the informed consent were obtained. The study protocol was approved by an Institutional Human Ethical Committee of Beni-Suef University.

Study Design and Groups

This study was done on 50 pregnant women. The PE cases were diagnosed according to general guidelines of the International Society for the Study of Hypertension in Pregnancy[15].

Study inclusion criteria included 20-40 years old singleton pregnant women, gestational age 20 weeks or more. Study exclusion criteria included twin or multiple pregnancies, any evidence of chronic hypertension, chronic renal disease, gestational diabetes mellitus, drugs likely increase blood pressure as corticosteroids and underlying medical diseases such as rheumatoid arthritis and systemic lupus erythematous.

After enrollment, participants were divided into two groups as follows:

1- **Preeclampsia group**: included 25 women that have been diagnosed complaining of preeclampsia (study group).

2- **Control group**: consisted of 25 normotensive women with uncomplicated pregnancies (control group).

Samples and immunoassays

The patient acquired a sample of venous blood and was permitted to form and retract a clot. The coagulated blood sample was centrifuged, collecting transparent serum. Serum was stored between 2°C and 8°C at temperature.

Considering that venous blood samples were gathered in the preeclampsiagroup before any medical therapy was initiated. Blood is collected for estimation of the serum levels of pro-inflammatory biomarker (TNF-α), and oxidative stress markers (Superoxide dismutase (SOD) and Malondialdehyde (MDA)).

Determination of Human TNF-alpha:

TNF-alpha was assayed using RayBio ® Human TNF-alpha ELISA kit (Ray Biotech Products)**.**

Principle:

Standards and samples are pipetted into the wells and TNF-alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human TNF-alpha antibody is added.

After washing away unbound biotinylated antibody, Horseradish Peroxidase (HRP) - conjugated streptavidin is pipetted to the wells. The wells are again washed, atetramethylbenzidine (TMB)substrate solution is added to the wells and color develops in proportion to the amount of TNF-alpha bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Determination of Human Malondialdehyde (MDA):

MDA is determined by lipid peroxidation assay using N-methyl-2- phenylindole[16].

Principle:

This Lipid Peroxidation Assay is based on the reaction of N-methyl-2-phenylindole (R1), a chromogenic reagent with 45°C MDA. With 2 Reagent R1 molecules, one molecule of either MDA or HAE responds to produce a stable chromophore with maximum absorption at 586 nm.

Determination of Superoxide dismutase (SOD)

SOD was determined by the use of the quantitative sandwich enzyme immunoassay technique[17].

Principle:

SOD1-specific antibody was pre-coated on a microplate. Standards and samples are piped into the reservoirs and the immobilized antibody binds any SOD1 present. A biotin-conjugated antibody particular to SOD1 is introduced to the reservoirs after removing any unbound substances. Horseradish Peroxidase (HRP) is added to the reservoirs after washing. A substratum solution is added to the wells after a wash to remove any unbound avidin-enzyme reagent and color develops in proportion to the amount of SOD1 bound in the initial step. The growth of color is halted and the color intensity is measured.

Statistics

Data were coded and entered using the statistical package SPSS version 23. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables.

Comparisons between groups were done using unpaired t-test in normally distributed quantitative variables while non-parametric Mann-Whitney test was used for non-normally distributed quantitative variables[18].

Results

This case-control study was conducted to assess the pro-inflammatory biomarkers and oxidative stress markers in PE. It included 50 pregnant women subdivided into 2 groups, 25 patients for each group as follow: Preeclampsia group and Control group.

Blood pressure among the studied groups: (Table 1 and Figure 1)

When comparing the blood pressure among the studied groups, the result of the present work showed that the mean systolic blood pressure (SBP) was significantly higher ($P< 0.001$) in preeclampsia group when compared to control group by (+ 34.04%). Also, the mean diastolic blood pressure (DBP) was significantly higher ($P<0.001$) in preeclampsia group when compared to control group by (+ 40.43%).

Table (1): Comparison of Blood pressure among the studied groups:

	Preeclampsia group	Control group
SBP (mmHg)	151.2 ± 7.39	112.8 ± 7.78
DBP (mmHg)	102.8 ± 7.78	73.2 ± 5.37

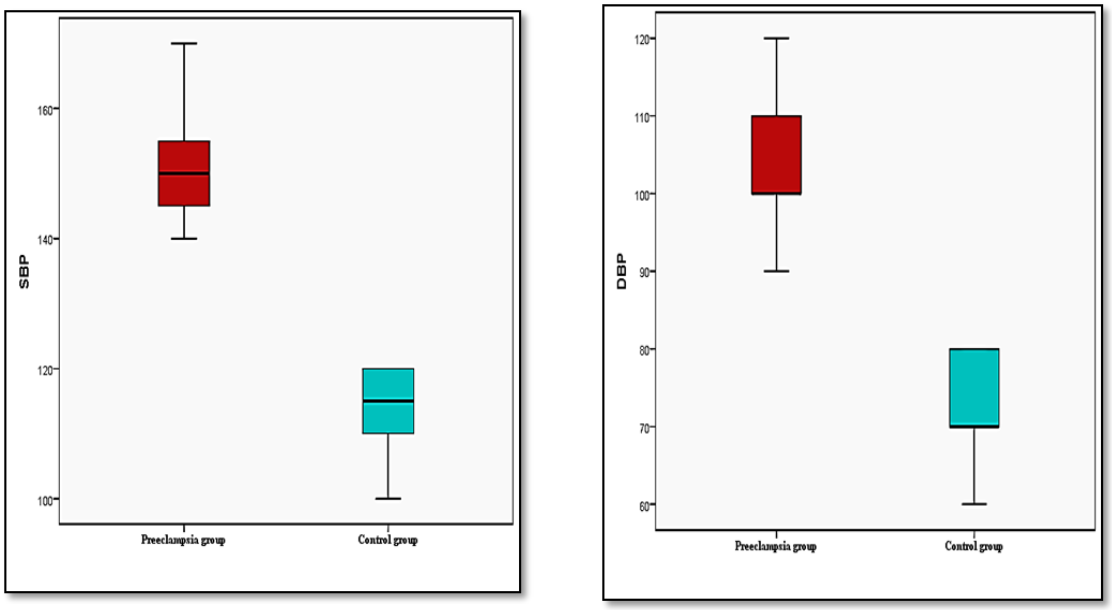


Figure (1): Comparison of Blood pressure among groups.

<u>Serum Tumor necrosis factor-alpha (TNF-α) level among the studied groups:</u>
(Table 2 and Figure 2)
When comparing the level of TNF-αamong the studied groups, the result of the present work showed that the mean values of serum TNFα were significantly higher in preeclampsia group when compared to control group by (+ 358.23%) (*P*< 0.001).

Table (2): Comparison of serum Tumor necrosis factor -alpha (TNF-α) level among the studied groups:

	Preeclampsia group	Control group
TNF-α (pg/L)	104.89 ± 15.94	22.89 ± 3.38

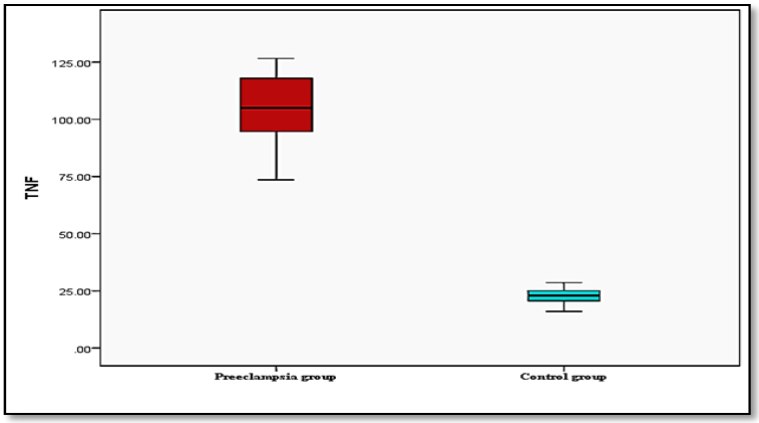


Figure (2): Comparison of serum Tumor necrosis factor -alpha (TNF-α) among groups

Serum Malondialdehyde (MDA) level among the studied groups: (Table 3 and Figure 3)
The result of the present work showed that the mean values of serum MDA were significantly increased in preeclampsia group when compared to control group by (+ 82.53%) (*P*<0.001).

Table (3): Comparison of serum level of MDA among groups:

	Preeclampsia group	Control group
MDA (µmol/L)	3.03 ± 0.44	1.66 ± 0.25

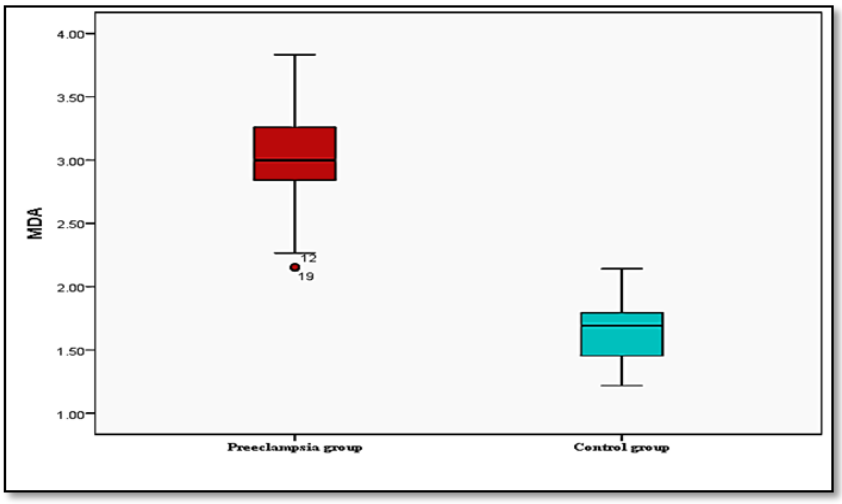


Figure (3): Comparison of Serum MDA among groups.

Serum Superoxide dismutase (SOD) level among the studied groups: (Table 4 and Figure 4)
When comparing the serum level of SOD among the studied groups, the result of the present work showed that the mean values of serum SOD were significantly lower (*P*< 0.001) in preeclampsia group when compared to control group by (- 36.38%).

Table (4): Comparison of serum level of SOD among the studied groups:

	Preeclampsia group	Control group
SOD (pg/L)	19.76 ± 2.75	40.23 ± 5.64

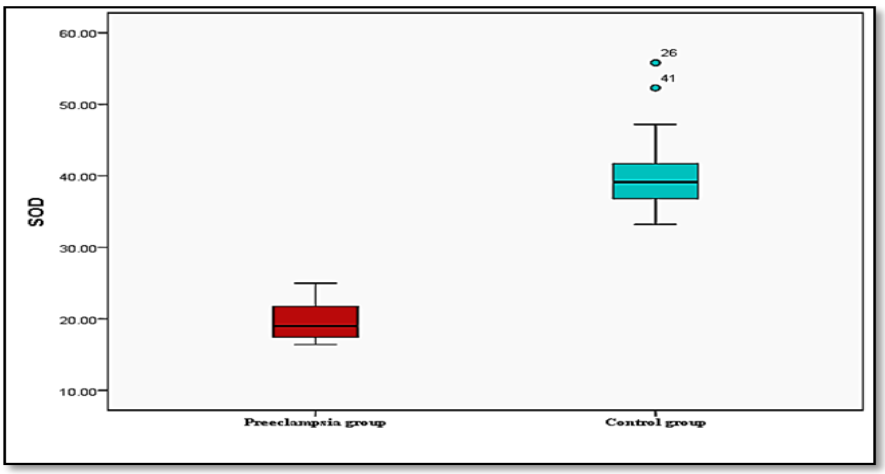


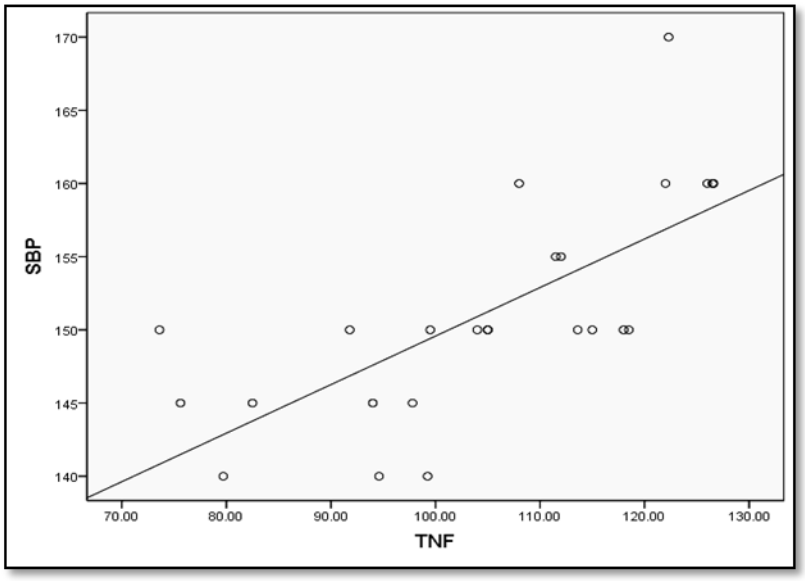
Figure (4): Comparison of Serum SOD among groups.

Correlations between TNF – α and blood pressure: (Table 5 and Figure 5)

The results of the present study showed that there wasa highly significant positive correlation between TNF-α and BP.

Table (5): Correlations between TNF – α and blood pressure:

		SBP	DBP
TNF-α	Pearson Correlation	0.714**	.636**
	Sig. (2-tailed)	< 0.001	< 0.001
	N	25	25
**. Correlation is significant at the 0.01 level (2-tailed).			



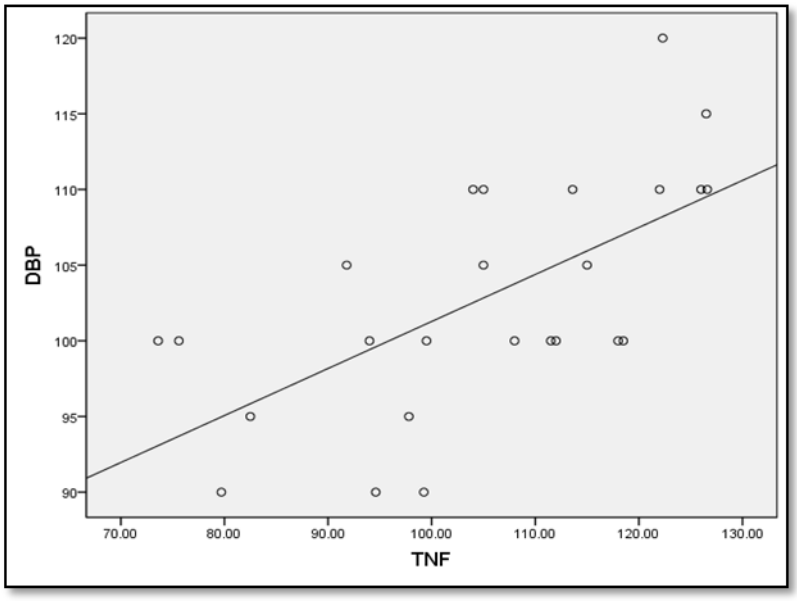


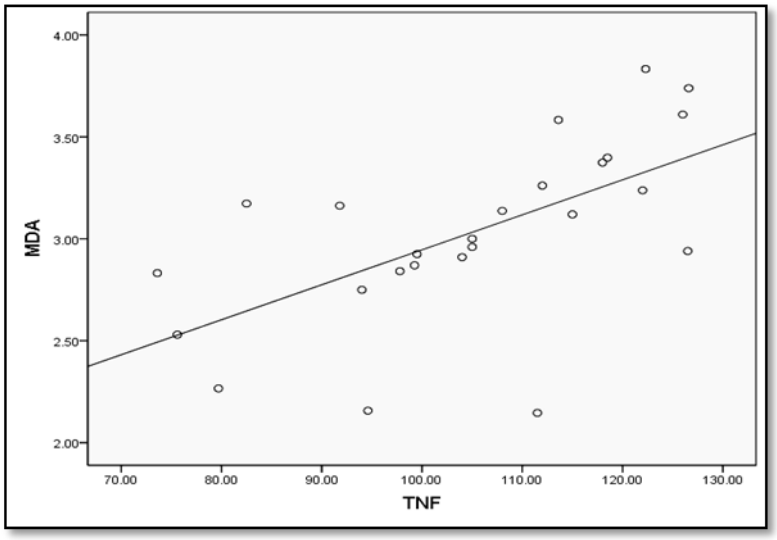
Figure (5): Correlations between TNF – α and blood pressure.

Correlations between TNF – α and oxidative stress markers: (Table 6 and Figure 6)

The results of the present study showed that there wasa highly significant positive correlation between TNF-α and MDA and a significant negativecorrelation between TNF-α and SOD.

Table (6): Correlations between TNF – α and oxidative stress markers:

		SOD	MDA
TNF- α	Pearson Correlation	-.682**	.612**
	Sig. (2-tailed)	< 0.001	< 0.001
	N	25	25
**. Correlation is significant at the 0.01 level (2-tailed).			



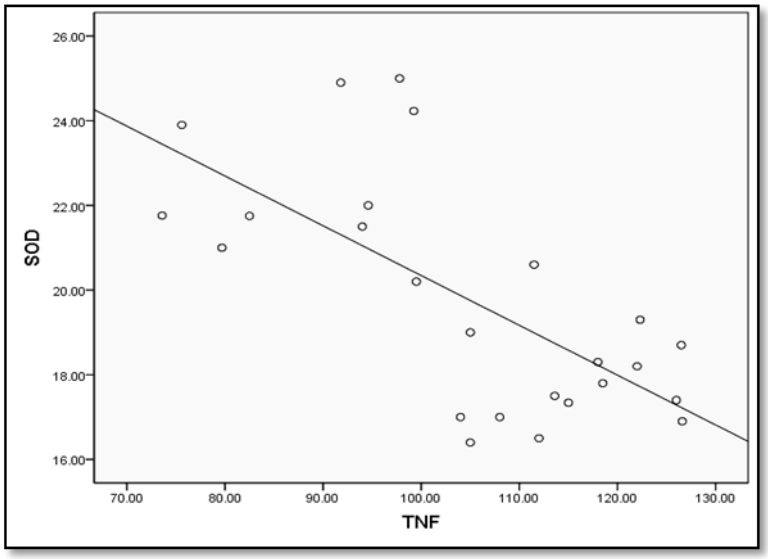


Figure (6): Correlations between TNF – α and oxidative stress markers.

Discussion

Preeclampsia (PE) is a common, and potentially severe, complication of pregnancy, characterrized by elevated blood pressure, proteinuria, and evidence of end-organ damage (such as liver dysfunction, renal dysfunction, central nervous system dysfunction, thrombocytopenia, or pulmonary edema). PE affects 5 to 8% of pregnancies and imposes a significant burden on the health care system with high maternal and fetal morbidity, intrauterine growth restriction and preterm birth. Without timely intervention, PE is conducive of a more grievous clinical picture of maternal seizures and convulsions, which could culminate into coma and death[19].

The pathophysiology of PE is still not well-understood; however, there are clear links between this disorder, oxidative stress, and a systemic inflammatory response.

The current study revealed increased mean values of both SBP and DBP in the preeclampsia group. The results of the present study were similar to findings in a previous study done by[20].

PE showed reduction in concentrations of vasodilators such as NO, prostaglandin I2, and reciprocal increases in vasoconstrictor concentrations such as ET1, thromboxane A2, Angiotensin II, and AT1R. The possible mechanism of hypertension in PE is an abnormal placentation owing to insufficient trophoblast invasion and failure of spiral artery remodeling is believed to result in inadequate blood flow to the placenta and a continuous cycle of repeated ischemia-reperfusion injury. The resulting hypoxic environment within the placenta stimulates oxidative stress and releases placental variables lead to PE vascular endothelial dysfunction, vasoconstriction, and hypertension[21].

The interaction of inflammation with endothelial activation in preeclampsia is extremely complex. Numerous studies reported a predominant immunity of the type Th2 and suppressed immunity of the type Th1 during normal pregnancy. In PE, the balance between the proinflammatory immune response and the tolerance of the immune system is repealed.

Increased T-helper1 (Th1) cytokines were also noted in PE females, including interleukin-2 (IL-2), IL-6, IL-8,IL-12, TNF-α, IFN-α, and IL-17, as well as downregulation in IL-10[22]. Proinflammatory cytokine TNF-α levels represent an important component of pregnancy blood pressure regulation. Placental PE ischaemia causes decreased blood flow and immune function to proinflammatory status with enhanced pro-inflammatory immune cells and cytocines and decreased regulatory cells and cytokines[23].

In the present study, the mean values of serum TNF-alpha showed a statistically significant increase in the preeclampsia group when compared to control group. This is in line with previous studies done by[24, 25].

In PE, cytokines such as TNF-α alter the expression of adhesion molecules in placental vessels, trophoblast apoptosis, and induce placental ischemia. Impaired placentation and decreased invasion of trophoblast cause decrease in uteroplacental perfusion pressure and placental hypoxia. This may stimulate the release of circulating bioactive factors causing asymmetries with the endothelial vascular growth factor of proangiogenic factors or the release of inflammatory cytokines, reactive oxygen species, hypoxia-induced factor1.The circulating bioactive factors attack endothelial cells and cause generalized endothelial dysfunction. Lower vasodilators with higher vasoconstrictors including endothelin-1 and thromboxane A2 result in generalized vasoconstriction[26].

Maternal circulation undergoes major physiological changes during pregnancy to satisfy the increasing metabolic demand of the growing fetus and the mother's health. The placenta's metabolic activity is high to support the development of both the placenta and the fetus, resulting in increased production of ROS during normal pregnancy. Pregnancy as a state of oxidative stress has been suggested[27].

During normal pregnancy there is a comparatively physiological balance between oxidants and antioxidants. Early pregnancy increased ROS production plays an important role in the proliferation, differentiation, invasion and

angiogenesis of the trophoblast. Placental SODs and catalases along with total antioxidant capacity also increase as gestation advances[28], that counters the increased generation of ROS.

It has been shown that both acute and chronic hypoxia increase the ROS. Oxidative stress occurs when ROS production obliterates the intrinsic antioxidant defense, either due to increased ROS formation or reduced ability to neutralize ROS, or both. Accumulating evidence suggests that ROS plays a key role in pregnancy pathogenesis complications[29].

The origins of lipid peroxides are not known in preeclampsia; however, the low-perfusion placental tissue could produce a free radical cascade to increase the prevalence of lipid peroxidation. The primary factors responsible for the production of free radicals were lipid peroxidation and oxidative stress through a poorly perfused placenta, resulting in platelets and leukocytes adhering to the vascular endothelium causing vasoconstriction and increased peripheral vascular resistance[30].

In the present study, the mean values of serum MDA were statistically significantly higher in preeclampsia group than in control group.

The findings of our study were similar to previous studies done by[31,32]. However, our study is in contrast with[33] case control study that showed that there was marked decrease of MDA in preeclampsia placentas.

The possible explanation of increased MDA in PE is that MDA may constitute a major pathological contributor to endothelial dysfunction and high blood pressure. It has been suggested that lipid peroxidation is causing oxidative stress in preeclampsia. A previous study found that free radicals cause lipid peroxidation in cell membranes by targeting polyunsaturated fatty acids[34].

Antioxidants, enzymatic or non-enzymatic, neutralize free radicals and inhibit oxidant assaults on proteins, lipids, carbohydrates and DNA. The most important enzymatic antioxidant is superoxide dismutase (SOD), which converts both the mitochondria and the cytosol of cells to hydrogen peroxide (H_2O_2). Other enzymes, such as catalase, peroxyredoxins and/or glutathione. Vitamin C, vitamin E and carotenoids such as carotene, which neutralize all free radicals, are non-enzymatic antioxidants. Antioxidant enzymes are particularly dependent on metal co-factors, which are capable of taking various valences as electrons are transferred during redox reactions, and metals such as copper, zinc, manganese, iron and selenium can therefore also be considered to be members of antioxidants.[35].

An endothelial dysfunction due to free radical oxidative damage is one of the predisposing parameters of preeclampsia and eclampsia. However, a sufficient antioxidant and free radical disposal program may be the most significant defense mechanism against preeclampsia. This leads to oxidative stress and preeclampsia in women who have low levels of defense against antioxidants[36].

In the present study, the mean values of serum SOD showed statistically significant decrease in the preeclampsia group than in control group.

This is in agreement with previous study that showed that SOD levels were significantly lowered in preeclamptic compared with normotensive patients[37]. The findings of our study were also similar to the meta-analysis of SOD activity that was done by[38] that was indicative of lower SOD activity in preeclamptic women. In the contrary, our study is in contrast to a previous study[39].

One explanation is that impairment of antioxidant activity can lead to increased lipid peroxidation and subsequent vascular endothelium damage during placentation. In addition, low-grade ischemia-reperfusion injury that occurred secondary to abnormal spiral artery remodeling during placement leads to oxidative stress[40].

It is believed that the use of antioxidants would increase the free radicals produced in preeclampsia. It is unclear whether endothelial dysfunction due to excess oxidative stress and antioxidant insufficiency is involved in preeclampsia growth or inappropriate function caused by existing preeclampsia. When superoxide anion is not detoxified, it reacts with

NO to peroxynitrite. Peroxynitrite is a highly oxidizing oxidant that can cause lipid peroxidation[38].

In conclusion, preeclampsia pregnant women have showed significant increase in SBP, DBP, serum pro-inflammatory cytokine (TNF-α), and oxidative stress marker (MDA).However, the mean serum values of antioxidant biomarker (SOD) were significantly lower in PE women.

Moreover, there was a significant positive correlation between TNF-α and SBP, DBP, and MDA. Also, there was a significant negative correlation between TNF-α and SOD.

The current study recommends that the increased serum TNF-α, MDA and the decreased SOD may be implicated in the pathogenesis of PE and the increase in the blood pressure. Further clinical studies should be designed to explain the exact link and the possible mechanism of protective effect of antioxidant in the pathophysiology of PE.

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