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Pre-eclampsia : An excessive maternal immune response in Egyptian women

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Abstract

Preeclampsia is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent.Although pre eclampsia causes high maternal /fetal morbidity, the etiology of this multisystem disorder is still unknown. We have detected the cytokine levels inpreeclamptic women compared to normotensive pregnant and non-pregnant women.This study aimed to understand immunological network , and physiological parameters for the pathogeneses of preeclampsia.Serum levels of tumor necrosis factor alpha (TNFα) and interleukin 10(IL-10)were measured by enzyme-linked immunosorbent assay (ELISA) . C reactive protein (CRP) was evaluated as inflammatory marker in preeclampsia.Our findings demonstrated that pre eclamptic state is associated with high levels of pro inflammatory cytokine TNFα ($p < 0.05$) and C.R.P. By contrast, normotensive pregnancy evolved high levels of regulatory cytokine Il-10 .The present study supports the hypothesis of altered immune response in preeclampsia.

Keywords: C-reactive protein (CRP)**,** pro inflammatory cytokine(TNFα)**,** regulatory cytokine (IL-10)**.**

1 Introduction

Preeclampsia (PE) is a common hypertensive disorder of pregnancy, affecting 5-10% of pregnancies. It is a pregnancy-specific disease in which hypertension,protein urea, vascular abnormalities, and often intrauterine growth retardation occur after 20 weeks of gestation, and if untreated can lead to eclampsia, a life-threatening disorder (Barak *et al* ., 2005).

Although the aetiologyof this syndrome remains unclear, it is certain that preeclampsia is triggered by placental factors given that occur only in pregnant women, and definitive treatment is delivery (Catarino*et al* ., 2012).The imbalance between pro- and anti-inflammatory factors has also been incriminated in the aetiology of this disorder (Xiao *et al* ., 2012**)**.

Preeclamptic women had elevated pro-inflammatory cytokines and decreased levelsof anti-inflammatory cytokinesthan normotensive non pregnant and pregnant women.This strongly implicates the maternal immune system as a major contributor to the pathogenesis of PE; however, whether excessive activation of the maternal immune system initiates the development of PE or participates at a later stage in PE or both is **(**Nguyen *et al* ., 2013)**.**The target of this study was to determine circulating levels of cytokines in a comprehensive manner involving a large number of healthy non-pregnant , pregnant women and preeclamptic patients. It is important to investigate whether serum cytokine levels were related to the clinical characteristics and laboratory parameters of the study participants.

2 Materials and Methods

2.1-Study Design

The study was designed using a case-controlled approachthirty preeclamptic patients, 20 healthy pregnant women with uncomplicated pregnancies and 20 healthy non-pregnant women were involved in the study. The study participants were enrolled in the Department of Obstetrics and Gynecology, at Mansoura University Hospital, Mansoura, Egypt.Exclusion criteria were multi fetal gestation, and fetal infection. None of the pregnant women were in active labor, and none had rupture of membranes.

Preeclampsia was defined by increased blood pressure (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 occasions at least 6 hours apart) that occurred after 20 week of gestation in a woman with previously normal

blood pressure, accompanied by proteinuria (≥0.3 g/24 h or ≥1 + on dipstick in the absence of urinary tract infection).

2.2-Blood Sample collection

Ten ml of blood was drawn from each subject. Three ml of this was transferred to an EDTA bottle for determination of the basic haematologicalindices .

Sevenml was dispensed in a sterile plain bottle for separation of serum in two aliquots. Both aliquots were stored in tubes containing drops of TrasylolTM (aprotinin) to inhibit degradation of cytokines and were subsequently stored at −20˚C for assay of cytokines . Blood samples were also taken from normal healthy controls after obtaining consent and treated similarly.

2.3-Parameters measured:

2.3.1Estimation of immunological Parameters.

Serum levels of IL10 and TNFα were assayed using an enzyme linked immunosorbent assay kit .C reactive protein(CRP) was estimated by Immunoturbidimetry. Turbidimetric Immunoassay is based on the principle of agglutination reaction for the ultrasensitive determination of C – reactive protein in human plasma(Vackova and Skokanova, 1992; Hayashi ,*et. al* ., 1970).

2.3.2 Estimation of physiological Parameters.

Basic haematological indices including complete blood count (CBC) ,haematocrit, blood sugar level as well as , creatinine , alanine amino transferase and aspartate amino transferase were assessed by standard haematological procedures (Kalplan et al.,1984; Bartels et *al.*,1971; Trinder 1969).

3-Statistical Analysis

The data were expressed as the mean ± SE. Statistical analysis while correlation analyses were undertaken using independent One-way ANOVA with post-hoc tukey test correlation coefficient test, respectively. A P-value <0.05 was accepted statistically significant. All the previous statistical analyses of data were carried out by SPSS software version. 18.

3 Results

The clinical characteristics of the control and preeclamptic groups are presented in Table 1. Patients with preeclampsia displayed significantly increased systolic (SBP) and diastolic blood pressure (DBP) relative to controls.

Immunological parameters (TNFα, IL10 and CRP)

In preeclamptic patient , TNFα (pg/ml) and CRP (mg/l) showed a significantly increased concentration (figure2,4). On the other hand IL10(pg/ml) significantly decreased in preeclamptic women as compared to control non pregnant and pregnant groups at P<0.05.

Physiological parameters

Complete blood cells (CBC)

A decline in haematocrite andhemoglobin values in preeclamptic women were noticed in comparison with the control non pregnant and pregnant groups at P<0.05 .

There was no significant deference in Red blood corpusclecount in preeclamptic women. On the other hand ,white blood cell showed a significant reduction in preeclamptic women compared with control non pregnant and pregnant women. A significant drop was found in platelet count of preeclampticpatient(Table2).

Alanine amino transferase, aspartate amino transferase, creatinine values and percentage changes in healthy non-pregnant ,pregnant women and preeclamptic patients

Data present in figure (5,6) showed a significant increase of creatinine value (mg/dl), ALAT(mg\dl) and ASAT(mg\dl) in pereclamptic women when compared with control and normal pregnant.

4 Discussion

In preeclampsia the initial trigger of the disease is local,and connected with the placenta. Erlebacher (2013)presented an attractive hypothesis that there is no specific abnormal reactivity in preeclampsia, but rather that it represents the extreme end of maternal inflammatory reactivity to normal pregnancy. Preeclamptic placenta secretes several inflammatory molecules as a result of the hypoxic state developed from a lack of vessel remodeling in the uterus (Harrison et al ., 2011; Serrano., 2006).

The results of this study showed that pro-inflammatory cytokine TNF-α level was present in higher concentrations in women with preeclampsia compared to control non pregnant women .Previous study suggested that TNF-α could be a marker for the severity of PE due to the correlation between plasma concentrations and different stages of the disease(Peracoli*et al* ., 2007).

Hypoxia promotes excess production of placental tumor necrosis factor (TNF-α), and has a potential cytotoxic effect to vascular endothelial cells, trophoblastic cells of placenta (Chen*et al* ., 2010).It induces apoptosis, inhibits proliferation of trophoblast cell (Xu et al ., 2011) associated with vasoconstriction (Zhao et al ., 2005).The level of TNF-α in the maternal circulation is increased prior to the clinical manifestation of preeclampsia (Pennington et al ., 2012).

Regarding IL10,the present result showed increased levels in normotensive pregnant compared to preeclamptic women and non - pregnant women . Studies in mice revealed that IL-10 deficiency in early pregnancy affects trophoblast growth and , differentiation ,causing placental failure and aabortion(Vitoratos *et al* ., 2010). Based on the aforementioned studies,administration of IL-10 alone, during gestation normalized blood pressure and endothelial function in mice. IL-10 treatment had the most beneficial effect on fetal development and renal function as well as decreased the levels of the pro-inflammatory cytokines IL-6,IFNγ,and TNFα (Chatterjee et al ., 2014).

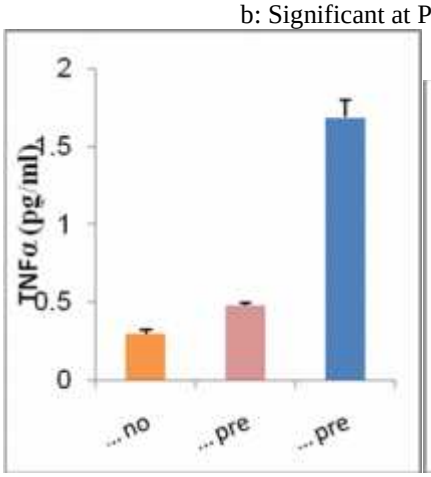
Our study detected increased CRP values in pregnant women who subsequently developed PE. The data obtained by Wolf *et al*.,(2001) and Tjoa *et al*.,(2001)showed CRP levels pregnancies that subsequently developed preeclampsia, are in accordance with our results .

There are also contradictory data resulting from the studies of Savvidou, who found no differences between CRP levels in pregnant women with preeclampsia compared to normal pregnancy (Guven et al ., 2009; Savvidou et al ., 2003).

Table1. Clinical characteristic of subjects.

Groups	Age (y)	Gestation age(w)	DBP(mm/Hg)	% of control	SBP(mm/Hg)	%
Non pregnant group	29±1.06		115.5±1.14	-	71±.69	--
pregnant group	29±.89	31±.81	110.5±1.35	-4.33	76±1.52	7.04
Pre eclamptic group	28±1.04	30±1.08	157.67±2.69[ab]	36.51	89.33±2.49[ab]	28.81

a:Significant at P<0.05 when compared to control group.
b: Significant at P<0.05 when compared to pregnant group



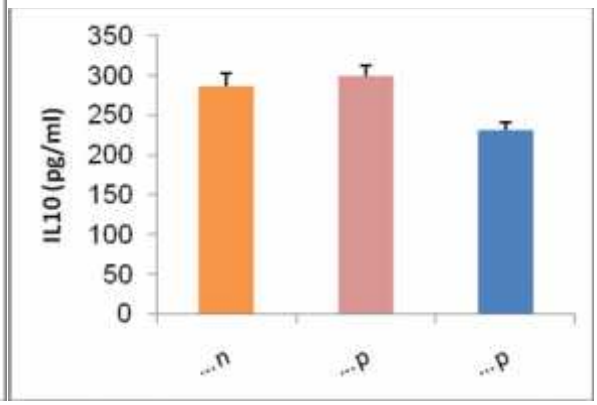


Fig.2.TNFα concentration in healthy non- pregnant ,pregnant and PE
Fig.3.IL10 concentration in healthy non-non- pregnant ,pregnant and PE patients.

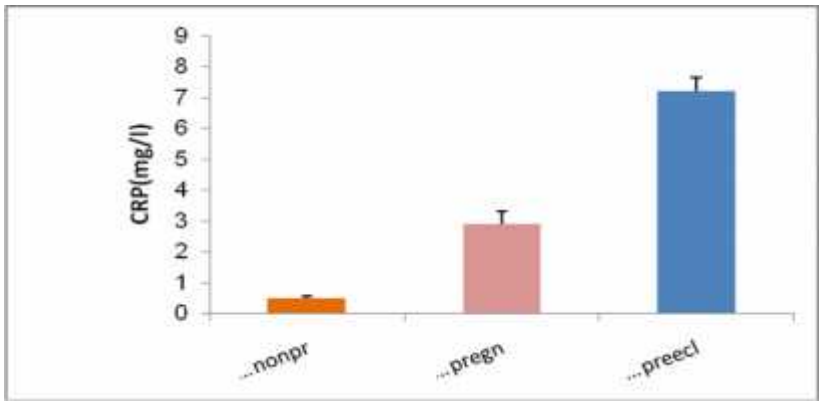
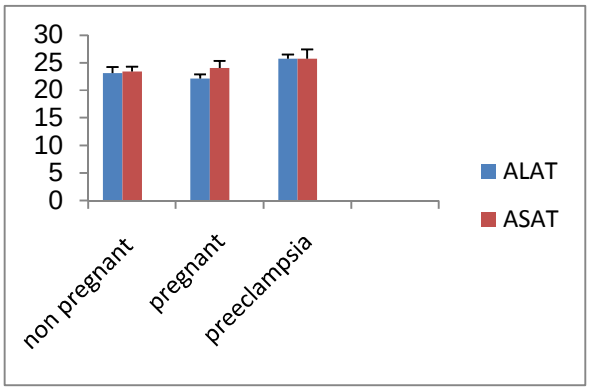


Figure4.C.reactiveprotien values in healthy non-pregnant ,pregnant and preeclamptic patients.

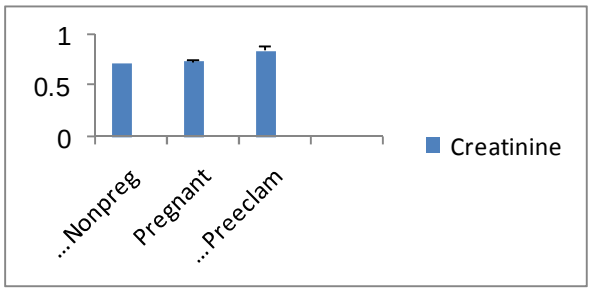
Table 2: Complete blood counts with percentages changes in healthy non-pregnant ,pregnant and preeclamptic patients.

Groups	**Hct(%)**	**%of control**	**HGB (g/dl)**	**% of control**	**RBCs (10^6/ul)**	**%of control**	**WBCs (10^3/ul)**	**% of control**	**PLTs (10^3/ul)**	**% of control**
Non pregnant group	34.36±.58	--	11.32±.15	--	4.36±.13	--	6.4±.04	--	206.6±6.27	--
pregnant group	34.62±.46	-6.55	10.87±.25	-3.98	4.03±.07	-7.57	7.07±.38	10.46	198.05±10.3	-4.13
Pre eclamptic group	32.11±.74[b]	.76	9.98±.19[ab]	-11.84	3.83±.09	-12.16	8.99±.58[ab]	40.47	136.23±3.53[ab]	-34.06

a:Significant at P<0.05 when compared to control group.
b: Significant at P<0.05 when compared to pregnant group.



(Fig.5)



(Fig.6)

Figure 5. ALAT and ASAT values in healthy non-pregnant ,pregnant and preeclamptic patients.

Fig. 6. Creatinine values in healthy non-pregnant ,pregnant and preeclamptic patients.

Increased vasoconstriction resulting in maternal hypertension and reduced uteroplacental blood flow, disturbed vascular endothelial integrity with increased vascular permeability, and activation of the coagulation cascade. C-reactive protein (CRP) is a marker of tissue damage and inflammation.

The results of our study indicate thathemoglobin was reduced. Although the reason for the relationship of low levels of hemoglobin with complications such as preeclampsia is still completely unknown, factors such as lack of other nutrients in people with low level of hemoglobinare among the proposed reasons which need further investigations . This study showed the decrease in hematocrit in preeclampsia , while white blood cell (WBC) was elevated during pregnancy.Platelet count recorded a decreased during the third trimesterassociated with high CRP levels in pregnancy with the development of preeclampsia.

Blood concentration is one of the main symptoms of preeclampsia and is probably caused by generalized vasoconstriction and endothelial dysfunction associated with increased vascular permeability. Depending on the

disease severity, blood concentration increases with preeclampsia, while in women with pregnancy induced hypertension, blood volume is usually normal.Also ,serum AST and creatinine levels were found to be significantly elevated in preeclampsia .Similar result had been reported by Benoit and Rey(2011).

The elevated serum transeaminase levels in PE may be due to arteriolar spam that occurs which involves the myocardium, liver and kidney. The arterioleconstriction in these tissues usually results in to hypoxia and damage to the hepatocellular integrity and thus release of AST tothe plasma .The concentration of hepatic disorder as cause of increased AST activities in PEcould be made by the determination of ALT activation which is more liver specific than AST (Benoit and Rey(2011).

Conclusion

Elevated amounts of TNFα,CRP and decreased amount of IL10 in the maternal circulation might play a central role in the generalized endothelial dysfunction characteristics of preeclampsia.

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