Neutrophil and Platelet to Lymphocyte Ratio in Prevailing the Oxidative Stress and Its Relation with the Endothelial Dysfunction in Preeclampsia

Article *in* Journal of Krishna Institute of Medical Sciences University · May 2020

CITATIONS
0

READS
16

5 authors, including:

Rajeev Gandham
Sri Devaraj Urs Medical College
9 PUBLICATIONS **15** CITATIONS

SEE PROFILE

Cd Dayanand
Sri Devaraj Urs Medical College
38 PUBLICATIONS **89** CITATIONS

SEE PROFILE

ORIGINAL ARTICLE

Neutrophil and Platelet to Lymphocyte Ratio in Prevailing the Oxidative Stress and Its Relation with the Endothelial Dysfunction in Preeclampsia

Rajeev Gandham[1], Sumathi ME[1], CD Dayanand[1], SR Sheela[2], Kiranmayee P[3]*

[1]Department of Biochemistry, [2]Department of Obstetrics and Gynaecology, [3]Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Medical College, SDUAHER, Tamaka, Kolar-563103 (Karnataka) India

Abstract:

Background: Preeclampsia (PE) is a pregnancy specific, hypertensive disorder. It affects 2-8% pregnancies. Oxidative stress and systemic inflammation are proposed to contribute significantly to the preeclampsia pathophysiology. The present study, aim is to determine and compare the markers of oxidative stress, endothelial dysfunction, systemic inflammatory markers Neutrophil/Lymphocyte Ratio (NLR) and Platelet/ Lymphocyte Ratio (PLR) in preeclampsia and gestational age matched healthy controls. *Material and Methods:* This study was conducted in the Department of Biochemistry and Department of Obstetrics and Gynecology, Sri Devaraj Urs Medical College, Kolar, Karnataka. The study included 98 preeclamptic women and 98 normotensive pregnant women. Five ml venous blood was collected from all the study subjects. Blood sample in EDTA vials was used for the complete blood count. NLR and PLR were calculated. Plasma was used for Ferric Reducing Ability of Plasma (FRAP) assay. Serum was used for the estimation of Malondialdehyde (MDA), nitric oxide, blood sugar, renal parameters and liver enzymes i.e., Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH) and magnesium. Corresponding urine samples were collected for urinary protein analysis by dipstick method. Fetal outcome was recorded. *Results:* Gestational age was significantly low in preeclamptic women as compared to those of controls. Blood pressure (Systolic and diastolic), mean arterial pressure, body mass index, pulse rate, serum creatinine, uric acid, AST, ALT, LDH, MDA and NLR were increased significantly in preeclamptic women as compared to those of controls. In subgroup analysis, NLR was increased significantly in severe preeclamptics as compared to mild preeclamptics. Serum Nitric Oxide (NO) and FRAP levels were decreased significantly in preeclamptic women as compared to those of controls. Significantly decreased birth weight was observed in babies born to preeclamptic mothers compared with controls. *Conclusion:* The present study results conclude that increased oxidative stress in terms increased MDA, decreased NO and reduced antioxidant status (FRAP) in preeclamptic women, results in adverse perinatal outcome. In addition, maternal NLR could be considered as a marker for severity of preeclampsia.

Keywords: Ferric Reducing Ability of Plasma, Fetal Outcome, Malondialdehyde, Nitric oxide

Introduction:

Preeclampsia (PE) is a pregnancy specific hypertensive (Blood pressure ⩾ 140/90 mmHg on two occasions, at least six hours apart) disorder associated with proteinuria (0.3g/day or a dipstick of 1+) after 20 weeks of gestation [1]. It affects 2 to 8% of all pregnancies [2]. The incidence of PE in India is around 8-10% [3]. Preeclampsia and eclampsia contributes significantly to morbidity and mortality of mothers (24%) and fetal morbidity and mortality rate is around 43/1000 live births [4]. The symptoms include persistent headache, blurred

vision, epigastric pain, vomiting and oedema [3]. The risk factors are first pregnancy, family history of previous preeclampsia/eclampsia, chronic hypertension, renal disorders, diabetes and obesity [5]. Even though exact cause of preeclampsia is unknown, a few studies evidenced that, oxidative stress is also one of the contributing factors in preeclampsia complications [4, 6].

In normotensive pregnancy, oxidative stress is a common phenomenon, but in PE, oxidative stress is elevated and hence may lead to endothelial dysfunction [7]. Endothelial cell injury and dysfunction is one of the preeclampsia cause, leads to increased systemic vascular resistance [8-9]. Preeclampsia is associated with abnormal trophoblastic differentiation and invasion that leads to altered vascular remodeling of spiral arteries, decreased placental perfusion and ischemia, results in oxidative damage, intravascular inflammation and endothelial dysfunction. Elevated Reactive Oxygen Species (ROS) may also trigger redox signaling process to induce cell apoptosis [10].

Nitric Oxide (NO) is a vasodilator, mediates endothelial function by regulating vascular tone, platelet aggregation, leukocyte adhesion and development of smooth muscle cells [10]. Several studies have reported controversial results on serum level of nitric oxide in preeclampsia [11-14]. Accelerated lipid peroxidation and lower anti-oxidant status were linked to the pathophysiology of preeclampsia by reduced perfusion and impaired fetal growth [8, 15]. Therefore, screening of oxidative stress markers facilitates to understand the balance between oxidants and antioxidants. Besides oxidative stress, inflammation also plays a crucial role in pathophysiology of preeclampsia [16, 17]. Systemic inflammatory and immuno-logical responses increase neutrophil count and modulate neutrophil function. Increased super-oxide decreased nitric oxide and inflammatory response, finally resulting in maternal endothelial dysfunction [18].

In preeclampsia, inflammatory status being evaluated by the markers such as C-reactive Protein (CRP), Neutrophil/Lymphocyte Ratio (NLR) and Platelet/Lymphocyte Ratio (PLR) have been studied as novel biomarkers in the prognosis of patients with preeclampsia [17, 19, 20]. The present study is aimed at to determine and compare the markers of oxidative stress, endothelial dysfunction, systemic inflammatory markers NLR and PLR in preeclampsia and gestational age matched healthy controls.

Material and Methods:

This prospective study was conducted in the Department of Biochemistry and Department of Obstetrics and Gynecology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India. Central ethics committee approval was obtained and written informed consent from study participants were obtained, the study population 196, consisted of 98 preeclamptic women and 98 normotensive pregnant women as control group. Preeclamptic pregnant women were sub grouped into mild (n=36) and severe preeclampsia (n=62) based on the criteria of blood pressure of $\geqslant$ 140/90 mmHg for mild and 160/110 mmHg for severe preeclampsia (ACOG guidelines 2002).

Age and gestation matched normotensive healthy pregnant women with singleton pregnancy, no fetal anomaly; nonsmokers were recruited as control group. For all the study subjects demographic, physical and clinical examinations were done. Pregnant women with history of renal disease, liver disease, thyroid disorder, chronic systemic

hypertension, gestational diabetics, epilepsy, hypertensive encephalopathy, cardio vascular diseases, pregnancy with fetal anomaly, smoking history and malignancy conditions were excluded from the study.

Sample Collection:

Sample size was estimated by using SPSS software version 22.0, with 80% power and 95% confidence interval. The sample size arrived for each group is 98, that is 98 preeclamptic subjects and 98 normotensive pregnant women. Five mL of venous blood sample was collected under aseptic conditions in plain (3 mL) and EDTA (2 mL) tubes from the preeclamptic and normotensive pregnant women visiting to the Department of Obstetrics and Gynecology, after 20 weeks of gestation period. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10 minutes to obtain the clear serum. Thus obtained clear serum samples were stored at -80°C for until analysis. Whole blood sample was used for the Complete Blood Count (CBC) in EDTA vials. NLR and PLR were calculated and plasma was used for the FRAP assay. Serum sample was used for the estimation of Malondialdehyde (MDA) by Thiobarbituric Acid (TBA) method, nitric oxide by Griess reaction method, blood sugar, renal parameters (urea, creatinine, uric acid) and liver parameters such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Lactate Dehydrogenase (LDH) and magnesium. Correspondingly five mL urine sample was collected for urinary protein analysis by dipstick method.

Statistical Analysis:

The results obtained were expressed as mean ± SD. Categorical variables were expressed as percentages (%). Mann-Whitney U test was used for continuous non-normally distributed variables. The level of significance was <0.05 considered. Data analysis was performed using SPSS software, version 22.0.

Results:

In the present study, gestational age (36.86 ± 3.55) was significantly low in preeclamptic women as compared to control group. Blood pressure [Systolic (156.18 ± 15.04) and diastolic (101.0 ± 10.59)], Mean Arterial Pressure (MAP) (119.39 ± 11.14), Body Mass Index (BMI) (27.03 ± 3.61) and pulse rate (87.42 ± 5.25) were significantly increased in preeclamptic women compared with control group as shown in table 1. Serum Creatinine (0.53 ± 0.15), uric acid (5.61 ± 1.74), AST (24.64 ± 14.65), ALT (19.11 ± 8.26), LDH (300.16 ± 142.20) were increased significantly in preeclamptic women compared with control group as depicted in table 1. However, serum magnesium levels were similar in both groups. Neutrophil to lymphocytes ratio (5.73 ± 2.10) was increased significantly in preeclampsia as compared to control group (Table 1). In subgroup analysis, NLR (5.14 ± 1.97) was increased significantly in severe preeclamptics compared with mild preeclamptic (Table 2). Platelet to lymphocyte ratio increased in preeclamptic and also in severe preeclampsia. Difference in mean values was not statistically significant. Serum MDA (18.12 ± 6.98) levels were increased significantly in preeclamptic women compared with control group. Serum NO (6.51 ± 1.25) and FRAP (1334.06 ± 533.84) levels were decreased significantly in preeclamptic compared with control group (Table 3).

Table 1: Baseline, Biochemical and Hematological Parameters between Preeclamptic and Normotensive Controls

Parameters	Control (n=98) Mean ± SD	Preeclampsia (n=98) Mean ± SD	*P*
Baseline Characteristics			
Age (years)	22.93±3.05	23.17±3.66	0.864
Gestational age (weeks)	38.39±1.77	36.86±3.55	0.000
BMI (kg/m^2)	25.82±3.10	27.03±3.61	0.02
SBP (mmHg)	114.88±13.05	156.18±15.04	0.000
DBP (mmHg)	73.95±6.52	101.0±10.59	0.000
MAP (mmHg)	87.93±6.07	119.39±11.14	0.000
Pulse rate (bpm)	85.05±7.38	87.42±5.25	0.002
Biochemical parameters			
RBS (mg/dL)	82.66±15.66	83.62±21.09	0.639
Serum Urea (mg/dL)	14.53±4.66	15.46±6.39	0.473
Serum Creatinine (mg/dL)	0.49±0.12	0.53±0.15	0.01
Serum Uric acid (mg/dL)	4.81±1.45	5.61±1.74	0.000
Serum AST (IU/L)	21.27±8.20	24.64±14.65	0.03
Serum ALT (IU/L)	14.54±7.51	19.11±8.26	0.000
Serum LDH (IU/L)	163.46±60.09	300.16±142.20	0.000
Serum Magnesium (mg/dL)	2.19±0.62	2.10±0.52	0.439
Hematological parameters			
Hemoglobin (%)	11.35±1.77	10.90±2.08	0.229
WBC ($10^3/\mu L$)	14.15±4.66	12.98±3.58	0.05
Neutrophils (%)	75.56±6.10	74.86±7.85	0.545
Lymphocytes (%)	16.30±4.69	15.00±5.67	0.008
Platelets, x ($10^9/L$)	245.12±61.99	236.51±84.61	0.624
Neutrophil to lymphocyte ratio	5.21±2.46	5.73±2.10	0.008
Platelet to lymphocyte ratio	16.63±7.10	18.01±8.13	0.139

**Significant, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, RBS: Random blood sugar, AST: Aspartate transaminase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, WBC: White blood cells*

Table 2: Inflammatory Markers in Mild and Severe Preeclampsia

Parameters	Mild Preeclampsia (n=36) Mean ± SD	Severe Preeclampsia (n=62) Mean ± SD	*P*
Neutrophil to lymphocyte ratio	5.14±1.97	6.07±2.11	0.042
Platelet to lymphocyte ratio	16.24±7.60	19.04±8.30	0.108

**Significant*

Table 3: Oxidative Stress Parameters in Preeclampsia and Control

Parameters	Control (n=98) Mean ± SD	Preeclampsia (n=98) Mean ± SD	*P*
Serum MDA (µmoles/L)	10.24±5.70	18.12±6.98	0.000
Plasma FRAP (mmol/L)	1978.08±142.95	1334.06±533.84	0.000
Serum NO (µmoles/L)	12.03±4.60	6.51±1.25	0.000

** Significant, MDA: Malondialdehyde, FRAP: Ferric reducing ability of plasma, NO: Nitric Oxide*

Birth weight (2.46 ± 0.64) of the babies born to preeclamptic mother was decreased significantly compared with control group. Intra uterine growth restriction (IUGR), Respiratory Distress Syndrome (RDS), Intrauterine Death (IUD) were observed in 21 (21.42%), 22 (22.44%) and 6 (6.12%) of babies. In normotensive mothers, RDS was observed in 9 (9.1%) babies (Table 4).

Table 4: Birth Weight and Fetal Complications of Neonates on Delivery of Preeclampsia and Control

Parameters	Control (n=98) Mean ± SD	Preeclampsia (n=98) Mean ± SD
Birth weight (Kg)	2.82±0.509	2.46±0.64
IUGR	Nil	21(21.42%)
RDS	9 (9.1%)	22 (22.44%)
IUD	Nil	6 (6.12%)

** Significant, IUGR: Intra uterine growth restriction, RDS: Respiratory distress syndrome, IUD: Intrauterine death*

Discussion:

Preeclampsia is observed with increased oxidative stress and endothelial dysfunction; this plays a significant role in pathophysiology of preeclampsia [8]. Elevated lipid peroxidation and reduced antioxidant status allows for endothelial cell injury and dysfunction in preeclampsia [21]. During normal pregnancy, normal ROS prevailing condition facilitates the proper placental development and angiogenesis [22, 23].

In preeclampsia, elevated ROS, increased inflammatory status adds to failure of spiral artery remodeling and improper invasion of trophoblast, an inadequate blood perfusion occurs, which leads to ischemia and reperfusion injury, which increases ROS generation [10]. A few studies have reported that lipid peroxidation products, MDA levels were increased in preeclamptic women [7-8]. In our study, serum MDA levels were increased significantly in preeclamptic women when compared to control group. Our study is in accordance with Adetunji *et al*. where MDA levels were significantly increased in preeclamptic subjects compared to normotensive controls [24, 25]. Total antioxidant status was decreased significantly in preeclamptic women compared with control group. This indicates that increased lipid peroxidation is associated with decreased antioxidant protective/compensatory adaptation in preeclampsia and buttressed the oxidative stress mechanism in preeclampsia [7, 24].

Nitric oxide is a potent vasodilator, involves in relaxation of smooth muscle and it mediates endothelial function. In our study, serum nitric oxide levels were significantly low in preeclampsia when compared to control group. Our study results are in accordance with Choi *et al*. who have reported that nitric oxide production increases during normal pregnancy and the levels were decreased in preeclampsia [26].

However, the present study focuses on markers of inflammation i.e. neutrophil to lymphocyte ratio was increased significantly in preeclamptic women compared with control group whereas platelet to lymphocyte ratio was not significant, increased levels were observed in preeclamptic subjects. In subgroup analysis, neutrophil to lymphocyte ratio was increased significantly in severe preeclamptic women as compared to mild preeclampsia. Neutrophils are usually thought to be the first line of defense against infection [16]. Studies have reported that increased activation of inflammatory cells and immunologic response in preeclampsia, led to release of inflammatory cytokines, auto-antibodies and increased production of superoxide, resulting in endothelial dysfunction [18, 27]. Studies by Kurtoglu *et al.* and Serin *et al*. have found a significantly increased NLR in preeclamptic women and women with severe preeclampsia, respectively, as compared to healthy pregnant women [28, 29]. In our study, NLR was increased significantly in severe preeclamptic women compared to mild preeclampsia, which indicates that elevated NLR could be serve as a predictor of severity of preeclampsia. Abd-Alazim *et al*. have studied the relationship between NLR in patients with preeclampsia and severity of preeclampsia. They found significantly increased NLR in preeclampsia as compared to healthy pregnant and significantly increased levels were seen in severe preeclampsia as compared to mild preeclampsia [30].

A few research reports indicated the increased PLR is a sensitive marker of inflammation and a prognostic marker in breast cancer, ovarian and colorectal cancers [31-32]. Although association of PLR with inflammation and other disease conditions were reported, seldom studies evidenced the onset of association of PLR and preeclampsia [33, 34].

During normal pregnancy, activation of physiological inflammatory pathway occurs and is activated extensively in preeclampsia. Increased concentration of soluble factors initiates activation of platelets, inflammatory cytokine production and endothelial dysfunction [35]. Activated platelets release various soluble and adhesion molecules, which trigger the interactions between platelets, leukocytes and endothelial cells. A few studies reported that platelets play a major role in preeclampsia pathophysiology [36, 37]. In our study, PLR was increased in preeclampsia compared with controls, but the levels were not statistically significant. In subgroup analysis, the levels were increased in severe preeclampsia compared with mild preeclampsia, though statistically insignificant. Similar to our study findings, Yavuzcan *et al*. reported that, there is no significant difference of PLR between severe preeclampsia and healthy pregnant women [34].

Birth weight was significantly decreased in babies born to preeclamptic mothers as compared to controls. Nadkarni *et al*. reported that the incidence of low birth weight to be 52% among mothers with pregnancy-induced hypertension [38]. Odegard *et al*. reported that, preeclampsia and severe preeclampsia were associated with a 5% and 12% reduction in birth weight, respectively [39]. IUGR was observed in 21.42% of babies which is supported by other studies reported, 15% to 50% in preeclampsia [40, 41]. In the present study, RDS was observed in 22.44% of babies, supported by Nadkarni *et al.* reported 10% incidence of RDS among babies born to preeclamptic mothers [38]. In our study, IUD was reported as 6.12%.

On summary of the results of the present study, indicated that the increased oxidative stress, inflammatory conditions are the forms/ phenomenon in contributing to pathophysiology of preeclampsia.

Conclusion:

This study concludes that increased oxidative stress decreased nitric oxide and decreased total antioxidant capacity in preeclampsia with adverse fetal complications and also increased neutrophil to lymphocyte ratio in preeclamptics in comparison with normotensive pregnancies.

Limitations:

Further research is required to confirm and implement the results in clinical practice.

References

1. Kar M. Role of biomarkers in early detection of preeclampsia. *J Clin Diagn Res* 2014; 8(4):BE01-BE04.
2. Jeyabalan A. Epidemiology of preeclampsia: Impact of obesity. *Nutr Rev* 2013; 71(Supp1):S18-S25.
3. Bambrana V, Dayanand CD, Kotur PP. Is xanthine oxidase, a marker in pre-eclampsia? A case-control study. *J Clin Diagn Res* 2015; 9(10):BC01-BC03.
4. Petla LT, Chikkala R, Ratnakar KS, Kodati V, Sritharan V. Biomarkers for the management of pre-eclampsia in pregnant women. *Indian J Med Res* 2013; 138:60-67.
5. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Preeclampsia: Pathophysiology, diagnosis and management. *Vasc Health Risk Manag* 2011; 7:467-474.
6. Vanitha Gowda MN, Aroor AR, Krishna L. Studies on oxidative stress in preeclampsia. *Biomed Res* 2010; 21(1):71-79.
7. Dhananjaya BS, Venkatesh G, Sendil KD. Study of correlation between oxidative stress parameters and severity of preeclampsia. *Int J Biol Med Res* 2012; 3(1):1260-1262.
8. Rukmini MS, Kowsalya R, Pai B, Das P, Perriera J, Nandini M, *et al*. Plasma Adenosine deaminase activity and antioxidant status in preeclampsia comapred to healthy pregnant and nonpregnant women. *Biomed Res* 2009; 20(1):15-20.
9. Oztas E, Ozler S, Tokmak A, Erel O, Ergin M, Uygur D, *et al*. Oxidative stress markers in severe preeclampsia and preeclampsia-related perinatal morbidity-preliminary report. *Ginekol Pol* 2016; 87(6):436-441.
10. Sanchez-Aranguren LC, Prada CE, Riano-Medina CE, Lopez M. Endothelial dysfunction and preeclampsia: role of oxidative stress. *Front Physiol* 2014; 5:1-11.
11. Gonzalen-Garrido Chem. JA, Olivares-Corichi IM, Tovar-Rodriguez JM, Hernandez-Santana NA, Mendez-Bolaina E, Ceballos-Reyes GM *et al*. Influence of the AT (2) receptor on the L-arginine-nitric oxide pathway and effects of(-)-epicatechin on HUVECs from women with preeclampsia. *J Hum Hypertens* 2013; 27:355-361.
12. Vural P. Nitric oxide/endothelin-1in preeclampsia. *Clin Chim Acta* 2012; 317(1-2):65-70.
13. Shaamash AH, Elsnosy ED, Makhlouf AM, Zakhari MM, Ibrahim OA, Him EL-D. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, preeclampsia and eclampsia. *Int J Gynecol Obstet* 2000; 68:207-214.
14. Diejomaoh FM, Omu AE, Al-Busiri N, Taher S, Al-Othman S, Fatinikum T *et al*. Nitric oxide production is not altered in preeclampsia. *Arch Gynecol Obstet* 2004; 269(4):237-243.
15. Negi R, Pande D, Karki K, Khanna RS, Khanna HD. Oxidative stress and Preeclampsia. *Adv Life Sci* 2011; 1(1):20-23.
16. Sachan R, Patel ML, Sachan VP, Shyam R. Diagnostic accuracy of neutrophil to lymphocyte ratio in prediction of nonsevere preeclampsia and severe preeclampsia. *J Curr Res Sci Med* 2017; 3:79-83.
17. Toptas M, Asik H, Kalyoncuoglu M, Can E, Can MM. Are neutrophil/lymphocyte ratio and platelet/lymphocyte ratio predictors for severity of preeclampsia? *J Clin Gynecol Obstet* 2016; 5(1):27-31.
18. Gogoi P, Sinha P, Gupta B, Firmal P, Rajaram S. Neutrophil-to-lymphocyte ration and platelet indices in preeclampsia. *Int J Gynecol Obstet* 2019; 144(1):16-20.
19. Dogan K, Guraslan H, Senturk MB, Helvacioglu C, Idil S, Ekin M. Can platelet count and platelet indices predict the risk and the prognosis of preeclampsia? *Hypertens Pregnancy* 2015; 34(4):434-442.
20. Han L, Liu X, Li H, Zou J, Yang Z, Han J, *et al*. Blood coagulation parameters and platelet indices: changes in normal and preeclamptic pregnancies and predictive values for preeclampsia. *PLoS One* 2014; 9(12):e114488.
21. Sharma JB, Sharma A, Bahadur A, Vimala N, Satyam A, Mittal S. Oxidative stress markers and antioxidant levels in normal pregnancy and preeclampsia. *Int J Gynaecol Obstet* 2006; 94(1):23-27.
22. Bogavac M, Jakovljevic A, Stajic Z, Nikolic A, Milosevic-Tosic M, Dejanovic J, *et al*. Preeclmapsia and level of oxidative stress in the first trimester of pregnancy. *Vojnosanitetski Pregled* 2017; 74(7):633-638.
23. Basu J, Bendek B, Agamasu E, Salafia CM, Mishra A, Benfield N, *et al*. Placental oxidative status throughout normal gestation in women with uncomplicated pregnancies. *Obstet Gynecol Int* 2015; 2015:276095.
24. Adeniji AO, Oparinde DP. Comparison of lipid peroxidation and anti-oxidant activities in preeclmaptic and normal pregnancies in Nigerian population. *Res J Med Sci* 2013; 7(2):40-43.

25. Karacay O, Sepici-Dincel A, Karcaaltincaba D, Sahin D, Yalvac S, Akyol M, *et al*. A quantative evaluation of total antioxidant status and oxidative stress markers in preeclampsia and gestational diabetic patients in 24 -36 weeks of gestation. *Diab Res Clin Pract* 2010; 89:231-238.
26. Choi JW, Im MW, Pai SH. Nitric oxide increases during normal pregnancy and decreases in preeclampsia. *Ann Clin Lab Sci* 2002; 32(3):257-263.
27. Laresgoiti-Servitje E. A leading role for the immune system in the pathophysiology of preeclampsia. *J Leukoc Biol* 2013; 94(2):247-257.
28. Kurtoglu E, Kokcu A, Celik H, Tosun M, Malatyalioglu E. May ratio of neutrophil to lymphocyte be useful in predicting the risk of developing preeclampsia? A pilot study. *J Matern Fetal Neonatal Med* 2015; 28(1):97-99.
29. Serin S, Avcl F, Ercan O, Kostu B, Bakacak M, Kiran H. Is neutrophil/lymphocyte ratio a useful marker to predict the severity of preeclampsia? *Pregnancy Hypertens* 2016; 6(1):22-25.
30. Abd-Alazim M, Mohammad AH, Radwan MS, Shokr AA. Is neutrophil/lymphocyte ratio a useful marker to predict the severity of pre-eclampsia? *Egypt J Hosp Med* 2018; 73(5): 6621-6625.
31. Cakmak B, Gulucu S, Aliyev N, Ozsoy Z, Nacar M, Koseoglu D. Neutrophil lymphocyte and platelet-lymphocyte ratios in endometrial hyperplasia. *Obstet Gynecol Sci* 2015; 58(2):157-161.
32. Turkmen K, Erdur FM, Ozcicek F, Ozcicek A, Akbas EM, Ozbicer A, *et al*. Platelet-to-lymphocyte ratio better predicts inflammation than neutrophil-tolymphocyte ratio in end-stage renal disease patients. *Hemodial Int* 2013; 17(3):391-396.
33. Proctor MJ, Morrison DS, Talwar D, Balmer SM, Fletcher CD, O'Reilly DS, *et al*. A comparison of inflammation-based prognostic scores in patientswith cancer. A Glasgow Inflammation Outcome Study. *Eur J Cancer* 2011; 47(17):2633-2641.
34. Yavuzcan A, Caglar M, Ustun Y, Dilbaz S, Ozdemir I, Yildiz E, *et al*. Mean platelet volume, neutrophil-lymphocyte ratio and platelet-lymphocyte ratioin severe preeclampsia. *Ginekol Pol* 2014; 85(3):197-203.
35. Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. *Clin J Am Soc Nephrol* 2007; 2(3):543-549.
36. Ahmed R, Dunford J, Mehran R, Robnson S, Kunadian V. Preeclampsia and future cardiovascular risk among women: A review. *J Am Coll Cardiol* 2014;63(18):1815-1822
37. Ceyhan T, Beyan C, Baser I, Kaptan K, Gungor S, Ifran A. The effect of preeclampsia on complete blood count, platelet count and mean platelet volume. *Ann Hematol* 2006; 85(5):320-322.
38. Nadkarni J, Bahl J, Parekh P. Perinatal outcome in pregnancy associated hypertension. *Ind Pediatr* 2001; 38(2):174-178.
39. Odegard RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. Preeclmapsia and fetal growth. *Obstet Gynecol* 2000; 96 (6):950-965.
40. Robert CL, Algert CS, Morris JM, Ford JB, Henderson-Smart DJ. Hypertensive disorders in pregnancy: a population based study. *Med J Aust* 2005:182(7):332-335.
41. Sivakumar S, Bhat BV, Badhe BA. Effect of pregnancy induced hypertension on mothers and their babies. *Indian J Pediatr* 2007; 74(7):623-625.

[*] ***Author for Correspondence:*** *Dr. Sumathi ME, Department of Biochemistry,*
Sri Devaraj Urs Medical College, SDUAHER, Tamaka, Kolar-563103 (Karnataka) India
Email: drsuma_bio@yahoo.co.in Cell: 9740371006