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Application of cell-free fetal DNA for early evaluation of preeclampsia to reduce maternal mortality by low-cost method – A prospective cohort study

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Adverse pregnancy outcomes such as preeclampsia are the leading cause of maternal morbidity and mortality in the world and its incidence is increasing. It has been observed in some studies that cell-free fetal DNA (cff DNA) is increased in maternal serum associated with preeclampsia. In the present study, we have tested whether the elevated amount of cff DNA in maternal plasma is associated with PE and development of new marker by the low-cost method to predict preeclampsia. Twenty-one pregnant women within the age group of 20–30 years attending for routine antenatal checkups at (G & O) antenatal OPD after 20 weeks with fulfilling the diagnostic criteria of preeclampsia were included in our study. Age-matched pregnant women without hypertension were included as controls. A complete clinical history and anthropometric observation showed that gravida (total number of pregnancy in a patient including present pregnancy), gestational age, gestational age at birth, birth weight in preeclampsia subjects were non-significantly lower than normotensive subjects. Blood analysis showed lower platelet count and higher creatinine level, bilirubin level, and liver enzyme activities in preeclampsia subjects in comparison to normotensive subjects. Identification of cell-free fetal DNA (cff DNA) in maternal plasma by using two in-house methods (phenol-chloroform-isopropanol and NaI) was found comparable and its content (GE/μL) in preeclampsia subjects were significantly higher than the normotensive subjects. Correlation analysis showed that APGAR score was significantly negatively correlated with both systolic and diastolic blood pressure and significantly positively correlated with gestational age and gestational age at birth; whereas, cff DNA was significantly positively correlated with blood pressure but significantly negatively correlated with platelet count. In conclusion, our study demonstrated that APGAR score, which is one of the indicators of physiologic maturity of the infant is severely affected by the causative factors of preeclampsia and cell-free fetal DNA quantification may be a promising marker for future adverse pregnancy outcome.

Keywords: APGAR score, Cell-free fetal DNA, Platelet count, Preeclampsia

Adverse pregnancy outcomes are the leading cause of maternal morbidity and mortality in the world and its incidence is increasing. On an average 830 pregnant women die every day from preventable causes worldwide, which accounts for maternal mortality ratio (MMR) 239 per 100000 live births in developing countries and 12 per 100000 live births in developed countries[1]. In 2015, the global MMR stood at 216 maternal deaths per 100000 live births[2], while it was reported 174 in India contributing up to 20% maternal death worldwides[1]. Most maternal deaths can be prevented[2]. Sustainable development goals (SDG) replacing the millennium development goals (MDG) have been aimed to reduce MMR below 70 per 100000 live births by the year 2030[1]. Therefore, antenatal screening is essential as preventive measures.

Preeclampsia (PE), a multisystem pregnancy-related hypertensive disorder of unknown etiology[3], is one of the leading causes, which may complicate pregnancy resulting in adverse impact on maternal health and often ended with maternal mortality. It is hypothesized that preeclampsia is a two-stage disease. In the first stage, trophoblast invasion and artery modification lead to placental insufficiency[3]. Defective implantation and placentation reduce uteroplacental perfusion, placental ischemia, hypoxia and oxidative stress, which activate intracellular signaling cascades, secretion of growth factors, antiangiogenic factors, vasoconstrictors, and cytokines. This leads to increased systemic vascular resistance, enhanced platelet aggregation,

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Abbreviations: cff DNA, Cell-free fetal DNA; PE, Preeclampsia; MMR, Maternal mortality ratio

activation of the coagulation system, and endothelial cell dysfunction[3]. Imbalance of vasodilators and vasoconstrictors results in a muscular spasm. Acute atherosis and spiral artery thrombosis have also been implicated in causing severe placental ischemia and infarction[4]. The endothelial dysfunction leads to the clinically recognized symptoms of the preeclampsia, including hypertension, proteinuria, thrombocytopenia, impaired liver function *etc*.[3]

Preeclampsia is a disorder of pregnancy typically characterized by new-onset hypertension and proteinuria after 20 weeks of gestation. Placental ischemic events and the release of placental factors appear to play a critical role in the pathophysiology. These factors contribute to a generalized systemic vascular endothelial dysfunction and result in increased systemic vascular resistance and hypertension[5].

Preeclampsia is one of the major health problems during pregnancy. It complicates 3-8% of pregnancies and causes a marked the increase in perinatal, maternal morbidity, and mortality[6-9]. Although the exact pathophysiology of preeclampsia is not completely understood, certain factors have been attributed to it, which include deficient trophoblastic invasion of the maternal vascular bed with subsequent reduction of placental blood flow[10,11]. Placental under perfusion initiates widespread systemic, maternal endothelial dysfunction, and increased vascular permeability[12].

It has been observed in some studies that cell-free fetal DNA (cff DNA) is increased in maternal serum associated with preeclampsia. Pregnancy with preeclampsia often complicated and causes adverse pregnancy outcomes, maternal end organs damage, eclampsia and even maternal mortality in its severe form if untreated. Early detection of the conditions may lead to appropriate intervention which may prevent preeclampsia among women with high or moderate risk of PE[13-15] and reduces the relative risk of preeclampsia by 53%[16]. Detection of cell-free fetal DNA using markers like RASSF1A, DSCR3 are useful in evaluating preeclampsia[17]. RASSF1A promoter is hypomethylated and sensitive to digestion of the above restriction endocluses. Similarly, CpG sites in the promoter region of the DSCR3 gene are hypermethylated in fetal DNA but hypomethylated in maternal DNA. The higher concentration of cff DNA in maternal serum is found in preeclampsia and particularly cff DNA and cf DNA ratio are twofold higher in severe preeclampsia group[18].

In the present study, we have evaluated whether cff DNA in maternal plasma is associated with causative factors of PE.

Materials and Methods

Preeclampsia (PE) is defined as new onset of elevated blood pressure more than 140/90 mm Hg as measured on two separate occasions after 20 weeks of pregnancy associated with significant proteinuria (>300 mg/day)[19]. Pregnant women attending for routine antenatal checkups at antenatal OPD of Department of Obstetrics and Gynaecology (OBG), College of Medicine & JN Medical Hospital, The West Bengal University of Health Sciences, Kalyani, Nadia, West Bengal with fulfilling the diagnostic criteria of preeclampsia were included in our study. They were interviewed for their demographic, past obstetrical, medical background as per performed structured questioners. Patients with the previous eclampsia, autoimmune disease, chronic hypertension, renal disease were excluded from the study. The study was approved by the Institutional Ethics Committee (No.F-24/Pr/COMJNMH/IEC/16/1210).

Methods

Twenty-one preeclampsia patients within 20-30 years were included in this study. Age-matched pregnant women without hypertension were included as controls. A complete clinical history and anthropometric measurements, including systolic and diastolic blood pressure were recorded. Venous blood was collected and plasma and serum were separated and stored at −4°C for analysis. Post-delivery conditions in term of gestational age at delivery, birth weight of babies, Apgar score, Stillbirth were also followed up. Hematological examination including hemoglobin and platelet count and biochemical analysis including liver enzymes and renal function tests were performed by commercially available kits using cell counter (Sysmax) and auto analyzer (ERBA 360), respectively.

Cell-free DNA in maternal plasma was extracted using in-house methods (phenol-chloroform-isopropanol and NaI) and commercially available Kit (QIAGEN) for comparison. Comparison of the PCI and NaI extraction protocols with the commercially-available kits was done to standardize the most efficient and economical method of cff DNA isolation which could give consistent quality and quantity of cff DNA. Methods of isolation, storage conditions and time before isolation of DNA were compared and standardized.

cff DNA is typically fragmented hypermethylated DNA of about 150-200 bp[20]. However, maternal cfDNA also remain present with cff DNA in the sample. The promoter of RASSF1A is hypermethylated in trophoblast resulting in resistance to digestion by methylation-sensitive restriction endonuclease HhaI, HpaII, Bstu1(New England Biolabs). On the contrary, RASSF1A promoter is hypomethylated in serum and sensitive to digestion of the above restriction endonucleases. Thus cell-free DNA purified from maternal plasma as stated above was digested with the above methylation-sensitive restriction enzymes. Subsequent PCR amplification with specific primers around the promoter detected the quantity of fetal DNA. For internal control, a specific primer for amplification of β-actin was used[21]. Cell-free DNA concentration after extraction comprises of both fetal and maternal origin of cf-DNA (Total cf-DNA). But after treatment with restriction enzymes, the maternal cf-DNA lyses and measurement by Nanodrop spectrophotometer (ND 100, Fisher) would reveal the concentration of cff DNA in Genomic Equivalent (GE/μL).

Statistical analysis

Results were expressed as mean ± SE (standard error). All statistical analysis was performed by one-way analysis of variance (ANOVA) with bivariate correlation tests and Student's '*t*' test using the Statistical Package for Social Sciences, version 25 (SPSS, Chicago, Illinois). A *'P'* value of <0.05 was considered significant. Receiver operating characteristic (ROC) curve analysis of cff DNA was done by MedCalc version 15.8 (MedCalc Software bvba; 2015).

Results

In this study, we found that gravida (total number of pregnancy in a patient including present pregnancy), gestational age, gestational age at birth, birth weight in preeclampsia subjects were non-significantly lower than normotensive subjects (Table 1). However, the APGAR score was found to be significantly lower in preeclampsia subjects in comparison to the normotensive subjects (Table 1). Hematological profile analysis showed that, though hemoglobin content was comparable in both groups, but platelet count was significantly lower in preeclampsia subjects than normotensive subjects (Table 1). Biochemical analysis showed significantly higher creatinine level, bilirubin level, and liver enzyme activities in preeclampsia subjects in comparison to normotensive subjects, though remained within the normal level (Table 1). Identification of cell-free fetal DNA (cff DNA) in maternal plasma by both the methods was found to be comparable (Fig. 1). However, cell-free fetal DNA content (GE/μL) in preeclampsia subjects were significantly higher than the normotensive subjects (Table 1).

Receiver operating characteristic (ROC) curve analysis (Fig. 2) of cff DNA content to identify a cut-off for preeclampsia was done and it showed that values more than 116.4 (GE/μL) in serum has the sensitivity of 85.71 % and specificity of 100% in

Table 1—Comparison of demographic, laboratory, pregnancy outcomes parameters between preeclampsia and control group.

Parameters	Case (n =21)	Control (n =21)
Age	25.9 ± 0.98	24.87 ± 0.82
Gravida	1.67 ± 0.17	1.76 ± 0.21
Gestational Age	250.05 ± 5.97	255.90 ± 4.48
Gestational age at Birth	261.14 ± 4.47	263.33 ± 3.8
Birth Weight	2.1 ± 0.15	2.4 ± 0.11
Systolic blood pressure	149.71 ± 3.5*	109.43 ± 2.6
Diastolic blood pressure	95.14 ± 3.1*	70.38 ± 2.1
Hemoglobin (g%)	10.52 ± 0.3	10.96 ± 0.25
Platelet Count (10^9/L)	201.7 ± 7.7*	265.4 ± 6.1
Creatinine (mg%)	0.99 ± 0.06*	0.72 ± 0.02
Serum Bilirubin (mg%)	0.89 ± 0.05$^{@}$	0.77 ± 0.03
SGPT (IU/L)	29.29 ± 1.7$^{\#}$	23.05 ± 0.6
SGOT (IU/L)	33.81 ± 1.96$^{\#}$	26.86 ± 0.62
APGAR out of 10	5.33 ± 0.38$^{@}$	6.33 ± 0.14
cff DNA Content (GE/uL)	7572.16 ± 1722.18*	32.27 ± 7.3

Values are mean ± SD of number of observation (n).
P values: *<0.001, $^{\#}$<0.01, $^{@}$<0.05 compared to healthy control subjects;

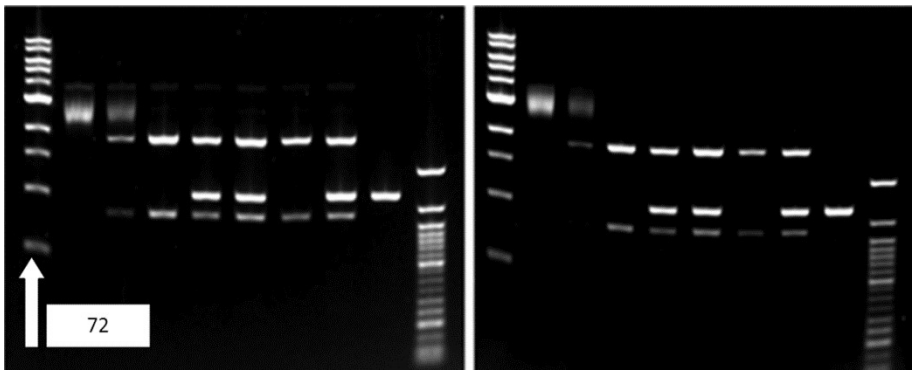

Fig. 1—PCR amplification using gender independent chromosome specific locus RASSF1A. Lanes 1 (from left to right) ϕX174 digested with restriction enzyme Hae III; from lowest to highest bands were of sizes 72, 118, 194, and 234 bp (other are not mentioned). Lane 2 shows negative control (without template). Lane 3, 4, 5, 6, 7 and 8 were from different samples. Lane 9 is the positive control with the primer-dimer. The band intensities visibly vary indicating that different samples had a different amount of cell-free fetal DNA as mothers.

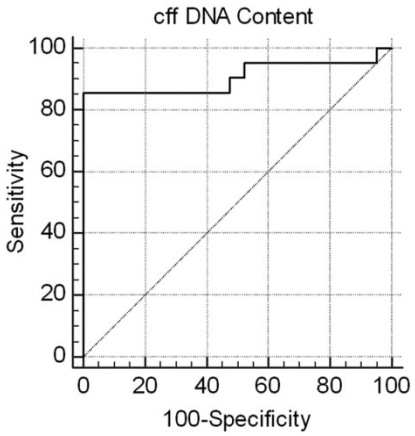

Fig. 2—Receiver operating characteristic (ROC) curve analysis of cff DNA content to identify cutoff for preeclampsia, [AUC = 0.907, SE ± 0.0552, 95% Confidence interval = 0.777 to 0.975, Significance level *P* (Area =0.5) = <0.0001]

predicting preeclampsia with AUC of 0.907. Descriptive analysis of cff DNA (Table 2) has revealed that the interquartile range of cff DNA in case was 2134.5000 to 11707.5000 (GE/μL) (95% CI for the median = 2212.0907 to 10276.1124) and in control of interquartile range was 7.6650 to 52.2000 (95% CI for the median = 7.7400 to 44.0745).

The correlation analysis showed (Table 3) that APGAR score was negatively correlated with systolic blood pressure (r = –0.361, *P* <0.05) and diastolic blood pressure (r= -0.413, *P* <0.01); whereas positively correlated with gestational age (r =0.392, *P* <0.01) and gestational age at birth (r =0.398, *P* <0.01). On the other hand, cff DNA was positively correlated with systolic blood pressure (r =0.371, *P* <0.05) and diastolic blood pressure (r= 0.423, *P* <0.01); whereas negatively correlated with platelet count (r = –0.474, *P* <0.01).

Table 2—Descriptive statistics of cff DNA content

Variables	Case	Control
Sample size	21	21
Lowest value	3.6600	3.1800
Highest value	25056.0000	116.4000
Median	3762.0000	19.9800
95% CI for the median	2212.0907-10276.1124	7.7400-44.0745
Interquartile range	2134.5000 to 11707.5000	7.6650 to 52.2000

Table 3—Comparison of correlation of APGAR score and cff DNA with other variables among total study population (n =42)

Variable	APGAR score	cff DNA
Age	−0.095 (0.550)	0.215(0.172)
SBP	−0.361* (0.019)	0.371* (0.016)
DBP	−0.413** (0.007)	0.423** (0.005)
Platelet	0.276 (0.077)	−0.474** (0.002)
Creatinine	−0.285 (0.067)	0.138 (0.384)
Gestational age	0.392** (0.010)	0.102 (0.522)
Gravida	0.020 (0.899)	−0.024 (0.882)
Gestational age at birth	0.398** (0.009)	0.117 (0.461)
APGAR	1	0.098 (0.536)
Cff DNA	0.098 (0.536)	1

*correlation is significant at the 0.05 level (2-tailed)
**correlation is significant at the 0.01 level (2-tailed)

Discussion

Although the exact cause of preeclampsia remains unclear, the syndrome may be initiated by placental factors that enter the maternal circulation and cause endothelial dysfunction resulting in hypertension and proteinuria[22-25]. There is extensive evidence that pre-pregnancy chronic hypertension is associated with a high risk of development of severe hypertension and

preeclampsia and birth of small-for-gestational-age neonates[26].

Changes in several hematological parameters may be associated with the preeclampsia that may affect mothers and their newborns[27]. Platelet count is decreased by vascular endothelial damage in preeclampsia, as observed in our study, leads to increased turnover of platelets[28]. Therefore, measuring the platelet parameters could better reveal early-stage severe preeclampsia[29].

Serum creatinine and platelet count were identified as independent factors in predicting severe features of preeclampsia[30]. This endothelial disease affects kidney function during pregnancy[31]. Though serum creatinine level in preeclamptic patients in our study was higher than normotensive, yet it was within the normal level. Earlier studies suggested that renal function in preeclamptic patients is significantly impaired and highly correlated with systolic or diastolic blood pressure[3,32]. One study suggested that serum creatinine is independent risk factors for hypertensive disorders of pregnancy[33]. Another study suggested that there is a sizable association between preeclampsia and ESRD[34].

In the present study, significantly higher activities of serum transaminases (AST and ALT) in the preeclamptic patients than those of the normotensive group were in agreement with other studies[3,35,36]. Pregnancy-specific disorders are the leading cause of abnormal liver function test during pregnant state, particularly in the third trimester[37].

Preeclampsia has a great implication on the adverse neonatal outcome. Appearance, pulse, grimace, activity, respiration (APGAR) score is one of the indicators of physiologic maturity of the infant. Preeclampsia has a great implication on adverse neonatal outcome[38]. Our study revealed for the first time that the APGAR score was negatively correlated with the blood pressure, while positively correlated with gestational age and gestational age at birth.

Usually, placentation involves apoptosis of trophoblast cells. During this process, fetal DNA is released into maternal circulation through the feto-placental barrier. Constituting about 10% of cell-free (cf) DNA, cff DNA can be detected as early as 5 weeks of gestation and cleared rapidly from maternal circulation within 2 h after deliver[1]. Hence, results are not affected by previous pregnancy complications[39]. Both in-house and kit based methods receiver operating characteristic (ROC) curve analysis of cff DNA content was done to identify cutoff for preeclampsia. Though it offers potential marker for prenatal diagnosis for various genetic conditions, such as achondroplasia, autosomal recessive disorders, fetal thalassemia, aneuploidy, RhD genotyping[1]; yet it has been shown that total cell free fetal DNA increased significantly among women with PE in our study by both in house and kit based methods. It has also been observed that elevated total cell-free DNA and cff DNA were also significantly higher among women with preterm labor and adverse fetal outcome groups compared with the term and favourable outcome groups[40]. In a study woman with preeclampsia and normotensive control with pregnancy between 28 and 32 gestational weeks, it has been shown that cell-free fetal DNA concentrations were higher in early preeclamptic women than control subjects[41]. In a meta-analysis of 13 studies, in 11 studies, elevated cff DNA was observed in preeclampsia, while in two studies no significant association was observed[42]. The correlation analysis in our study showed that cff DNA was positively correlated with blood pressure; whereas negatively correlated with platelet count further indicated that higher cff DNA may predict the adverse fetal outcome.

Conclusion

Our study demonstrated that APGAR score, which is one of the indicators of physiologic maturity of the infant is severely affected by the causative factors of preeclampsia and cell-free fetal DNA quantification may be a promising marker for preeclampsia prediction. However, it is necessary to use well-defined population to ascertain the efficacy of cff DNA quantification in different degree of preeclamptic patients. Isolation of cff DNA by phenol-chloroform-isopropanol and NaI are alternative low-cost methods without utilizing any commercial kit.

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References

1 Das SK & Saha MM, Cell free fetal DNA: marker for predicting pregnancy outcomes. *Indian J Clin Biochem*, 32 (2017) 251.

2 https://sustainabledevelopment.un.org/sdg3. Accessed on 28 May, 2018

3 Saha T, Halder M, Das A & Das SK, Role of nitric oxide, angiogenic growth factors and biochemical analysis in preeclampsia. *Indian J Biochem Biophys*, 50 (2013) 462.

4 Powe CE, Levine RJ & Karumanchi SA, Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. *Circulation*, 123 (2011) 2856.

5 Granger JP, Spradley FT & Bakrania BA, The endothelin system: a critical player in the pathophysiology of preeclampsia. *Curr Hypertens Rep,* 20 (2018) 32.

6 Adam GK, Bakheit KH & Adam I, Maternal and perinatal outcomes of eclampsia in Gadarif Hospital, Sudan. *J Obs Gynaecol*, 29 (2009) 619.

7 Anderson UD, Olsson MG, Kristensen KH, Åkerström B & Hansson SR, Review: Biochemical markers to predict preeclampsia. *Placenta,* 33 (Suppl) (2012) S42.

8 Redman CW & Sargent IL, Latest advances in understanding preeclampsia. *Science*, 308 (2005) 1592.

9 Walker JJ, Preeclampsia. *Lancet (London, England)* 356 (9237) (2000) 1260.

10 Burton GJ, Woods AW, Jauniaux E & Kingdom JCP, Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. *Placenta*, 30 (2009) 473.

11 Burton GJ, Charnock-Jones DS & Jauniaux E, Regulation of vascular growth and function in the human placenta. *Reproduction*, 138 (2009) 895.

12 Maynard SE & Karumanchi SA, Angiogenic factors and preeclampsia. *Semin Nephrol*, 31 (2011) 33.

13 Duley L, Henderson SD, Meher S & King J, Antiplatelet agents for preventing preeclampsia and its complications. *Cochrane Database Syst Rev*, 2 (2007) CD004659.

14 Sibai BM, Caritis SN, Thom E, Shaw K & McNellis D, National Institute of Child Health and Human Developmental Maternal-Fetal Medicine Network. Low-dose aspirin in nulliparous women: safety of continuous epidural block and correlation between bleeding time andmaternal-neonatal bleeding complications. *Am J Obstet Gynecol*, 172 (1995) 1553.

15 CLASP (Collaborative Low-dose Aspirin Study in Pregnancy Collaborative Group), CLASP: a randomised trial of low-dose aspirin for theprevention and treatment of preeclampsia among 9364 pregnant women. *Lancet*, 343 (1994) 619.

16 Roberge S, Nicolaides KH, Demers S, Villa P & Bujold E, Prevention of perinatal death and adverse perinatal outcome using low-doseaspirin: a meta-analysis. *Ultrasound Obstet Gynecol*, 41 (2013) 491.

17 Drury S, Hill M & Chitty LS, Cell-free fetal DNA testing for prenatal diagnosis. *Adv Clin Chem*, 76 (2016) 1.

18 Kim HJ, Kim SY, Lim JH, Kwak DW, Park SY & Ryu HM, Quantification and Application of Potential Epigenetic Markers in Maternal Plasma of Pregnancies with Hypertensive Disorders. Baker PN, ed. *Int J Mol Sci*, 16 (2015) 29875.

19 Gupte S & Wagh G, Preeclampsia–Eclampsia. *J Obst Gynaec India*, 64 (2014) 4.

20 Yu SC, Chan KC, Zheng YW, Jiang P, Liao GJ, Sun H, Akolekar R, Leung TY, Go AT, van Vugt JM, Minekawa R, Oudejans *CB, Nicolaide*s KH, Chiu RW & Lo YM, Size-based molecular diagnostics using *plasma DNA for non* invasive prenatal testing. *Proc Natl Acad Sci U S A*, 111 (2014) 8583.

21 White HE, Dent CL, Hall VJ, Crolla JA & Chitty LS, Evaluation of a novel assay for detection of the fetal marker RASSF1A: facilitating improved diagnostic reliability of noninvasive prenatal diagnosis. *PLoS One*, 7 (2012) e45073.

22 Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP & Karumanchi SA, Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med*, 350 (2004) 672.

23 Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP & Karumanchi SA, Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*, 111 (2003) 649.

24 Maynard S, Epstein FH & Karumanchi SA, Preeclampsia and angiogenic imbalance. *Annu Rev Med*, 59 (2008) 61.

25 Page NM, Woods RJ, Gardiner SM, Lomthaisong K, Gladwell RT, Butlin DJ, Manyonda IT & Lowry PJ, Excessive placental secretion of neurokinin B during the third trimester causes preeclampsia. *Nature*, 405 (2000) 797.

26 Nzelu D, Dumitrascu-Biris D, Nicolaides KH & Kametas NA, Chronic hypertension: first-trimester blood pressure control and likelihood of severe hypertension, preeclampsia, and small for gestational age. *Am J Obstet Gynecol*, 218 (2018) 337.e1.

27 Elgari MM, Khabour OF & Alhag SM, Correlations between changes in hematological indices of mothers with preeclampsia and umbilical cord blood of newborns. *Clin Exp Hype tens*, (2018) 1-4. doi: 10.1080/10641963.2018. 1441861.

28 Özdemirci Ş, Başer E, Kasapoğlu T, Karahanoğlu E, Kahyaoglu I, Yalvaç S & Tapısız Ö, Predictivity of mean platelet volume in severe preeclamptic women. *Hypertens Pregnancy*, 35 (2016) 474.

29 Wang ZM, Zhu QY, Zhang JF, Wu JL, Yang R & Wang DM, Changes of platelet parameters in early severe preeclampsia. *Clin Exp Obstet Gynecol*, 44 (2017) 259.

30 Cui L, Shu C, Liu Z, Tong W, Cui M, Wei C, Tang JJ, Liu X, Hai H, Jiang J, He J, Zhang DY, Ye F & Li Y, Serum protein marker panel for predicting preeclampsia. *Pregnancy Hypertens*, pii: (2018) S2210-7789(17)30482.

31 Lopes van Balen VA, Spaan JJ, Cornelis T & Spaanderman MEA, Prevalence of chronic kidney disease after preeclampsia. *J Nephrol*, 30 (2017) 403.

32 Seow KM, Tang MH, Chuang J, Wang YY & Chen DC, The correlation between renal function and systolic or diastolic blood pressure in severe preeclamptic women. *Hypertens Pregnancy*, 24 (2005) 247.

33 Yalamati P, Bhongir AV, Betha K, Verma R & Dandge S, Relationship of serum uric acid, serum creatinine and serum cystatin C with maternal and fetal outcomes in rural Indian pregnant women. *Int J Reprod Contracept Obstet Gynecol*, 4 (2015) 1505.

34 Kattah AG, Scantlebury DC, Agarwal S, Mielke MM, Rocca WA, Weaver AL, Vaughan LE, Miller VM, Weissgerber TL, White W & Garovic VD, Preeclampsia and ESRD: The Role of Shared Risk Factors. *Am J Kidney Dis*, 69 (2017) 498.

35 Mondal BR, Ahmed S, Saha S, Perveen SI, Paul D, Sultana T, Rahman MQ, Sarker UK & Ahmed AN, Alanine

Aminotransferase and Total Bilirubin Concentration in Preeclampsia and Eclampsia. *Mymensingh Med J*, 25 (2016) 85.

36 Wu SW, Wu LF, Wang Q & Zhang WY, Risk factors of adverse pregnancy outcomes during expectant management of early onset severe preeclampsia. *Zhonghua Fu Chan Ke Za Zhi*, 45 (2010) 165.

37 Mishra N, Mishra VN & Thakur P, Study of Abnormal Liver Function Test during Pregnancy in a Tertiary Care Hospital in Chhattisgarh. *J Obstet Gynaecol India*, 66 (Suppl 1) (2016) 129.

38 Ayaz A, Muhammad T, Hussain SA & Habib S, Neonatal outcome in pre-eclamptic patients. *J Ayub Med Coll Abbottabad*, 21 (2009) 53.

39 Lo YM, Corbetta N & Chamberlain PF, Presence of fetal DNA in maternal plasma and serum. *Lancet*, 350 (1997) 485.

40 Abdel Halim RM, Ramadan DI, Zeyada R, Nasr AS & Mandour IA, Circulating maternal total cell-free DNA, cell-free fetal DNA and soluble endoglin levels in preeclampsia: predictors of adverse fetal outcome? A cohort study. *Mol Diagn Ther*, 20 (2016) 135.

41 Seval MM, Karabulut HG, Tükün A & Koç A, Cell free fetal DNA in the plasma of pregnant women with preeclampsia. *Clin Exp Obstet Gynecol*, 42 (2015) 787.

42 Martin A, Krishna I, Badell M & Samuel A, Can the quantity of cell-free fetal DNA predict preeclampsia: a systematic review, *Prenat Diagn*, 34 (2014) 685.

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