

## Association of inflammation with endothelial dysfunction in pregnancy induced hypertensive women

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### Abstract

**Introduction:** Pregnancy induced hypertension is considered as the major cause of maternal and perinatal mortality. Even though occurrence of PIH is due to abnormal placentation, endothelial dysfunction (ED) plays a pivotal role in the genesis of the multisystem disorder that develops in pre eclampsia and eclampsia. Excessive inflammation might lead to ED. So, a study has been designed to analyse some inflammatory markers (TNF- $\alpha$ , HsCRP, IL-6) and their association with endothelial dysfunction (NO) in PIH women.

**Materials and Methods:** Study group consisted of Normotensive pregnant women (N) preclamptic women (PE) and eclamptic women (E) with 100 subjects in each group in the 3rd trimester of pregnancy. They were investigated for TNF- $\alpha$ , IL-6, HsCRP & NO. Statistical analysis was done using ANOVA

**Results:** When compared to controls TNF- $\alpha$ , IL-6, HsCRP levels were found to be significantly high & NO levels were significantly low in PE, E group. Eclamptic women showed a significantly high level of TNF- $\alpha$ , and low levels of NO when compared to PE group.

**Conclusion:** Early careful monitoring of TNF- $\alpha$  along with NO might be helpful to predict the origination and advancement of the disease. It may help in emerging approaches for diagnosis and prevention of maternal and fetal complications.

**Keywords:** PIH- Pregnancy induced hypertension, TNF- $\alpha$  – Tumor necrosis factor-alpha, IL-6 – Interleukin-6, HsCRP-High sensitive C-reactive protein, NO- Nitric oxide.

### Introduction

Pregnancy induced hypertension (PIH) is the second most common medical disorder seen during pregnancy.<sup>1</sup> Preeclampsia (PE) is pregnancy related disorder characterized by hypertension and proteinuria that occurs after 20 weeks of gestation.<sup>2</sup> Expectant mothers with hypertension are predisposed towards the development of potentially lethal complications. The causes of hypertension during pregnancy, particularly preeclampsia (PE), remain unknown. PE is a complication of pregnancy with significant morbidity and mortality for both mother and fetus.<sup>3</sup> It effects 5-7% of pregnancies worldwide. It is a multi-organ disorder usually recognized by new onset of hypertension and proteinuria appearing in the 2nd half of pregnancy. Eclampsia is classified as presence of seizures, non-attributable to other causes, in a women diagnosed with PE.<sup>4</sup> Placenta plays a principal role in the pathogenesis of the disease.<sup>5</sup> The abnormal cytotrophoblast differentiation is an early defect that may lead to reduced placental perfusion and ischemia.<sup>6</sup> Reduced placental perfusion leads to widespread endothelial dysfunction<sup>7</sup> and it is considered as a classic hallmark of preeclampsia<sup>8</sup> which likely affects the cerebral endothelium as well leading to cerebral edema and seizures seen in eclampsia.<sup>9</sup> The contribution of endothelial dysfunction can be viewed in a superior context as part of the inflammatory network.<sup>10</sup>

During pregnancy a generalized maternal inflammatory response exists.<sup>11</sup> But, it has been hypothesized that systemic inflammation gets exaggerated in preeclampsia.<sup>11</sup> Tumor necrosis- $\alpha$  (TNF- $\alpha$ ) is a pro-inflammatory cytokine with a pleiotropic effect on the immune system, tissue homeostasis, embryonic development and placentation. When released in large amounts, TNF- $\alpha$  induces enhanced activation and injury to the vascular endothelium. IL-6 (Interleukin-6) is a proinflammatory cytokine which is involved in immune activation, vascular wall function and modulation of TNF- $\alpha$  production.<sup>12</sup> HsCRP It is an acute phase reactant produced by the liver in response to pro inflammatory cytokines, especially.<sup>13</sup> Therefore a study has been designed to analyse some inflammatory markers (TNF- $\alpha$ , HsCRP, IL-6) and their association with endothelial dysfunction (NO) in PIH women.

### Materials and Methods

A cross sectional analytical study was conducted in the inpatient ward of the Department of Obstetrics and Gynecology, Annapoorana institute of medical sciences, Salem, Tamilnadu from August 2012 to April 2016. The study was approved by the Institutional Ethics Committee of AMC&H and informed consent was obtained from all participants. PIH patients were defined according to the NHBPEP (National high blood pressure education programme) guidelines.<sup>14</sup>

### Patients and controls

Hundred each normotensive pregnant women who served as control (Group1), PE (Group2) & E (Group3) patients were selected. The mean SBP in 3 groups were recorded as (116 ± 5.45 vs. 162.18 ± 18.26 vs. 170 ± 15.52) mm Hg. The mean DBP in 3 groups were recorded as (75 ± 5.99 vs. 107.5 ± 11.35 vs. 112.28 ± 10.59) mm Hg. The urine albumin levels in 3 groups were measured as (150.92 ± 33.4 vs. 436 ± 96 vs. 432 ± 101) mg/d. Preeclampsia was defined as having a systolic blood pressure 140 mm Hg or a diastolic blood pressure 90 mm Hg with proteinuria 300 mg/d. Sign and symptoms of PIH like swelling in the hands, face and feet, severe headaches, abdominal pain, reduced output of urine or no urine, blood in the urine, a change in reflexes, convulsion/seizures, coma, dizziness, excessive vomiting, nausea and rapid gain in weight were recorded. Eclamptic complications like cerebrovascular, cardio vascular, visual, pulmonary, renal, hepatic, haemostatic and obstetrical were also recorded. Individuals with past history of cardiac, renal, hepatic illness, diabetes, and hypertension were excluded.

### Statistical Analysis

The quantitative variables like Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), serum TNF- $\alpha$ , IL-6, HsCRP, and NO were compared between PE, E women & Normotensive controls. The same was also compared between PE & E. The data was processed on computer software package SPSS version

20. The numerical data was presented as Mean ± SD. A value of P < 0.05 at 95% CI was considered as statistically significant.

### Results

**Table 1** shows base line characteristics like maternal age, gestational age, SBP, DBP, urine albumin between 3 groups. The SBP, DBP, urine albumin levels were significantly higher in PE & E women (P= 0.000, P= 0.000, P= 0.000) than controls and the levels were significantly high in eclamptic women compared to PE patients (P=0.001, P=0.000, P=0.000). The maternal age and gestational age was almost comparable between all the groups.

**Table 2** shows mean TNF- $\alpha$ , IL-6, HsCRP, and NO values between 3 groups. Preeclamptic and eclamptic patients showed a significantly higher serum TNF- $\alpha$ , IL-6, HsCRP levels and significantly lower NO than controls (P=0.000, P=0.000, P=0.000, P=0.000) and the TNF- $\alpha$  levels were significantly high (P=0.000) and NO levels were significantly low (P=0.000) in eclamptic women than preeclampsia whereas IL-6 & HsCRP levels were not significant between PE & E.

**Table 3** shows the correlation analysis between inflammatory markers (TNF- $\alpha$ , IL-6, HsCRP) and endothelial dysfunction (NO). The inflammatory markers were negatively correlated with Nitric oxide (NO).

**Table 1: Comparison of baseline characteristics between the groups**

Parameters	Controls		Pre-eclampsia		P value	Eclampsia		P value	P-value between PE & E
	Mean	SD	Mean	SD		Mean	SD		
Age (Years)	23.97	3.30	24.62	4.07	NS	25.71	3.71	<0.01	NS
Gestational Age (weeks)	31.57	2.67	32.42	3.12	NS	31.05	2.90	NS	NS
SBP (mm Hg)	116	5.45	162.18	18.26	<0.01	170	15.52	<0.01	<0.01
DBP (mm Hg)	75	5.99	107.5	11.35	<0.01	112.28	10.59	<0.05	<0.01
Urine albumin (mg/day)	150.9	33.4	436	96	<0.01	432	101	<0.01	<0.01

\*Data expressed as Mean±SD. A p value of <0.05 is considered as significant.

\*\*Data expressed as Mean±SD. A p value of <0.01 is considered as highly significant.

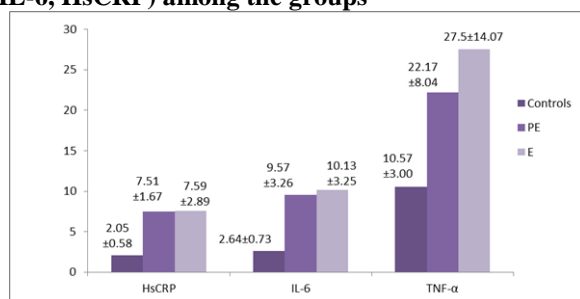
**Table 2: Comparison of levels of inflammatory markers and NO between the groups**

Parameters	Controls		Pre-eclampsia		P value	Eclampsia		P value	P-value between PE & E
	Mean	SD	Mean	SD		Mean	SD		
TNF- $\alpha$ (pg/ml)	10.57	3.00	22.17	8.04	<0.01	27.50	14.07	<0.01	<0.01
IL-6 (pg/ml)	2.64	0.73	9.57	3.26	<0.01	10.13	3.25	<0.01	NS
HsCRP (mg/L)	2.05	0.58	7.51	1.67	<0.01	7.59	2.89	<0.01	NS
NO ( $\mu$ mol/L)	117.37	14.77	43.8	6.13	<0.01	38.6	9.94	<0.01	<0.01

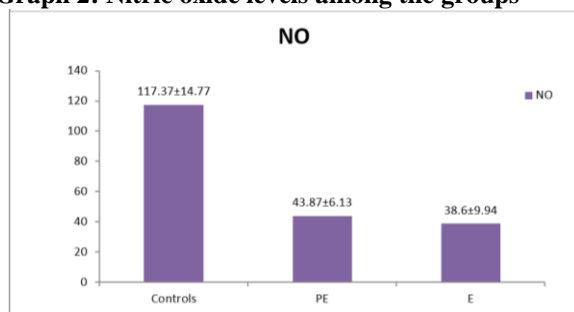
\*Data expressed as Mean±SD. A p value of <0.05 is considered as significant.

\*\*Data expressed as Mean±SD. A p value of <0.01 is considered as highly significant.

**Graph 1: Levels of Inflammatory markers (TNF- $\alpha$ , IL-6, HsCRP) among the groups**



**Graph 2: Nitric oxide levels among the groups**



**Table 3: Correlation analysis between inflammatory markers and endothelial dysfunction**

Parameter	NO (µmol/L) 'r' value
TNF- $\alpha$ (pg/ml)	-0.586**
IL-6 (pg/ml)	-0.766**
HsCRP(mg/L)	-0.760**

**Discussion**

Pregnancy-Induced Hypertension (PIH) remains to be a main obstetric problem in existing healthcare practice. It affects maternal health and also puts fetal development at risk.<sup>15</sup> Normal pregnancy is characterized by a mild systemic inflammation, but the inflammation may become damaging if dysregulated.<sup>16</sup> Increased inflammatory changes during pregnancy may be explained by different stimuli occurring at different phases of pregnancy such as implantation, monocyte/macrophage production stimulated by interleukin-6 (IL-6) and the necrotic process associated with placental ageing.<sup>17</sup> Added to these reasons, in PE because of the placental hypo perfusion, ROS and cytokines are released from the placenta which may induce oxidative stress, inflammatory response and endothelial cell dysfunction in mother.<sup>18</sup> An excessive inflammatory reaction has been associated with recurrent miscarriage or other pregnancy complications such as pre-eclampsia or premature labor <sup>19</sup>. In this respect our study displayed significant higher levels of TNF- $\alpha$ , IL-6, HsCRP in PE & E women compared to the normotensive pregnant women. But, only TNF-  $\alpha$  levels were significantly higher in eclamptic women

compared to pre-eclamptics whereas IL-6 & HsCRP were not significant.

TNF- $\alpha$  and IL-6 are some of the pro-inflammatory cytokines playing an important role in activation of immune system among pre-eclamptic women and are associated with disease severity.<sup>20</sup> TNF-alpha is produced by monocytes, induces apoptosis, and inhibits proliferation of trophoblast cells in preeclampsia.<sup>21</sup> Pathologically secreted TNF- $\alpha$  damage the vascular endothelial cells by causing occlusion of vessels there by reducing the regional blood flow leading to the increase in the permeability of endothelium.<sup>22</sup> The displayed high levels of TNF – alpha in our study can be explained as a consequence of placental hypoxia which exists in PIH.<sup>23</sup> The ROS generated by hypoxia /reoxygenation insults of placenta might also play a central role in placental expression and production of TNF-alpha by direct activation of p38 MAP kinase (mitogen activated kinase) and NF-KB (Nuclear factor).<sup>24</sup> Furthermore, in preeclampsia the placenta derived factors might stimulate monocytes and neutrophils to produce TNF- $\alpha$  that lead to endothelial disturbances.<sup>25</sup> Generally the monocytes are the main reservoir for proinflammatory cytokines and therefore can be good candidates for excessive TNF- $\alpha$  synthesis in preeclampsia.<sup>26</sup>

Unlike most cytokines, circulating TNF- $\alpha$  can cross the blood brain barrier (BBB) through receptor-mediated endocytosis. The binding of TNF- $\alpha$  to its receptors on the BBB upsurges paracellular permeability that can promote vasogenic edema.<sup>27</sup> TNF- $\alpha$  up regulates endothelial cell adhesion molecules such as E-selectin, ICAM-1 and VCAM-1 that facilitate passage of leukocytes into the brain. Leukocyte infiltration of the BBB has been revealed to be seizure provoking by activating microglia that can then produce TNF- $\alpha$ . In the brain, the production of TNF- $\alpha$  lower the seizure threshold and cause seizures <sup>28</sup> by altering the balance of excitation AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and inhibition (GABAA ( $\gamma$ -amino butyric acid receptor) receptors.<sup>28</sup>

Many reports indicated that the plasma of pre-eclamptic patients contain elevated levels of IL-6, a multifunctional cytokine that regulates hematopoiesis along with acute phase reaction. It controls both pro- and anti-inflammatory events. In the present study elevated IL-6 levels might be due to the characteristic decidual secretion. TNF-alpha, markedly up-regulates the IL-6 mRNA and its protein expression by the resident decidual cells.<sup>26</sup>

Moreover plasma from pre-eclamptic women activates vascular endothelial cells through an NF- $\kappa$ B-mediated mechanism, these cells could be a potential source of increased circulating IL-6 that is seen in this disease.<sup>29</sup> Elevated IL-6 interferes with endothelial cell function by increasing the endothelial cell permeability by changing the cell shape and rearrangement of

intracellular actin fibers.<sup>23</sup> It increases the thromboxane A2 to prostacyclin ratio; reduce prostacyclin (PG I2) synthesis by inhibiting the cyclooxygenase enzyme and stimulating the platelet derived growth factor. It could also trigger the neutrophil activation, expression of von Willebrand factor and cell adhesion on the endothelium resulting in vascular damage.<sup>26</sup> The inflammatory mediators TNF- $\alpha$ , IL-8 and IL 1- $\beta$  synergizes with elevated plasma IL-6 levels to promote systemic vascular damage, particularly in the kidney, that results into a characteristic proteinuria and hypertension of the maternal syndrome of PE.<sup>30</sup>

C-reactive protein (CRP) is an acute phase protein which is increased in systemic inflammation. During the challenges like severe tissue injury, microbial infections, systemic autoimmune disease and malignant tumors it is mainly synthesized by hepatocytes. Our results regarding HsCRP were in consistent with several studies.<sup>17,18</sup> The reason might be due to the regulation of production by the correspondent gene located on the long arm of chromosome 1, induced at the transcriptional level by IL-6 & tumor necrosis factor-alpha (TNF- $\alpha$ ).<sup>17,29</sup> The cytokines exert their biological effects on CRP by signaling through their receptors on hepatic cells there by activating different kinases and phosphatases. This leads to the translocation of various transcription factors on the CRP gene promoter and the production of CRP. The concentration of CRP doubles for every 8 hours and peaks at 36-50 hours, while it depends on the stimulus and its severity. CRP concentration can increase above 500 mg/l and this amounts to as much as a 1000-fold or more concentration variation in response to a inflammatory insult. CRP, in agreement with its proposed function, may play a role in eliciting the inflammatory response characteristics of preeclampsia.<sup>31</sup> However, the nonsignificant levels of IL-6 and HsCRP between PE & E groups displayed in our study need to be evaluated further.

NO is an endothelium derived factor responsible for vasodilatation and platelet activation inhibition. It is involved in various stages of pregnancy including implantation, maintenance of uterine acquiescence during pregnancy, control of uterine contractions and relaxation, basic physiological adaption for successful gestation and regulation of blood pressure.<sup>32</sup> The hallmark of ED is impaired NO bioavailability.<sup>33</sup> Our study displayed significantly low levels of NO in PE & E women than in controls. The levels of NO were significantly low in eclamptic women than in preeclamptic women. Our results were in consistent with other studies.<sup>34,35</sup> The inflammatory cytokines can also inhibit eNOS, causing reduction in NO levels leading to vasoconstriction in the peripheral circulation.<sup>36</sup> It has also been stated that arginine deficiency can also reduce NO and increase superoxide formation, leading to NO degradation and excess peroxynitrite formation.<sup>37</sup>

## Conclusion

Our results indicate that the women with PE & E exhibit markedly elevated concentrations of inflammatory cytokines (TNF- $\alpha$ , IL-6, HsCRP). However the high levels of TNF- $\alpha$  among eclamptics than preeclamptics in our study indicate that TNF- $\alpha$  is a better marker for predicting the onset and progression of disease. Moreover, the negative correlation between inflammatory cytokines with NO indicates that the inflammation is directly related with the severity of ED in PIH women. So, early careful monitoring of TNF- $\alpha$  along with NO might help to predict the origination and advancement of the disease.

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