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RESEARCH ARTICLE

EXPRESSION OF SERUM CYTOKINE(IL-17) AND OXIDATIVE STRESS MARKER (GSH) IN IRAQI PREGNANT WOMEN WITH PRE-ECLAMPSIA.

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Abstract

Background:- Pre-eclampsia is the second leading cause of maternal morbidity and mortality in the United States. Infants born to affected mothers face a five-fold increase in death rate. Sometimes referred to as toxemia by the lay public, Pre-eclampsia is defined as the presence of hypertension (systolic blood pressure [BP] ≥ 140 mmHg or diastolic BP ≥ 90 mmHg), and proteinuria exceeding 0.3 g/day after the twentieth week of pregnancy in a previously normotensive non protein uric woman. preeclampsia is associated with chronic inflammation and placental oxidative stress (ROS), chronic IL-17 increases blood pressure, which though exerts a host-defensive role in many infectious diseases, it promotes inflammatory pathology in autoimmunity and other settings.

Objectives:- To evaluate natural antioxidant level (GSH) & IL-17 in the sera of pregnant women with preeclampsia.

Patients / Methods:- The target subjects of this descriptive cross-sectional study consisted of 60 pregnant (30 pregnant women with preeclampsia (severe and mild to moderate) and 30 (non-preeclamptic) pregnant women (control) diagnosed by a specialist Obstetrics and Gynecology, aged 15 to 40 years who were recruited from the outpatient clinic of Obstetrics and Gynecology at the Al-Imamain Al-khadimain city hospital, between December of 2014 and the November 2015. Serum IL-17 was estimated using an Enzyme-Linked Immunosorbent Assay (ELISA) technique. while GSH was measured by Burtis and Ashwood.

Results:- Serum levels of IL-17 was statistically significantly elevated in the patients with preclampsia (severe and mild to moderate) compared to normotensive pregnant women ($p < 0.001$) and a significant difference was detected as well within preclamptic patients groups severe with mild to moderate ($p < 0.05$). A significant decrease ($P < 0.001$) is observed in the level of serum Glutathione (GSH) of preclampsia pregnancy (severe and mild to moderate) when compared with normotensive pregnant women , also a significant difference within preclamptic patients ($P < 0.01$) was observed.

Conclusions:- increased level of IL-17 in the sera of pregnant women with preeclampsia & Reduced levels of glutathione. These results support that these markers are involved in the pathogenesis of preeclampsia. These findings suggest that severe preeclamptic women have higher cytokine, in this study pregnant women with Preeclamptic may indicate the presence of disturbance in immunological tolerance.

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Introduction:-

Preeclampsia is the most common major medical complication of pregnancy. It represents the leading cause of both fetal and maternal morbidity and mortality, affecting 5–10% of all pregnancies (1). Most commonly, it presents as de novo development of high blood pressure and proteinuria in the second half of pregnancy, but can variably affect the maternal renal, hepatic, neurological, hemopoietic, and cardiovascular systems as well as the placenta and fetus. The pathogenesis of preeclampsia remains poorly understood, although it has been suggested to result from a maternal immune response against the fetus (1, 2). Recently, regulatory T (Treg) cells were proposed as major contributors to the maintenance of tolerance during pregnancy (3, 4). It has been reported that normal human pregnancy is associated with elevated numbers of Treg cells (5, 6). Whether deficiencies in the number or function of Treg cells are implicated in the development of preeclampsia remains controversial. Two recent reports described decreased numbers of Treg cells in preeclampsia compared with normal pregnancy (7, 8), whereas others found comparable frequencies (9, 10). It has been reported that soluble endoglin, an inhibitor of TGF- β signaling (11), is increased in preeclampsia and that the level correlates with disease severity (12, 13). Because TGF- β is known to drive the differentiation of human Treg cells (14) and to inhibit that of Th17 cells (15) These Th17 cells, which secrete IL-17, are thought to play a role in chronic inflammation (16). Th17 cells produce proinflammatory cytokines, such as IL-17 and IL-22. The IL-17 family contains six members (IL-17A–F), with IL-17A being designated as the prototypic of IL-17 cytokine (17). The transcriptional factor to develop Th17 cells is acid-related orphan receptor γ t (ROR γ t) in humans. Numerous studies of recurrent pregnancy loss, reproductive failure, and pregnant failure in humans have revealed a close association of these conditions with the increased production of Th17 cells (18–20). It is hypothesized that preeclamptic condition could be explained by alterations in the function of vascular endothelium and placenta, likely in response to reduced perfusion, produces circulating factor(s) that alters endothelial function. All these factor(s) are products of oxidative stress (21,22). However, oxidative stress describes the damage that occurs when reactive oxygen species (ROS) overwhelm the antioxidant defenses of the host. Oxidant stress may play an important role in the pathogenesis of hypertension in pregnancy & may be a final common pathway leading to tissue damage (21). Oxidative stress occurs as a result of excessive free radicals interacting with antioxidants (23.). Superoxide dismutase (SOD), catalase (Cat) and glutathione peroxidase (GSH) play important roles in counteracting oxidative stress (24,25). Oxidative agents are overproduced due to ischaemic-reperfusion injury and may overwhelm potential antioxidant activity (26,27,28). Additionally, adhesion molecules, such as E-selectin (produced by endothelial cells) and P-selectin (found in a-granules of platelets), have been shown to correlate with the level of oxidative stress in pregnancy (29). To the best of our knowledge, many pregnancy complications and birth defects have been linked to oxidative stress, free radical damage and low GSH levels in the mother and fetus. GSH and other antioxidants attenuate oxidative stress in pregnant women with inflammation or maternal conditions like PE, The GSH also prevents or minimizes the oxidative stress that occurs during labor and the birth process (30).

The aim of the present study was to compare the serum levels of GSH and IL-17 in pre-eclamptic and normotensive pregnant women.

Subjects, Material and Methods:-

Subjects:-

This study comprised sixty(60) pregnant women ;30 pregnant women with preeclampsia (17 had severe PE and 13 had mild to moderate PE) and 30 non-preeclamptic pregnant women (control), diagnosed by a specialist in Obstetrics and Gynecology , aged 15 to 40 years who were recruited from the outpatient clinic of the department of Obstetrics and Gynecology at the Al-Imamain Al-khadimain city hospital, between December of 2014 and the November of 2015. Serum IL-17 was estimated using an Enzyme-Linked Immunosorbant Assay (ELISA) technique. while GSH was measured by Burtis and Ashwood.

Exclusion Criteria:-

Younger than 15 years and older than 40 years. Pre conception hypertension, diabetes mellitus, proteinuria. Other chronic or autoimmune diseases as well as those with endocrine disorders & women who develop HT secondary to other disorders e.g renal diseases. Pregnant women with multiple pregnancies.

Blood Sampling:-

Blood samples (10 ml) were collected from patients and control subjects in serum separator vacutainers (BD Vacutainer Systems, Plymouth, UK). Sera were separated and immediately stored at -20°C until analysis.

Serum Cytokine Measurement:-

The quantitative determination of IL-17 level was conducted by an Enzyme-Linked Immunosorbent Assay (ELISA) technique, using a commercial available kit (The RayBio Human IL-17 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-17 in serum), Every sample was run in duplicate, measurements differed by less than 10 %, and the mean value was calculated and used for statistical analysis.

Serum glutathione Measurement:-

Serum levels of GSH was measured by Burtis and Ashwood(31). Serum (0.2 ml) were used in the assay. The GSH was made to react with 5'- dithiobis (2-nitrobenzoic acid) [DTNB], which reacts with sulfhydryl groups, to develop a stable color. The absorbance was measured at 412 nm and GSH content was expressed as $\mu\text{mol/gHb}$.

Statistical analysis:-

All data were coded and entered using the program statistical package for social sciences (SPSS) version 20 under windows XP. Descriptive data was summarized using mean, standard deviation (SD). P values < 0.05 were considered statistically significant.

Results:-

Serum Oxidative stress markers (GSH), and Cytokine profile (IL-17) levels were estimated in 60 patients 30 women with preeclampsia, compared to 30 pregnant control group, gestational age matched.

As expected, the patients with preeclampsia (severe and mild to moderate) had significantly higher level of IL-17 levels than the controls (pregnant ladies) ($p < 0.001$), also a significant difference was found between pregnant women with severe PE compared to those with mild to moderate PE ($p < 0.05$) as shown in table (1) and figure (1-1).

a significant decrease in GSH in preeclampsia patients (severe and mild to moderate) as compared with normal pregnant women, ($p < 0.001$) our statistical analysis also show a significant difference within the pre-eclamptic patients (Severe PE x mild-moderate PE : ($p < 0.01$)). see table (1) & figure (1-2).

The level of IL-17 and Oxidative stress (GSH) in normal healthy subjects (pregnant women and preeclampsia subjects (severe and mild to moderate)) was depicted in Table 1.

Table 1:- The Anthropometric and biochemical variables among the three studied groups.

Parameters	Normotensive Pregnant (NE)	Mild to moderate Preeclampsia (PE)	severe Preeclampsia (PE)	P(ANOVA) (T-Test)
NO.	30	13	17
IL-17 (pg/ml)	34.68 \pm 11.53	61.32 \pm 11.87	84.18 \pm 15.33	Severe PE x mild-moderate PE : $p < 0.05$ PE x NE: $p < 0.001$
GSH ($\mu\text{mol/l}$)	396.55 \pm 66.0	311.46 \pm 53.0	265.23 \pm 58.0	Severe PE x mild-moderate PE : $p < 0.01$ PE x NE: $p < 0.001$

Values are Mean \pm SD, X=VS.

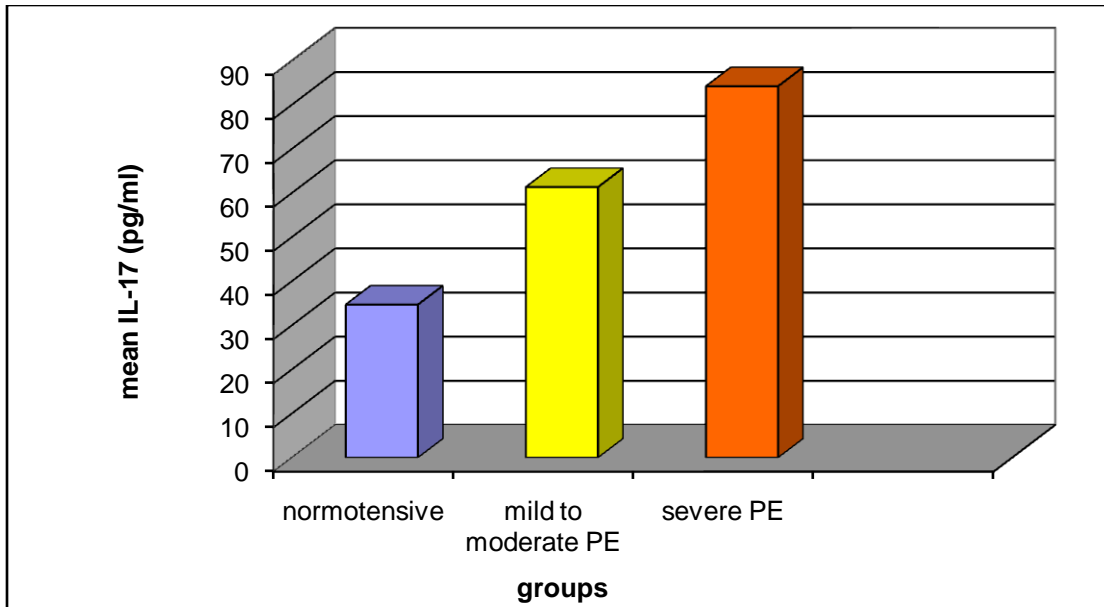


Figure 1-1:- Serum levels of IL - 17 in patients with normal pregnancy and preeclmpsia women.

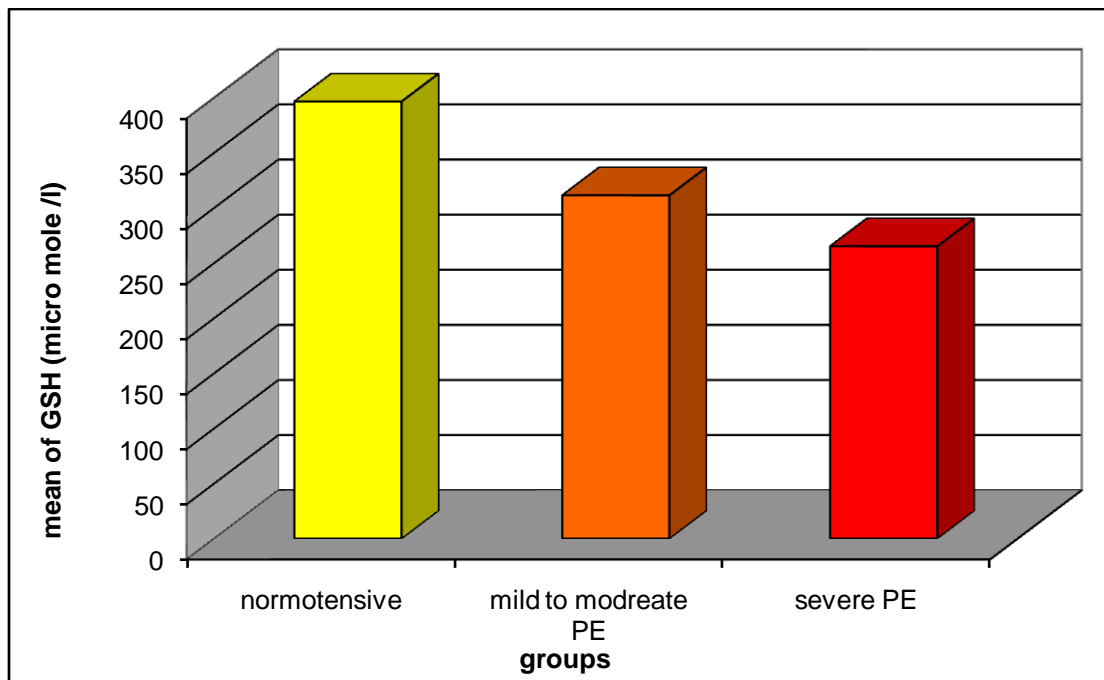


Figure 1-2:- Serum levels of GSH in patients with normal pregnancy and preeclmpsia women.

Discussion:-

Preeclampsia is one of the most feared pregnancy complications, with a risk of maternal and fetal death and with no ideal therapy readily available. The cause of this strictly pregnancy-related disease is still unknown and is therefore a great challenge to all researchers in the field of pregnancy-related pathophysiology.

Endothelial cell injury and dysfunction may play a central role in the pathogenesis of PE, this dysfunction associated with PE characterized by an enhanced inflammatory response and altered cytokine production [32]

IL-17 was significantly increased in patients with severe preeclampsia, and less in women with mild to moderate PE compared with control groups. This findings suggests that endothelial activation results in the increased production of chemokines in women with preeclampsia. Also it may be due to many pathological conditions such as apoptosis, inflammation, neutrophil activation, endothelial cell damage and dysfunction, and increased endothelial permeability.

We investigated the expression level of IL-17 protein in the sera of PE patients and normal pregnant women. We observed significantly increased proinflammatory cytokines IL-17 levels in sera of PE patients compared with control groups ., these results are agreement with other results done by Y. Sasaki, et al .,2007 who suggest a potential predominant role of Th17 cells in the PE patients [33]. cytokine profile in peripheral blood of PE patients may provide suitable environment for the differentiation of Th17 cells, but not suitable for Treg cells. Thus, preeclampsia is characterized as a state of the excessive maternal inflammatory response with a predominance of the production of Th17 cells, suggesting systemic inflammation is a dominant component in the pathogenesis of preeclampsia [34, 35]. These results not only help to understand the etiology of preeclampsia, but also provide a novel rationale for the prevention or intervention of PE via regulation of the balance of Th17/Treg cells. Our results are consistent with recent data by Santner-Nanan et al. demonstrating that it is the balance between Tregs and Th17 cells that is critical to maintain tolerance to the fetus and prevents PE patients [36–38].

In contrast to normal pregnancy, there are indications of increased inflammatory responses and also of an immune deviation toward Th1 in the established preeclamptic pregnancy (39). (40) were one of the first to suggest that mediators released from the preeclamptic placenta are responsible for the endothelial damage seen in preeclampsia. Subsequent to the damage, the injured endothelium initiates a dysfunctional cascade of coagulation, vasoconstriction and intravascular fluid redistribution that results in the clinical syndrome of preeclampsia. As reviewed by Redman and Sargent et al in (2003) (41), TNF- α can activate the endothelial cells and present damage similar to that seen in preeclampsia. Furthermore, Redman and Sargent et al in (2003) (41) suggest that preeclampsia is an excessive maternal inflammatory response to pregnancy. Indeed, preeclampsia is associated with systemic maternal inflammation (reviewed by Redman et al in 1999 (42) which at the adaptive level has been suggested to be dominated by T-helper (Th) type 1 responses, as reviewed by Saito and Sakai et al in (2003). (43).

Pre-eclampsia is a hypertensive disorder associated with oxidative stress during pregnancy. Excessive production of oxidants in maternal placenta in response to poor tissue perfusion has been proposed as an explanation, but there is no consensus yet on the exact mechanism (44).

The mean serum GSH level for severe PE women were found to be ($265.23 \pm 58.0 \mu\text{mol/l}$) and for mild to moderate PE ($311.46 \pm 53.0 \mu\text{mol/l}$) lower than that of normotensive pregnant women ($396.55 \pm 66. \mu\text{mol/l}$).these results gose with previous study done by Chen et al. 1993 who observed that GSH is a general scavenger of oxidative agents (45), which was shown to have lower levels in our pre-eclamptic patients. Moreover, while total antioxidant levels were lower in pre-eclamptic patients older than 30 years,and this result agreement with other results done by Vanderlelie et al. (2005) also showed that the level of SOD and GSH was lower among pre-eclamptic patients compared with normotensive pregnant women (46). In the present study, we have observed significant decreases in the activity of glutathione (GSH) in hypertensive patients as compared with controls.

The results of the present study conclude that in hypertensive pregnant women have a high oxygen free radical productions & decreased catalase activity supports the higher oxidative stress hypothesis in pregnancy induced hypertension. In some previous study, the increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress (47,48,49,50).In present study the serum GSH concentration was very significantly lowered in hypertensive nonpreeclamptics pregnant women, The present findings regarding serum GSH in agreement with previous work by Padmini et al in 2008 (51). The present study reveals a decreased level of serum GSH activity (Table 1) in PE compared to NT pregnancy. It is shown that the uncontrolled lipid peroxidation is a key contributing factor to pathophysiologic condition of PE. It has also been hypothesized that reduction in the antioxidants in PE is due to their consumption by the body to overcome the free radicals generation (Sharma et al in 2006 (52).In PE, studies have demonstrated a significant increase in (O_2^-) production by neutrophils in the placental tissue of PE women (53) al., 2001). GSH plays a role in detoxification of ROS such as (O_2^-) and ($\text{OH}\cdot$) which are accumulated in PE state . Therefore the level of GSH in PE pregnant decreases (54). This has also been shown in the present study and in consistency with the result reported by Kharb et al ., 2000(55),Pyska et al ., 2002,(56) and AL-Shammary et al., 2002(57) also found that there were imbalance between

oxidants and antioxidants and indicated that enhanced superoxide generation and impaired glutathione metabolism may be involved in the pathogenesis of PE .

58,59,60,61,62 Glutathione and glutathione related enzymes are one of the major antioxidant systems within body. It conveys an antioxidant power through the direct inactivation of reactive oxygen species or by acting as an electron donor for glutathione peroxidase that reduces hydrogen peroxide to water.(63)The present study clearly demonstrated that erythrocyte GSH levels in PE decreased significantly when compared to control. The decrease in this non enzymatic antioxidant parameter may be due to increased turnover, for preventing oxidative damage in these patients. These findings are supported by works done earlier by MohdSuhail 2008 (64) and Krishna mohan S 2007.(65)Despite of continuous advances in research, preeclampsia remains a major challenge in both understanding its pathophysiology and management. Many pregnancy complications and birth defects have been linked to oxidative stress, free radical damage and low GSH levels in the mother and fetus.GSH and other antioxidants attenuate oxidative stress in pregnant women with inflammation or maternal conditions like PE. The GSH also prevents or minimizes the oxidative stress that occurs during labor and the birth process (66).In conclusion, increased level of IL-17 in the sera of pregnant women with preeclampsia & Reduced glutathione. These data support the view that serum IL-17 and GSH are involved in the pathogenesis of preclampsia, Reduced glutathione could be associated with increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of preeclampsia.The increased generation of free radicals can lead to alteration in serum GSH to prevent oxidative damage.These findings suggest that severe preeclamptic women have higher cytokine Preeclamptic pregnant women, in this study may indicate the presence of disturbance in immunological tolerance. Expression of cytokine (IL-17) may facilitate the potential development of therapeutic targeting for the treatment of the PE patients.

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