Association of Inflammatory Cytokines, Lipid Peroxidation End Products and Nitric Oxide with the Clinical Severity and Fetal Outcome in Preeclampsia in Indian Women

Devika Tayal · Binita Goswami · S. K. Patra ·
Reva Tripathi · Alka Khaneja

Received: 18 January 2013 / Accepted: 24 March 2013 / Published online: 13 April 2013
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Abstract Preeclampsia is a multisystem disorder associated with maternal hypertension, placental abnormalities and adverse fetal outcomes. The various pathways involved in its etiology include endothelial dysfunction, inflammatory milieu, lipid peroxidation and immunological imbalance. The present study was conducted to evaluate the causative and predictive role of nitric oxide, lipid peroxidation end products (MDA) and inflammatory cytokines (IL-6, TNF- α) in clinical presentation, severity and fetal outcome in preeclampsia. The study population was divided into 3 groups- Non- pregnant females comprising the control population; G1 and G2 groups included normal pregnant and pregnant females with preeclampsia with 50 patients in each group. Nitric Oxide and MDA levels were found to be highest in the preeclamptic patients as compared to other two groups. ROC curve analysis shows the superiority of the inflammatory markers as determinants of

severity of preeclampsia which suggests the emerging role of pro inflammatory markers in the various pathological changes in preeclampsia. TNF- α emerged as the best marker in multivariate analysis and thus, has the potential for being used as a marker for PIH. Our study illustrates the multifactorial etiology of preeclampsia involving oxidative stress, proinflammatory milieu and endothelial dysfunction.

Keywords Preeclampsia · Inflammation · IL-6 · TNF- α · Oxidative stress · Lipid peroxidation · MDA · Endothelial dysfunction · NO

Introduction

Preeclampsia is a disorder observed in 7–10 % of all pregnancies. It is a multisystem disorder associated with maternal hypertension, placental abnormalities and adverse fetal outcomes. It is diagnosed by the appearance of hypertension and proteinuria after 20 weeks of gestation [1]. The pathophysiological hallmarks of preeclampsia include generalized vasoconstriction, increased vasoactivity, reduced perfusion to organs, and platelet activation [2].

The etiology behind preeclampsia is debatable and still under intense research. The various pathways implicated from time to time include endothelial dysfunction, inflammatory milieu, lipid peroxidation, immunological imbalance among others [3]. Nitric oxide is an important modulator of endothelial reactivity. Studies have shown that NO levels are altered in patients with preeclampsia. The results have been equivocal with studies showing elevated, decreased as well as similar NO levels in preeclamptic patients as compared to controls [4].

Oxidative stress has been shown to be involved in the pathogenesis of a number of diseases. Elevated reactive

D. Tayal
Department of Biochemistry, Lala Ram Swaroop Institute, New Delhi, India

B. Goswami (✉) · S. K. Patra
Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India
e-mail: binita.dr@gmail.com

S. K. Patra
e-mail: drsurajeetlhm@gmail.com

R. Tripathi
Department of Obstetrics & Gynaecology, Maulana Azad Medical College & Associated LN Hospital, New Delhi, India
e-mail: linkbinita@rediffmail.com

A. Khaneja
Department of Biochemistry, Maulana Azad Medical College & Associated LN Hospital, New Delhi, India
e-mail: binitadr@yahoo.co.in

oxygen species (ROS) have been observed in preeclampsia [5]. The underlying probable mechanism is the stimulation of neutrophils by shed syncytiotrophoblast membranes into the maternal circulation. Oxidative stress involving the generation of free radicals, lipid peroxides and ROS further aggravates the underlying endothelial dysfunction [6].

Recently pro inflammatory response in response to a multitude of placental and maternal factors has been attributed to the development and aggravation of preeclampsia. Some researchers hypothesized that the inadequate remodeling of uterine arteries characteristic of preeclampsia is responsible for the focal ischemia and generation of pro inflammatory cytokines such as Interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) by the placenta [7–9]. IL-6 enhances endothelial cell permeability, aberrant angiogenesis and inhibits prostacyclin synthesis thereby modifying vascular physiology profoundly [10]. TNF- α inhibits decidual invasion by cytotrophoblasts, stimulates cell proliferation and hypoxia-mediated cell activation. It also induces structural and functional alterations in endothelial cells, endothelin-1 (ET-1) production and inhibition of acetylcholine induced vasodilatation [11, 12].

The present study was conducted to evaluate the causative and predictive role of NO, lipid peroxidation end products (MDA) and inflammatory cytokines (IL-6, TNF- α) in the determination of clinical presentation, severity and fetal outcome in preeclampsia.

Materials and Methods

The study was conducted by the Department of Biochemistry in collaboration with the Department of Obstetrics & Gynaecology, Maulana Azad Medical College and associated hospitals. The study was approved by the Institutional Ethical Committee and the enrollment of the study population was commenced after informed consent.

The study population was divided into 3 groups with G0 comprising of non pregnant females comprising the control population; G1 and G2 groups included normal pregnant and pregnant females with preeclampsia respectively. There were 50 subjects in each group. The subjects belonging to groups G1 and G2 were recruited from the antenatal OPD after detailed history taking and examination. The control population (G0) comprising of age matched non pregnant healthy females was recruited from among the healthy volunteers in the Departments of Biochemistry and Obstetrics & Gynaecology, LNH. Subjects with history of any renal, liver and metabolic disease were excluded from the study. History of any chronic inflammatory process, acute infections and any surgical intervention in the recent 3 months also resulted in exclusion from the study population.

Preeclampsia was defined by the criteria of hypertension, proteinuria and hyperuricemia. Hypertension was defined as an increase of 30 mmHg systolic or 15 mmHg diastolic pressure compared with values obtained before 20 weeks of gestation, or an absolute blood pressure greater than 140/90 mmHg after 20 weeks of gestation if earlier blood pressures were not known. Proteinuria was defined as greater than 500 mg/24 h collection or greater than 2+ on a voided or greater than 1+ on a catheterized random urine specimen. Hyperuricemia was defined as more than 1 SD above usual values (at term, >5.5 mmol/L) [13, 14].

Severe preeclampsia was defined based on the American College of Obstetricians and Gynecologists criteria: gestational age >20 weeks, blood pressure of 160 mmHg systolic or higher or 110 mmHg diastolic or higher on 2 occasions at least 6 h apart, and/or proteinuria of at least 5 g in a 24-hour urine specimen or 3+ or greater on 2 random urine samples collected at least 4 h apart. Other elements of the diagnosis included: IUGR (<10 th percentile), cerebral or visual disturbances (headache, visual changes), epigastric or right upper-quadrant pain, pulmonary edema or cyanosis, oliguria (urinary output less than 500 mL/24 h), impaired liver function, and thrombocytopenia ($<100,000$ cells/ μ L) [15].

The serum samples were collected after overnight fast in appropriate vacutainers maintaining appropriate aseptic conditions. The baseline investigations—hemogram, liver function and kidney function tests and lipid profile were conducted in the study population. The specialized tests included- malondialdehyde (MDA), NO, IL-6 and TNF- α . MDA was analysed in blood by the method described by Yagi [16]. NO was estimated by the modified Griess method [17]. IL-6 and TNF- α were estimated by commercially available ELISA by Diaclone, France.

Statistical Analysis

The data were expressed as the mean $\pm$ standard deviation. Anova test with Tukey's post hoc analysis was used to compare the values between the three groups. Spearman's correlation analysis was used to find the association between the various parameters of our study. Receiver operating characteristics (ROC) curves were plotted to determine the superiority of the markers under study. A P value of <0.05 was accepted as statistically significant. All statistical analyses were performed with the program Statistical Package for the Social Science 12.0 (SPSS Inc, Chicago, Illinois).

Results

The demographic data of the study population is depicted in Tables 1 and 2. The subjects in all the three groups were

age matched. The body weight was significantly higher in the subjects with pregnancy induced hypertension. The parameters under evaluation- MDA, NO, IL-6 and TNF were significantly higher in the group comprising of pre-eclamptic patients. The levels of these parameters were significantly higher in the pregnant females except IL-6. The fetal weight was significantly lower in the subjects with preeclampsia.

The correlation analysis between the different parameters is depicted in Table 3. A significantly positive correlation was observed between NO and uric acid, MDA and urinary proteins. A statistically significant negative correlation was observed between NO levels and fetal weight. A similar association was observed between MDA levels with fetal weight with correlation coefficient of -0.869 . Severe proteinuria was also an indicator of adverse fetal outcome with correlation coefficient of -0.664 . A statistically significant correlation was observed between MDA, urinary proteins and uric acid.

ROC curve analysis is depicted in Table 4. The data shows the superiority of the inflammatory markers as determinants of severity of preeclampsia. They were closely followed by NO and MDA with area under the curves (AUC) as 0.995 and 0.981 respectively. Multivariate analysis is depicted in Table 5. The analysis illustrates the superior predictive role of TNF in assessing the severity of preeclampsia. This was followed by IL-6.

Discussion

Preeclampsia is a major determinant of maternal and fetal morbidity and mortality. A number of diverse etiological pathways and mediators have been implicated in its pathogenesis. The following study was planned to assess the role of NO, MDA, IL-6 and TNF as prognostic determinants.

Uric acid is considered to be a marker of PE. It consistently rises from the first trimester in women predisposed to preeclampsia. Elevated uric acid levels lead to reduced production of NO and endothelial dysfunction. It has also been implicated in stimulation of pro inflammatory cytokine release from monocytes [18].

The role of NO in preeclampsia is controversial with studies reporting increased, decreased as well as equivocal values of NO in pre eclampsia as compared to normal subjects. Variations in the subject ethnicity, methodology may account for these discrepancies [19]. The NO levels were found to be highest in the preeclamptic patients as compared to normal pregnant as well as non pregnant controls. Our findings are in concordance with Palmer et al. [20] who reported high NO levels in preeclamptic patients. The increase in NO levels may be due to over expression of nitric oxide synthase (NO) and consequent increased production. NO inflicts maximum damage by forming the free radical—peroxynitrite—which leads to the microvascular damage observed in preeclampsia [4]. It also interferes with normal protein function by modifying tyrosine residues in proteins resulting in nitrotyrosine residues. It may promote vascular dysfunction in PE by increasing the degradation of HIF-1 α and stimulation of matrix metalloproteinases. Peroxynitrite also plays a role in mediating apoptosis and modulating eicosanoid biosynthesis. DNA breaks caused by peroxynitrite cannot be repaired by poly(ADP- ribose) polymerase and further leads to depletion of NAD and ATP stores. Immunostaining for eNOS, SOD and NO in the blood vessels of preeclamptic patients resulted in increased eNOS and NO staining and decreased SOD staining [21]. It can be concluded that initially there is compensatory increase in NO levels to maintain uteroplacental and fetoplacental blood flow and nitric oxide being double edged sword later acts as an oxidant and causes further free radical injury in preeclampsia.

Table 1 Comparison of the various epidemiological and biochemical parameters in the study population

Parameter	G0	G1	G2
Age (years)	26 $\pm$ 4.6	26.3 $\pm$ 4.4	26.1 $\pm$ 4.3
Weight (kg)	53.0 $\pm$ 3.3	55.1 $\pm$ 6.67	64.7 $\pm$ 7.64
Urea (mg/dL)	24.7 $\pm$ 4.67	20.7 $\pm$ 3.5	26.2 $\pm$ 14.8
Creatinine (mg/dL)	0.74 $\pm$ 0.14	0.77 $\pm$ 0.16	0.84 $\pm$ 0.43
Uric Acid (mg/dL)	2.83 $\pm$ 0.31	3.0 $\pm$ 0.45	3.6 $\pm$ 1.14
Calcium (mg/dL)	8.7 $\pm$ 0.97	8.5 $\pm$ 0.9	8.3 $\pm$ 1.0
MDA (nmol/mL)	1.76 $\pm$ 0.26	2.93 $\pm$ 0.57	4.66 $\pm$ 0.94
NO (μ mol/L)	29.1 $\pm$ 5.37	66.1 $\pm$ 11.1	115.8 $\pm$ 17.9
IL6 (pg/mL)	1.3 $\pm$ 0.16	2.4 $\pm$ 0.18	31.7 $\pm$ 3.9
TNF (pg/mL)	24.6 $\pm$ 2.2	43.9 $\pm$ 1.9	271.1 $\pm$ 14.3
Fetal weight (kg)	–	2.7 $\pm$ 0.26	2.3 $\pm$ 0.95

G0 non pregnant, G1 normal pregnant, G2 pregnant with pregnancy induced hypertension

Table 2 Comparison of various parameters among the three groups

Parameter	G0 vs. G1	G0 vs. G2	G1 vs. G2
Age (years)	NS	NS	NS
Weight (kg)	NS	<0.001	<0.001
Urea (mg/dL)	NS	NS	NS
Creatinine (mg/dL)	NS	NS	NS
Uric Acid (mg/dL)	NS	<0.001	<0.001
Calcium (mg/dL)	NS	NS	NS
MDA (nmol/mL)	<0.001	<0.001	<0.001
NO (μ mol/L)	<0.001	<0.001	<0.001
IL6 (pg/mL)	NS	<0.001	<0.001
TNF (pg/mL)	<0.001	<0.001	<0.001

Table 3 Correlation among the various parameters under study in the subjects with preeclampsia

Parameter	r	P
NO vs. MDA	0.412	0.024
NO vs. uric acid	0.438	0.016
NO vs. urinary proteins	0.552	0.002
NO vs. fetal age	−0.545	0.002
MDA vs. fetal weight	−0.869	<0.001
MDA vs. uric acid	0.608	<0.001
MDA vs. urinary protein	0.563	0.001
Urinary protein vs. fetal weight	−0.664	<0.001
Urinary protein vs. uric acid	0.472	0.009
Uric acid vs. fetal weight	−0.457	0.011
IL-6 vs. fetal weight	−0.667	<0.001

Table 4 ROC characteristics of the parameters under study

Parameter	AUC	95 % CI	P
Uric acid (mg/dL)	0.984	0.957–1.011	<0.001
MDA	0.981	0.960–1.003	<0.001
NO	0.990	0.984–1.006	<0.001
IL-6	0.992	0.986–1.008	<0.001
TNF	0.995	0.987–1.010	<0.001
Urinary protein	0.783	0.667–0.900	<0.001

Table 5 Multivariate analysis of the various parameters under study

Parameter	Coefficient of determination (R^2)	Regression coefficient β	P value
MDA	0.560	−0.002	NS
TNF	0.992	0.848	<0.0001
IL6	0.966	0.128	0.039
NO	0.741	0.028	NS

Lipid profile is also deranged in preeclampsia with increased serum concentrations of triglycerides and LDL-cholesterol. These serve as substrates for the generation of lipid peroxides which lead to endothelial damage and oxidative damage [22]. One of the first biomarkers of lipid peroxidation found to be elevated in the plasma of women with preeclampsia was MDA, a major metabolite of lipid

peroxide breakdown. MDA may modify the amino acid side chains resulting in the formation of additional carbonyl groups. The lipid peroxides lead to vasoconstriction by inhibiting prostacyclin synthase but not thromboxane synthase enzyme [23].

Another striking finding in our study is the increased pro inflammatory cytokine levels in the patients with pre

eclampsia as compared to normal healthy controls. Similar results were reported by Founds et al. [11]. In fact TNF emerged as the best marker for the disease in multivariate analysis. This suggests the emerging role of pro inflammatory milieu in instigating the various pathological changes that are pathognomonic of preeclampsia-endothelial dysfunction, prothrombotic environment and the various metabolic perturbations in PE. It has been hypothesized that inadequate remodeling of the uterine spiral arteries leads to the generation of these pro inflammatory cytokines by the placenta. TNF- α modulates growth, differentiation, lipid metabolism, coagulation, insulin resistance and inflammation [9]. It also leads to structural and functional alterations in ECs, enhances the formation of ET-1, reduces acetylcholine-induced vasodilatation and destabilizes eNOS mRNA [24]. TNF- α leads to apoptosis of trophoblastic cells and inhibits their migration. The resultant defective placentation is the hallmark of PE [9, 25].

IL-6 is a pleiotropic cytokine that plays a role in acute phase reaction. It has been shown to interfere with endothelial function by altering ultrastructural distribution of tight junctions which is the underlying pathology in preeclampsia [26]. It also induces aberrant angiogenesis and inhibits endothelial prostacyclin synthesis [27]. Similar to our findings, Page et al. [28] and Tosun et al. [29] have reported high levels of IL-6 and TNF in patients with PE.

Our study illustrates the multifactorial etiology of preeclampsia involving a multitude of factors such as oxidative stress, proinflammatory milieu and endothelial dysfunction. Our study also demonstrated the prognostic implications of these factors on fetal outcome. A better insight into the pathophysiology of this condition will help in early detection and better management of this disorder.

References

1. Davidge ST, Signorella AP, Hubel CA, Lykins DL, Roberts JM. Distinct factors in plasma of preeclamptic women increase endothelial nitric oxide or prostacyclin. *Hypertension*. 1996;28:758–64.
2. Friedman SA, Taylor RN, Roberts JM. Pathophysiology of preeclampsia. *Clin Perinatol*. 1991;18:661–82.
3. Sheppard SJ, Khalil RA. Risk factors and mediators of the vascular dysfunction associated with hypertension in pregnancy. *Cardiovasc Hematol Disord Drug Targets*. 2010;10(1):33–52.
4. Baker PN, Davidge ST, Roberts JM. Plasma from women with preeclampsia increases endothelial cell nitric oxide production. *Hypertension*. 1995;26:244–8.
5. Redman CW, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review. *Placenta*. 2003;24:S21–7.
6. Tsukimori K, Fukushima K, Tshushima A, Nkano H. Generation of reactive oxygen species by neutrophils and endothelial cell injury in normal and preeclamptic pregnancies. *Hypertension*. 2005;46:696.
7. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. *Am J Reprod Immunol*. 1997;37:240–9.
8. Conrad KP, Miles TM, Benyo DF. Circulating levels of immunoreactive cytokines in women with preeclampsia. *Am J Reprod Immunol*. 1998;40:102–11.
9. Benyo DF, Smarason A, Redman CWG, Sims C, Conrad KP. Expression of inflammatory cytokines in placentas from women with preeclampsia. *J Clin Endocrinol Metab*. 2001;86(6):2505–12.
10. Lockwood CJ, Yen CF, Basar M, Kayisli UA, Martel M, Buhimschi I, et al. Preeclampsia-related inflammatory cytokines regulate interleukin-6 expression in human decidual cells. *Am J Pathol*. 2008;172(6):1571–9.
11. Founds SA, Powers RW, Patrick TE, Ren D, Harger GF, Markovic N, Roberts JM. A comparison of circulating TNF alpha in obese and lean women with and without preeclampsia. *Hypertens Pregnancy*. 2008;27(1):39–48.
12. Vitoratos N, Economou E, Iavazzo C, Panoulis K, Creatsas G. Maternal serum levels of TNF-alpha and IL-6 long after delivery in preeclamptic and normotensive pregnant women. *Mediators Inflamm*. 2010;2010:908649.
13. Brown MA, Lindheimer MD, de Swiet M, van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertens Pregnancy*. 2001;20:IX–XIV.
14. Curtin WM, Weinstein L. A review of HELLP syndrome. *J Perinatol*. 1999;19:138–43.
15. Lewis DF, Canzonieri BJ, Wang Y. Maternal circulating TNF- α levels are highly correlated with IL-10 levels, but not IL-6 and IL-8 levels, in women with pre-eclampsia. *Am J Reprod Immunol*. 2009;62(5):269–74.
16. Yagi K. Assay for blood plasma or serum. *Methods Enzymol*. 1984;105:328–31.
17. Mathew B, Johnson GJ, Lancaster JR. Quantitation of nitrate and nitrite in extracellular fluid. *Methods Enzymol*. 1996;268:237–45.
18. D'Anna R, Baviera G, Scilipoti A, Leonardi I, Leo R. The clinical utility of serum uric acid measurements in pre-eclampsia and transient hypertension in pregnancy. *Panminerva Med*. 2000;42:101–3.
19. Sandrim VC, Palei ACT, Metzger IF, Gomes VA, Cavalli RC, Tanus-Santos JE. Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia. *Hypertension*. 2008;52:402–7.
20. Palmer RMJ, Bridge L, Foxwell NA, Moncada S. The role of nitric oxide in endothelial cell damage and its inhibition by glucocorticoids. *Br J Pharmacol*. 1992;105:11–2.
21. Roggensack AM, Zhang Y, Davidge ST. Evidence for peroxynitrite formation in the vasculature of women with preeclampsia. *Hypertension*. 1999;33:83–9.
22. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during preeclampsia linking placental ischemia with endothelial dysfunction. *Hypertension*. 2001;38:718–22.
23. Rajmakers MTM, Dechend R, Poston L. Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension*. 2004;44:374–80.
24. LaMarca BB, Bennett WA, Alexander BT, Cockrell K, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of tumor necrosis factor-alpha. *Hypertension*. 2005;46(4):1022–5.
25. Meekins JW, McLaughlin PJ, West DC, McFadyen IR, Johnson PM. Endothelial cell activation by tumour necrosis factor-alpha (TNF- α) and the development of pre-eclampsia. *Clin Exp Immunol*. 1994;98(1):110–4.

26. Desai TR, Leeper NJ, Hynes KL, Gewertz BL. Interleukin-6 causes endothelial barrier dysfunction via the protein kinase C pathway. *J Surg Res.* 2002;104:118–23.
27. Maruo N, Morita I, Ishizaki Y, Murota S. Inhibitory effects of interleukin 6 on prostaglandin I2 production in cultured bovine vascular endothelial cells. *Arch Biochem Biophys.* 1992;292: 600–4.
28. Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, et al. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. *Nature.* 2000;405(6788):797–800.
29. Tosun M, Celik H, Avci B, Yavuz E, Alper T, Malatyalioglu E. Maternal and umbilical serum levels of interleukin-6, interleukin-8, and tumor necrosis factor- α in normal pregnancies and in pregnancies complicated by preeclampsia. *J Matern Fetal Neonatal Med.* 2010;23(8):880–6.