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A study on maternal and fetal cell free DNA (cffDNA) for predicting the adverse pregnancy outcomes

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Abstract

This gender-independent detection of cell-free fetal DNA in maternal plasma using RASSF1A/beta-actin has curtained off a new dimension regarding its utility to predict the adverse pregnancy outcomes. Recent efforts have been directed at developing sequences from cell-free fetal DNA (cffDNA) as markers for pregnancy outcomes. The utility of cffDNA using the methylation-dependent DSCR3 and RASSF1A markers along with total cell-free DNA (cf-DNA) in maternal serum by HYP2 marker are useful in predicting adverse pregnancy outcomes. Indigenously developed low-cost method of the gender-independent sequence markers from cffDNA was investigated and evaluated with the standardized commercial kits as predictive markers for adverse pregnancy outcomes. In the present study, we have tested whether the elevated amount of cffDNA in maternal plasma is associated with adverse pregnancy outcomes and development of new marker by the low-cost method to predict adverse pregnancy outcomes. 210 pregnant women within the age group of 20 – 30 years attending for routine antenatal checkups after 20 weeks with fulfilling the diagnostic criteria of adverse pregnancy outcomes were included in our study. Age-matched pregnant women without adverse pregnancy outcomes were included as controls (n=210). Identification of cell-free fetal DNA (cffDNA) in maternal plasma by using in-house methods (Guanidium isothiocyanate) was found comparable with commercial kit and its content (GE/ µl) in adverse pregnancy outcomes subjects were significantly higher than the normotensive subjects. Our results indicated that indigenously developed method for detection of gender-independent cffDNA can be applicable for screening test of adverse pregnancy outcome.

Keywords: APGAR score; Cell-free Fetal DNA; Platelet count; Preeclampsia

1. Introduction

Worldwide approximately 830 pregnant women die every day from preventable causes 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]During Pregnancy a mother often faces many unexplained complications which are very difficult to establish by conventional methods. Approximately 20-25% percent of pregnant women will have some bleeding per vagina before 20 weeks' gestation which is known as threatened miscarriage, and roughly one half of these pregnancies will end in spontaneous misscarriage[2]. Overall 15-20 % of recognized pregnancies will end in miscarriage. However, when women are followed with serial serum human chorionic gonadotropin (hCG) measurements, the actual miscarriage rate is higher and found to be 60% which includes preclinical losses[3].First-trimester bleeding in a pregnant woman has an extensive differential diagnosis and should be evaluated with a full history and physical examination to exclude the

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other causes of bleeding like:Cervical abnormalities (e.g., excessive friability, malignancy, polyps, and trauma), Ectopic pregnancy, Idiopathic bleeding in a viable pregnancy, Infection of the vagina or cervix, Molar pregnancy, Vaginal trauma. It is hypothesized that first trimester bleeding may indicate an underlying placental dysfunction, which may manifest later in pregnancy causing adverse outcomes such as increased risk of preeclamptic toxemias (PET), preterm delivery, prelabour rupture of membranes (PROM), and intrauterine growth restriction and even intrauterine fetal death[4].

Preeclampsia (PE) is a multisystem disorders of unknown etiology which is defined as blood pressure more than 140/90 mm Hg as measured on two separate occasions after 20 weeks of pregnancy associated with significant proteinuria (>300mg/day).[5] In some studies it has been observed that cell free fetal DNA (cffDNA) 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.[6] Tough cffDNA already recommended for screening of fetal aneuploidy, but its use to evaluate preeclampsia is a newer concept to predict adverse pregnancy outcomes[7].

Preeclampsia (PE), intrauterine growth restrictions (IUGR) and preterm labor (PTL) may often complicate a pregnancy and result in adverse pregnancy outcomes in terms of maternal as well as fetal health. Complication in pregnancy includes preterm birth, low weight birth, stillbirth, spontaneous abortion, and induced abortion. Preterm birth (<37 weeks gestation) affects 5-19% of pregnancies worldwide and is one of the leading causes of child death before 5 years.[8] About 75% of preterm birth occurs after spontaneous labor onset; preeclampsia or intrauterine growth restriction may contribute to the rest of pre-term birth.[9]Preeclampsia, increased arterial hypertension during pregnancy, previously normotensive, complicates 2%-8% of pregnancy worldwide. Intrauterine growth restriction (IUGR), where the estimated weight of unborn baby is below the 10th percentile, may result in low birth weight and other complications like hypoglycemia, low resistance to infection. Spontaneous preterm labor is caused by diverse pathological processes including infection induced inflammation, preterm prelabour rupture of membranes (PROM), vaginal bleeding, due to defective decidual hemostasis and compromised immunotolerance. Preterm labor is thus considered to be syndromic and recently reviewed.[10]Early detection of the conditions may lead to appropriate intervention. For example, preeclampsia and eclampsia are preventable to some extent if detected in early stage of pregnancy. Several randomized controlled trials have been carried out to show that aspirin, an anti-platelet agent, prevents preeclampsia effectively and safely among women with high or moderate risk of PE.[11,12,13] It has been shown that aspirin reduces relative risk of preeclampsia by 53% when administered at 12-16 weeks' of gestation.[14] Evidence that magnesium sulfate treatment is able to control and prevent eclamptic seizures has been provided.[15] Magnesium sulfate has been shown to reduce more than 50% risk of preeclampsia as well as eclampsia.[16] However, early detection or possible predictions for PE, IUGR and PTL are lacking. Several studies, even though with small sample sizes, reported that increased cffDNA in maternal plasma is elevated in PE, IUGR and PTL.[17,18] Contradictory result of no increase in cffDNA is also available. Given the observation that cffDNA in maternal plasma can be detected in early weeks of gestation and elevated in some studies, we shall test the hypothesis that increased cffDNA is associated with PE, IUGR and PTL. We shall further establish relationship between elevated amount of cffDNA and adverse outcome of pregnancy in terms of preterm birth, low birth weight, stillbirth and spontaneous abortion among pregnant women.

2. Material 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 (>300mg/day)[19]. Pregnant women attending for routine antenatal checkups at antenatal OPD of Department of Obsteterics and Gynaecology (OBG), College of Medicine & JNM Hospital, WBUHS, 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 preformed 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).

2.1. Methods

Two hundred ten preeclampsia patients within 20 – 30 years were included in this study. Age-matched pregnant women (n=210) 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 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 cffDNA isolation which could give consistent quality and quantity of cffDNA.Methods of isolation, storage conditions and time before isolation of DNA were compared and standardized.

cffDNA is typically fragmented hypermethylated DNA of about 150-200 bp[20]. However, maternal cfDNA also remain present with cffDNA in the sample. Promoter of RASSF1A is hypermethylated in trophoblast resulting in resistance to digestion by methylation sensitive restriction endonuclease HhaI, HpaII, Bstu1. 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].

2.2. Statistical analysis

Results were expressed as mean ± SE (standard error). All statistical analysis were 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 cffDNA was done by MedCalc version 15.8 (MedCalc Software bvba; 2015).

3. Results

In this study, 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 (Table 1). However, APGAR score was found 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 normal level (Table 1). Identification of cell free fetal DNA (cffDNA) in maternal plasma by both methods was found comparable (Fig. 1). However, cell free fetal DNA content (GE/ µl) in preeclampsia subjects were significantly higher than the normotensive subjects (Table 1).

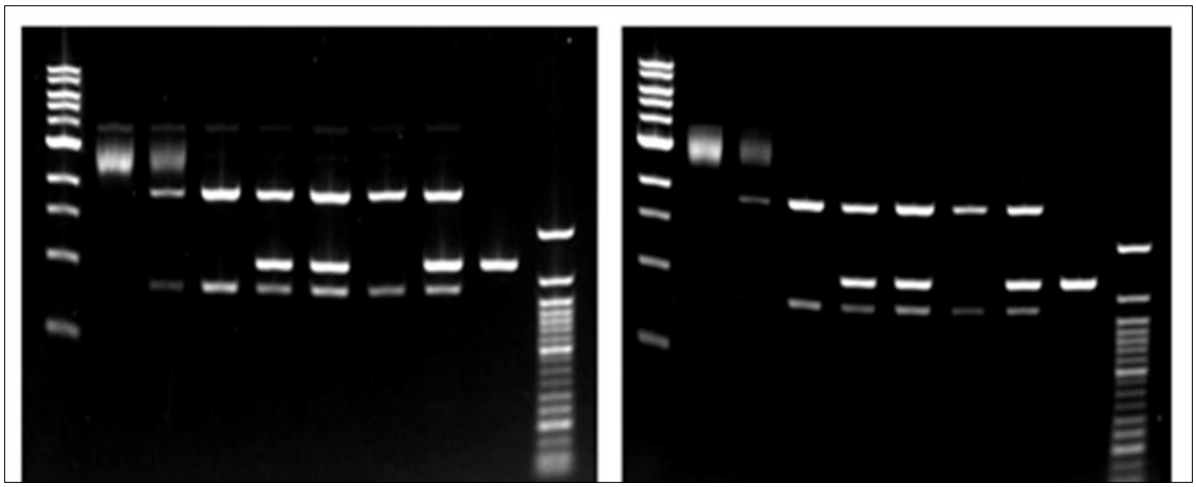

Figure 1 PCR amplification using gender independent chromosome specific locus RASSF1A. Lanes 1 (from left to right) ϕX174 digested with restriction enzyme Hae III; from lowest to highest bands were of sizes 72bp, 118bp, 194bp, 234bp (other are not mentioned). Lane 2 shows negative control (without template).Lane 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 different amount of cell free fetal DNA as mothers.

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

Parameters	Case (n=210)	Control (n=210)
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;

Receiver operating characteristic (ROC) curve analysis (Fig.2)of cffDNA content to identify cut-off for preeclampsia was done and it showed that values more than116.4 (GE/ µl) in serum has the sensitivity of 85.71% and specificity of 100% in predicting preeclampsia. Descriptive analysis of cffDNA (Table 2) has revealed that the interquartile range of cffDNA in case was 2134.5000 to 11707.5000 (GE/ µl) (95% CI for the median = 2212.0907 to 10276.1124) and in control the interquartile range was 7.6650 to 52.2000 (95% CI for the median = 7.7400 to 44.0745).

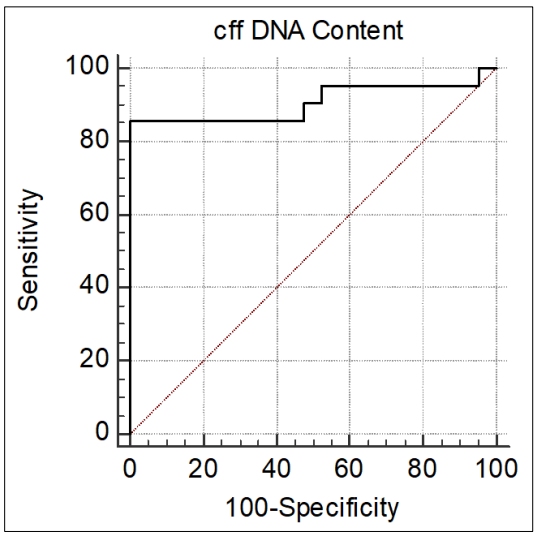

Figure 2 Receiver operating characteristic (ROC) curve analysis of cffDNA content to identify cut off for preeclampsia, [AUC = 0.907, SE ± 0.0552, 95% Confidence interval = 0.777 to 0.975, Significance level P (Area=0.5) = <0.0001]

Table 2 Descriptive statistics of cffDNA content

Variables	Case	Control
Sample size	210	210
Lowest value	3.6600	3.1800
Highest value	25056.0000	116.4000
Median	3762.0000	19.9800
95% CI for the median	2212.0907 to 10276.1124	7.7400 to 44.0745
Interquartile range	2134.5000 to 11707.5000	7.6650 to 52.2000

Correlation analysis showed (Table 3) that APGAR score was significantly negatively correlated with systolic blood pressure (r= -0.361, p<0.05) and diastolic blood pressure (r= -0.413, p<0.01); whereas significantly positively correlated with gestational age (r=0.392, p<0.01) and gestational age at birth (r=0.398, p<0.01). Interestingly, cffDNA was significantly positively correlated with systolic blood pressure (r= 0.371, p<0.05) and diastolic blood pressure (r= 0.423, p<0.01); whereas significantly negatively correlated with platelet count (r= -0.474, p<0.01).

Table 3 Comparison of correlation of APGAR score and cff DNA with other variables among total study population (n=420)

Variable	APGAR score	cffDNA
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)

4. Discussions

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 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 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 great implication on adverse neonatal outcome. Appearance, pulse, grimace, activity, respiration (APGAR) score is one of the indicators of physiologic maturity of the infant. Preeclampsia has great implication on adverse neonatal outcome[38]. Our study revealed for the first time tharAPGAR score was significantly negatively correlated with the blood pressure, while significantly 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, cffDNA can be detected as early as 5 weeks of gestation and cleared rapidly from maternal circulation within 2 hours after delivery of the baby[39]. Hence, results are not affected by previous pregnancy complications[40]. Both in house and kit based methods receiver operating characteristic (ROC) curve analysis of cffDNA 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[39,41]; 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 cffDNA were also significantly higher among women with preterm labour and adverse fetal outcome groups compared with the term and favourable outcome groups[42]. 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[43]. In a meta-analysis of 13 studies, in 11 studies, elevated cffDNA was observed in preeclampsia, while in two studies no significant association was observed[44]. Correlation analysis in our study showed that cffDNA was significantly positively correlated with blood pressure; whereas significantly negatively correlated with platelet count further indicated that higher cffDNA may predict adverse fetal outcome.

Abbreviations

- cffDNA, cell free fetal DNA;
- PE, Preeclampsia;
- MMR, Maternal Mortality Ratio

5. 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 efficacy of cffDNA quantification in different degree of preeclamptic patients.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Maternal mortality. Facts sheet. World health Organization. http://www.who.int/ media centre/factsheets/fs348/en, 2022. Accessed on 04.04.2022.

[2] Everett C. Incidence and outcome of bleeding before the 20th week of pregnancy: prospective study from general practice. BMJ. 1997; 315:32-4.

[3] Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med. 1988; 319:189-94.

[4] Weiss JL., Malone, E.D., Vidaver, J, et al. Threatened miscarriage: A risk factor for poor pregnancy outcome, a population-based screening study. Am J of Obstetrics &Gynecology. 2004; 190, 745-750.

[5] Karrar SA, Hong PL. Preeclampsia, [Updated 2022 Jun 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2022; Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK570611/ .

[6] Turbeville HR, Sasser JM. Preeclampsia beyond pregnancy: long-term consequences for mother and child. Am J Physiol Renal Physiol. 2020; 318(6):F1315-F1326.

[7] Kwak, DW, Kim, SY, Kim, HJ et al. Maternal total cell-free DNA in preeclampsia with and without intrauterine growth restriction. Sci Rep. 2020; 10: 11848.

[8] Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE . Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015; 385: 430-440.

[9] Goldenberg RL, Culhane JF, Iams JD, Romero R . Epidemiology and causes of preterm birth. Lancet. 2008; 371: 75-84.

[10] Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014; 345:760-765.

[11] Duley L, Henderson SD, Meher S, King J. Antiplatelet agents for preventing preeclampsia and its complications. Cochrane Database Syst Rev. 2007; 2: CD004659.

[12] 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 and maternal-neonatal bleeding complications. Am J Obstet Gynecol. 1995; 172: 1553-1557.

[13] CLASP (Collaborative Low-dose Aspirin Study in Pregnancy Collaborative Group. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. Lancet. 1994; 343: 619-629.

[14] Roberge S, Nicolaides KH, Demers S, Villa P, Bujold E. Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. Ultrasound Obstet Gynecol. 2013; 41: 491-499.

[15] Sheth SS, Chalmers I. Magnesium for preventing and treating eclampsia: time for international action. Lancet . 2002; 359: 1872-1873.

[16] Duley L, Gulmezoglu AM, Henderson-Smart DJ. Magnesium sulphate and other anticonvulsants for women with pre-eclampsia, Cochrane Database Syst Rev. 2003; CD000025.

[17] VanBoeckel SR, Davidson DJ, Norman JE, Stock SJ. Cell-free fetal DNA and spontaneous preterm birth. Reproduction. 2018; 155(3): R137-R145.

[18] Wu Y, Werlang A, Cheng W, Lanes A, Wen SW, Walker M. Association between Levels of Total Cell-Free DNA and Development of Preeclampsia-A Literature Review. AJP Rep . 2021; 11(1):e38-e48.

[19] Gupte S, Wagh G. Preeclampsia-eclampsia. J ObstetGynaecol India. 2014 Feb;64(1):4-13.

[20] Yu S C, Chan K C, Zheng Y W, Jiang P, Liao G J, Sun H, Akolekar R, Leung T Y, Go A T, van Vugt J M, Minekawa R, Oudejans C B, Nicolaides K H, Chiu R W & Lo Y M . Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. ProcNatlAcadSci U S A. 2014;111(23): 8583-8588.

[21] White H E, Dent C L, Hall V J, Crolla J A & Chitty L S. Evaluation of a Novel Assay for Detection of the Fetal Marker RASSF1A: Facilitating Improved Diagnostic Reliability of Noninvasive Prenatal Diagnosis. PLoS One. 2012; 7(9): e45073.

[22] Levine R J, Maynard S E, Qian C, Lim K H, England L J, Yu K F, Schisterman E F, Thadhani R, Sachs B P, Epstein F H, Sibai B M, Sukhatme V P &Karumanchi S A. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med. 2004; 350: 672-683.

[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. 2003 Mar; 111(5):649-58.

[24] Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med. 2008; 59:61-78.

[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 pre-eclampsia. Nature. 2000 Jun 15; 405(6788):797-800.

[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. 2018 Mar; 218(3):337.e1-337.e7.

[27] Elgari MM, Khabour OF, Alhag SM. Correlations between changes in hematological indices of mothers with preeclampsia and umbilical cord blood of newborns. ClinExpHypertens. 2019; 41(1):58-61.

[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. 2016 Nov; 35(4):474-482.

[29] Wang ZM, Zhu QY, Zhang JF, Wu JL, Yang R, Wang DM. Changes of platelet parameters in early severe preeclampsia. ClinExpObstet Gynecol. 2017; 44(2):259-263.

[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. 2018 Oct; 14:279-285.

[31] Lopes van Balen VA, Spaan JJ, Cornelis T, Spaanderman MEA. Prevalence of chronic kidney disease after preeclampsia. J Nephrol. 2017 Jun; 30(3):403-409.

[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. 2005; 24(3):247-57.

[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 ReprodContraceptObstet Gynecol. 2015 Sep-Oct; 4(5):1505-1510.

[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. 2017 Apr; 69(4):498-505.

[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. 2016 Jan; 25(1):85-90.

[36] Wu SW, Wu LF, Wang Q, Zhang WY. [Risk factors of adverse pregnancy outcomes during expectant management of early onset severe pre-eclampsia]. Zhonghua Fu Chan KeZaZhi. 2010 Mar; 45(3):165-9.

[37] Mishra N, Mishra VN, Thakur P. Study of Abnormal Liver Function Test during Pregnancy in a Tertiary Care Hospital in Chhattisgarh. J ObstetGynaecol India. 2016 Oct; 66(Suppl 1):129-35.

[38] Ayaz A, Muhammad T, Hussain SA, Habib S. Neonatal outcome in pre-eclamptic patients. J Ayub Med Coll Abbottabad. 2009 Apr-Jun; 21(2):53-5.

[39] Everett TR, Chitty LS. Cell-free fetal DNA: the new tool in fetal medicine. Ultrasound Obstet Gynecol. 2015 May;45(5):499-507.

[40] Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997 Aug 16; 350(9076):485-7.

[41] Drury S, Hill M, Chitty L. Recent Developments in Non-invasive Prenatal Diagnosis and Testing. Fetal and Maternal Medicine Review. Cambridge University Press; 2014;25(3-4):295–317.

[42] AbdelHalim 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. MolDiagnTher. 2016 Apr; 20(2):135-49.

[43] Seval MM, Karabulut HG, Tükün A, Koç A. Cell free fetal DNA in the plasma of pregnant women with preeclampsia. ClinExpObstet Gynecol. 2015, 42(6):787-91.

[44] Martin A, Krishna I, Badell M, Samuel A. Can the quantity of cell-free fetal DNA predict preeclampsia: a systematic review. PrenatDiagn. 2014 Jul, 34(7):685-91.