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Maternal Plasma Cell-Free Fetal DNA and Preeclampsia

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ABSTRACT

Preeclampsia(PE) is one of the serious complication of pregnancy which involves proteinuria and high blood pressure. The aim isto determine the changes in maternal serum fetal cell-free DNA (cff-DNA) in women with preeclampsia (PE) in comparisonto normotensive pregnant women.Twenty pregnant patients with PE at 28-32 weeks of gestation were included in the study. They have developed elevation of blood pressure after 24 weeks of gestation (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on at least two occasions, and detection of protein in urine (≥300 mg in a 24-hour urine collection or dipstick measurement ≥ 1). In addition, 30 normotensive pregnant women were considered as a control group. Serum obtained and nucleic acid in free condition for both groups is tested by direct fluorescent assay.Women with PE had low gestational age at delivery and low birth weight than those with normal pregnancies. In regard to Apgar scores both within 1^{st} and 5^{th} minute which was not significantly different.There are significant differences in the 2 groups in regard to mother blood test of DNA. Mother serum total cff-DNA level were higher in preeclampsia group in comparison to normotensive group. In conclusion, there is evidence

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suggesting that cff-DNA concentrations would become higher with onset of the disease. Thus, cff-DNA can be applied as a biomarker for PE.

Key words: Fetal DNA, Hypertension, Maternal plasma, Preeclampsia, Pregnant women.

INTRODUCTION

Preeclampsia(PE) is one of the serious complicatonof pregnancy which involves proteinuria and high blood pressure. There are over 30 000 women die worldwide each year because of PE, with 98% of the deaths in developing countries(Kassebaum*et al*, 2014).The total perinatal loss caused by PE occurs at a rate of 10-25%(Redman andSergent, 2000; Vogal*et al*, 2014).PE occurs in 5% primgravidae, with serious complications including cerebral hemorrhage, hepatic and renal dysfunction(Redman andSergent, 2000).Preeclampsiacanbemanifested as hypertension and proteinuria with or without fetal growth restriction(FGR) (Redman andSergent, 2000).

Preeclampsia is a placenta derived disease, with a series of pathophysiological changes before clinical symptoms, such as reactive hyperplasia of syncytiotrophoblasts caused by placental hypoxemia which could increase the HCG secretion (Lambert-Messerlian*et al*, 2000; Ahn*et al*, 2011).Many theories have implanted for the causes of PE as abnormal placental trophoblastic invasion, immunological defect and genetic predisposing factor. Therefore, PE regarded as multifactorial disorder.

The presence of circulating fetal DNA in maternal plasma and serum has been detected in 1997 (Lo *et al*, 1997). Then, it has been discovered that levels of fetal DNA are higher in pregnancies associated with preeclampsia in comparison to normal pregnancies (Ward & Taylor, 2015). So, it can be concluded that fetal DNAlevels in serum can be applied for the detection of PE.

Cell-free nucleic acids(cff-DNA) in plasma and serum are considered biomarkersfordifferent medical conditions as well as obstetrical aspect in prenatal status (Schwarzenbach*et al*, 2008).Placental or fetal-DNAmakes up a small fraction (5%) of the total cff-DNA in maternal plasma, and it is even lower in serum samples (Atamaniuk*et al*, 2011). The cff-DNA can be detected in the blood of the mother at the 5^{th}-6^{th}weeks of pregnancy which increases with advancing gestational age(Lau *et al*, 2002).

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Preeclampsia and fetal growth restriction (FGR) are the major obstetrical problem. These 2 conditions are associated with same pathogenesis and predisposed factors(Romero, 1996;Bibbins-Domingo *et al*, 2017;Nardozza*et al*, 2017). The mechanisms include abnormal placentation, defect in trophoblast invasion. chronic utero-placental ischemia, elevation of trophoblast apoptosis which lead to maternal inflammatory reaction. But beside of these similarities in the underlying mechanisms, the 2 conditions differ in their clinical features(Levine *et al*, 2006).

Previous study hadsuggested that women with history of placental dysfunction (preeclampsia [PE] and fetal growth restriction [FGR]) had high concentrations of cff-DNA(Fisher and Roberts, 2015).Both those conditions areaccompanying by placentalblood vessel disturbance which leads to ischemia of trophoblastic cells, which may increase the release of cff-DNA in maternal plasma (Madazil*et al*, 2000). A new study has observed thatcff-DNA as marker for PE its severity. They concludedthatcff-DNA levels weremore before the onset of the clinical PE (Erlebacher, 2013; Khodzhaeva*et al*, 2016).

It had been concluded that women with established PE had high level of plasma or serum concentrations of both total and fetal cell-free (cff-DNA)than in women with normal blood pressure and it is elevated in severe PE (Loisel*et al*, 2013; Redman *et al*, 2015). Such observation might be related to increase necrosis of trophoblastic cells due to infarction and decrease disappearance of the cff-DNA from the maternal blood in women with PE (Am. Coll. Obstet. Gynecol, 2002).

The aim of the study is to determine the changes in maternal serum fetal cell-free DNA (cff-DNA) in women with PE in comparisonto normotensive pregnant women.

PATIENTS AND METHODS

Ethical approval was obtained from the Committee of the Arabic Board for Medical Specialization. The women accept enrolled and involvement in the study.

Twenty pregnant patients with PE at 28-32 weeks of gestation were included in the study. They have developed elevation of blood pressure after 24 weeks of gestation (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on at least two occasions, and detection of protein in urine (≥300 mg in a 24-hour urine collection or dipstick measurement ≥ 1) (Sibai, 2003).

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In addition, 30 normotensive pregnant women were considered as a control group.Their pregnancies were ended at(37–42 weeks) as one baby with a 5-minute Apgar score ≥7 and normal birth weight. The women should be normal with uncomplicated pregnancies.Gestational estimation was detection of last menstrual period (LMP), beside ultrasound examination of fetal age in the first trimester.

Fetal growth retardation (FGR) was considered if the weight of the fetus less than the 10^{th} percentile associated with abnormal umbilical, and middle cerebral artery Doppler study (Caramelli*et al*, 2003).

Exclusion criteria:

a- Multiple pregnancies.

b-Pregnant with malformation.

c- Thyroid disorder.

d-Systemic lupus erythematosus.

e-Diabetes mellitus.

f-Placenta previa.

g-Vaginal bleeding.

Women of both groups were subjected to the following investigations:

a-Complete blood picture(CBP).

b-Renal function test.

c-Urine for albumin.

Venousserum samples were collected from all the women in both groups.Serum obtained and nucleic acid in free condition is tested by direct fluorescent assay.The assessment of cff-DNA is done by aspiration of 10 ml of venous blood in EDTA-K_2tubes,centrifuged at 1600g for 10 minutes. The supernatant plasma were collected into EppendorfDNAse free tubes. Then, it is recentrifuged again at 16000g for further 10 minutes. The final supernatant will be undergone DNA extraction by using special kit.High total cell-free DNA concentrations were defined as a serum concentration above 850ng/mL(Lo *et al*, 1999).

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Statistical evaluation was done with the SPSS package. P value <0.05 was considered statistically significant.

RESULTS

A total of 50 pregnant women were included in the study, they were divided into 20 patients with preeclampsia, and 30 women with normal pregnancies (Table 1).

The clinical features of the 2 groups were illustrated in Table (1). No significant differences were observed between the 2 groups concerning age, women body mass index, their number of pregnancy, number of children but there was significantly higher rate of intrauterine growth restriction in patients with PE than control group.

Table 1. Clinical features for both groups.

Variable	Precelampsia group: 20	normotensive group N :30	Test of significant
Women age	30± 52	28± 32	0.1
BMI	26.4 ± 5.6	26.1 ± 6.5	o.1
PARITY Primgravida 1-5 >5	 11 7 2	 13 7 10	o.1
Gestational Age at sample collection (weeks)	30.4 ± 4.6	30.9 ± 4	
History of IUGR	11	4	
History of help syndrome	1	-	0.1
Mode of delivery: Vaginal delivery	 8	 24	 0.05

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Caesarian section	12	6	0.05

Women with PE had low gestational age at delivery and low birth weight than those with normal pregnancies. In regard to Apgar scores both within 1st and 5th minute which was not significantly different (Table 2).

Table 2. Perinatal outcomes among the both groups.

Variable	Control	Preeclampsia	Test of significant
Duration of pregnancy at delivery (weeks)	38±1.5	35.3±3.7	<0.001
Neonatal Birthweight (gr)	3189.8±450.1	1232.6±753.1	<0.001
Apgar score <5 at 1'	3	9	0.05

There are significant differences in the 2 groups in regard to mother blood test of DNA. Mother serum total cff-DNA level were higher in preeclampsia group in comparison to normotensive group (Table 3).

Table 3- Total cf-DNA serum value in the two groups

	N	MEAN of DNA concentration	Range

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NORMAL PREGNANCY	30	1.67	(1.00- 2.35)
Mild PE	7	2.43	2.1- 4.2
Sever PE	13	2.55	1.98 ±4.7
P value		o.oo4	

The increment in the level of cff-DNA was higher in female with preeclampsia and fetal growth retardation than those with preeclampsia and normal growth fetus (P = 0.003) (Figure 1, Table 4).

Table 4- Maternal serum level in patient with IUGR

Variable	N	Level of serum DNA	Range
IUGR in PET group	11	1.19 ±4.7	1.05-3.2
Normal for gestational age in PET group	3	3.1± 4.2	1.97-3.56
P value		0.003	

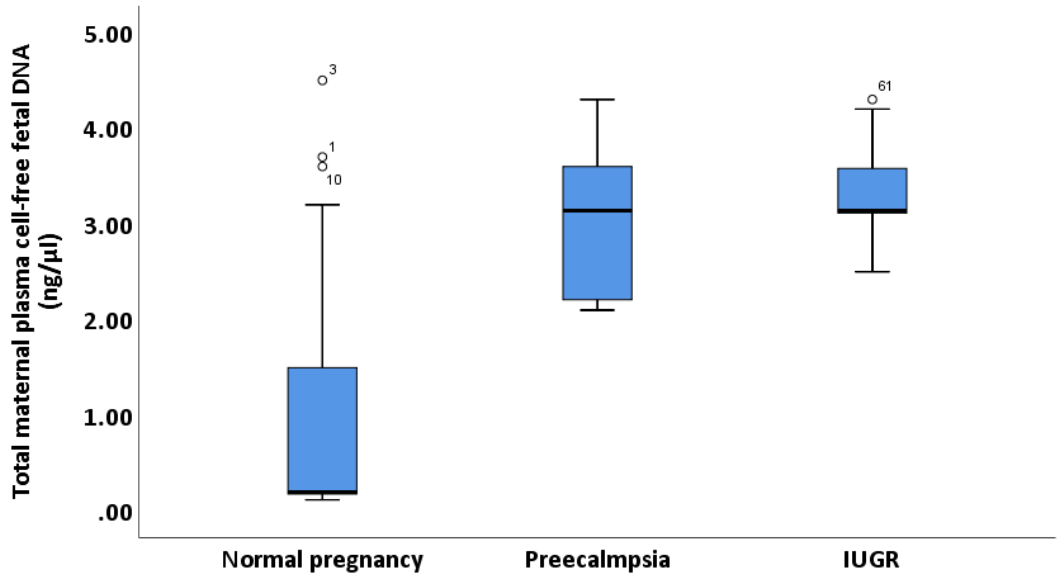

Fig 1. Maternal serum level in patients with IUGR in both groups.

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DISCUSSION

Preeclampsia is a disease affecting multiple system during the second half of pregnancy. nowadays focus on advance techniques in detection of hypertensive disorder. The new used cff-DNA inpatientsserum give chance for non- invasive fetal diagnosisin obstetrical management (Lo *et al*, 1999; Zhong*et al*, 2002).

During the present study, women with elevation of blood pressure had low period of ammenorrhea at delivery and birth weight than women with normotensive women. Apgar scores at the 1st and 5thminute were significantly different in both groups which is corresponding with past studies (Tubbergen*et al*, 1999; Lambert-Messerlian*et al*, 2000).

In the present study,serumcff-DNA were higher in hypertensive patients in comparison to normotensive women. This pattern was reported in other works (Zhong*et al*, 2002;Sibai, 2003; Caramelli*et al*, 2003;Nardozza*et al*, 2017). It has been found that the increased nucleic acid occurs at 16 weeks of pregnancy (Cotter *et al*, 2004).It has beenalso observed that some variation in the quantity of cff-DNA at 11-13 weeks in pregnant women who suffered PE (Papantonion*et al*, 2013). In contrast, it has been stated that high cff-DNA concentrations cannot be detected prior to 20 weeks of pregnancy(Bauer *et al*, 2006; Stein *et al*, 2013).But another report indicates no differences or there in cff-DNA in the second term with PE (Stein *et al*, 2013).

Furthermore, it has been confirmed that the high concentration of plasma cff-DNA was detected among pregnant women with PE (Leung *et al*, 2001; Zhong*et al*, 2002). Therefore, cff-DNA has been established as biomarker for diagnosis among risky women (Farina *et al*, 2004). Anyhow, this situationbecomes the main cause for high morbidity and mortality all over the world.

So, there are 2 mechanisms different and similar, which initiating PE and FGR and are related to each other. The similar one involves: 1) Dysfunction of trophoblast invasion. 2) The relationship between angiogenic and anti-angiogenic agents in mother circulation. 3) Ischemia of the placenta. 4) Apoptosis and necrosis of trophoblasts.5) Inflammatory reaction in the mother. Even so, these mechanisms are varying in their clinical features (Egbor*et al*, 2006; Nardozza*et al*, 2017).

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In the current study, the level cff-DNA was higher among patients with PE and IUGR in comparison to those with PE and ordinary fetal growth.

A study has proved that there is no difference between FGR gestations and normal pregnancies as far as cff-DNA values. In contrast to our study, however, the work could not found a significant variation in the serum level of cff-DNA between women with PE and those with FGR. This dissimilarity may due to usage of plasma(Sekizawa*et al*, 2003) rather than what we have done in serum sample. Similar pattern has been confirmed by many other researchers (Smid*et al*, 2006; Alberry*et al*, 2008; Al-Nakib*et al*, 2009) which was due to placenta dysfunction.

The main total fetal cff-DNA comes originally from maternal DNA. The maternal inflammation has a great role in increasing leukocytes number in PE (Alberry*et al*, 2008). The established clinical picture of PE might be related to placental hypoxia (Alberry*et al*, 2009; Al-Nakib*et al*, 2009). Placental ischemia and unavoidcff-DNA from women circulation with PE may have a role in apoptosis of trophoplastes (Lau *et al*, 2002).However, a recent review did not confirm the application of fetal cff-DNA in the diagnosis of PE before the start of the disease (Martin *et al*, 2014).

Patients with HELLP syndrome have higher plasma cff-DNA levels than those with no HELLP syndrome. This syndrome involves hepatic cellular necrosis and hemolysis indicating that cellular necrosis might have a relation to the high concentrations of cff-DNA in the maternal circulation. Thus, further work is needed to explain this mechanism(DiFederico et al, 1999).

CONCLUSION

However, there is evidence suggesting that cff-DNA concentrations would become higher before the onset of the disease. Thus, cff-DNA can be applied as a biomarker for PE. (The action is rather complicated and include apoptosis, hypoxia and inflammatory reactions).

REFERENCES

1. Ahn H, Park J, Gilman-Sachs A, Kwak-Kim J. Immunologic characteristics of preeclampsia, a comprehensive review. Am J ReprodImmunol. 2011; 65: 377–94.

2. Al-Nakib M, Desbriere R, Bonello N, Bretelle F, BoubliL, Gabert J, et al. Total and fetal cell-free DNA analysis in maternal blood as markers of placental insufficiency in intrauterine growth restriction. Fetal DiagnTher. 2009;26:24–28. doi: 10.1159/000236355.
3. Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, Abdel-Fattah SA, et al. Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. Am J Obstet Gynecol. 2009;200:e1–e6. doi: 10.1016/j.ajog.2008.07.063.
4. Alberry MS, Maddocks DG, Hadi MA, Metawi H, Hunt LP, Abdel-Fattah SA, et al. Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction. Am J ObstetGynecol. 2009; 200: 98.e1–6.
5. Am. Coll. Obstet. Gynecol. Comm. Obstet. Pract. Diagnosis and management of preeclampsia and eclampsia. ObstetGynecol. 2002; 99:159–67.
6. Atamaniuk J, Hsiao YY, Mustak M, Bernhard D, Erlacher L, Fodinger M, et al. Analysing cell-free plasma DNA and SLE disease activity. European J clin investigation. 2011;41(6): 579–83. Epub 2010/12/07. 10.1111/j.1365-2362.2010.02435.x.
7. Bauer M,Hutterer G, Eder M, Majer S, Leshane E, Johnson KL, et al. A prospective analysis of cell-free fetal DNA concentration in maternal plasma as an indicator for adverse pregnancy outcome. PrenatDiagn. 2006;26: 831-6.
8. Bibbins-Domingo K, Grossman DC, Curry SJ, Barry MJ, Davidson KW, Doubeni CA, et al. Screening for Preeclampsia: US Preventive Services Task Force Recommendation Statement. JAMA. 2017;317(16):1661–7. Epub 2017/04/27. 10.1001/JAMA. 2017.3439.
9. Caramelli E, Rizzo N, CouncuM,Simonazzi G, Carinei P, Bondavalli C, et al. Cellfree fetal DNA concentration in plasma of patients with abnormal uterine artery Doppler waveform and intrauterine growth restriction— a pilot study. PrenatDiagn. 2003; 23: 367-1.
10. Cotter AM, Martin CM, O'Leary JJ, Daly SF. Increased fetal DNA in the maternal circulation in early pregnancy is associated with an increased risk of preeclampsia. Am J ObstetGynecol. 2004; 191: 515-20.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 2924 - 2936
Received 08 November 2021; Accepted 15 December 2021.

11. DiFedericoE,Genbacev O, Fisher SJ. Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. Am J Pathol. 1999;155:293-301.
12. Egbor M, Ansari T, Morris N, Green CJ, Sibbons PD. Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. BJOG: Intern J ObstetGynaecol. 2006;113(5):580–9. Epub 2006/04/04. 10.1111/j.1471-0528.2006.00882.x .
13. Erlebacher A. Immunology of the maternal-fetal interface. Ann Rev Immunol. 2013; 31: 387.
14. Farina A, Sekizawa A, Sugito Y, Iwasaki M, Jimbo M, Saito H, et al. Fetal DNA in maternal plasma as a screening variable for preeclampsia. A preliminary nonparametric analysis of detection rate in low-risk. Prenatal Diagn. 2004; 24(2): 83-6.
15. Fisher S, Roberts JM. The placenta in normal pregnancy and preeclampsia. In Taylor RN, Roberts JM, Cunningham FG (eds): Chesley's Hypertensive Disorders in Pregnancy, 4th ed. Amsterdam, Academic Press, 2015.
16. Kassebaum NJ, Bertozzi-Villa A, Coggeshall MS, Shauckelford HA. Global, regional, and national levels and causes of maternal mortality during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet.2014;384:980–1004.
17. Khodzhaeva ZS, Kogan YA, ShmakovRG,KlimenchenkoN, AkatyevaAS, Vavina OV, et al. Clinical and pathogenetic features of early- and late-onset preeclampsia. J Matern Fetal Neonatal Med. 2016; 29(18): 2980-6
18. Lambert-Messerlian GM, Silver HM, Petraglia F, Luisi S, PezzaniI,Maybruck WM, et al. Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin a as predictiors of preeclampsia in the third trimester of pregnancy. J SocGynecol Invest. 2000; 7: 170–4.
19. Lambert-Messerlian GM, Silver HM,Petraglia F, Luisi S, Pezzani I, Maybruck WM, et al. Second-trimester levels of maternal serum human chorionic gonadotropin and inhibin a as predictiors of preeclampsia in the third trimester of pregnancy. J SocGynecol Invest. 2000; 7: 170–4.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 2924 - 2936
Received 08 November 2021; Accepted 15 December 2021.

20. Lau TW, Leung TN, Chan LY, Lau TK, Chan KC, Tam WH, et al. Fetal DNA clearance from maternal plasma is impaired in preeclampsia. ClinChem. 2002; 48(12):2141e6.
21. Lau TW, Leung TN, Chan LY, Lau TK, Chan KC, Tam WH, et al. Fetal DNA clearance from maternal plasma is impaired in preeclampsia. ClinChem. 2002; 48: 2141–6.
22. Leung TN, Zhang J, Lau TK, Chan LY, Lo YM. Increased maternal plasma fetal DNA concentrations in women who eventually develop preeclampsia. ClinChem. 2001;47: 137-9.
23. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, et al. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. The New England J Med. 2006;355(10):992–1005. Epub 2006/09/08. 10.1056/NEJMoa055352.
24. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997; 350: 485–7.
25. Lo YM, Leung TN, Tein MS, Sargent IL, Zhang J, Lau TK, Haines CJ, Redman CW: Quantitative abnormalities of fetal DNA in maternal serum in preeclampsia. ClinChem. 1999;45: 184-8.
26. Loisel DA, Billstrand C, Murray K, Patterson K, Chaiworapongsa T, Romero R, etal.The maternal HLA-G 1597 DC null mutation is associated with increased risk of pre-eclampsia and reduced HLA-G expression during pregnancy in African-American women. Mol Hum Reprod. 2013; 19(3): 144-52.
27. Madazli R, Budak E, CalayZ,Aksu M. Correlation between placental bed biopsy findings, vascular cell adhesion molecule and fibronectin levels in preeclampsia. BJOG. 2000; 107: 514-8.
28. Martin A, Krishna I, Martina B, Samuel A. Can the quantity of cell-free fetal DNA predict preeclampsia: a systematic review.PrenatDiagn. 2014; 34(7): 685-91.
29. Nardozza LM, Caetano AC, Zamarian AC, Mazzola JB, Silva CP, Marcal VM, et al. Fetal growth restriction: current knowledge. Arch gynecol obstet. 2017;295(5):1061–77. Epub 2017/03/13. 10.1007/s00404-017-4341-9.
30. Papantoniou N, Bagiokos V, Agiannitopoulos K, Kolialexi A, Destouni A, et al. RASSF1A in maternal plasma as a molecular marker of preeclampsia. PrenatDiagn. 2013;33: 682-7.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 26, Issue 1, 2022, Pages. 2924 - 2936
Received 08 November 2021; Accepted 15 December 2021.

31. plasma with Doppler status in pathological pregnancies. PrenatDiagn. 2006;26: 785–90. doi: 10.1002/pd.1504.
32. Redman CW, Sargent IL, Taylor RN. Immunology of abnormal pregnancy and preeclampsia. In Taylor RN, Roberts JM, Cunningham FG (eds): Chesley's Hypertensive Disorders in Pregnancy, 4th ed. Amsterdam, Academic Press, 2015.
33. Redman, CW. Sargent, I.L. Placental stress and preeclampsia: A revised view. Placenta. 2000; 30: S38–S42.
34. Romero R. The child is the father of the man. PrenatNeonat Med. 1996;1:8–11.
35. Schwarzenbach H, Stoehlmacher J, Pantel K, Goekkurt E. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. Ann New York Academy Sci. 2008;1137:190–6. Epub 2008/10/08. 10.1196/annals.1448.025.
36. SekizawaA,Jimbo M, Saito H, Iwasaki M, Matsuoka R, Okai T, et al. Cell-free fetal DNA in the plasma of pregnant women with severe fetal growth restriction. Am J Obstet Gynecol. 2003;188(2):480–4. Epub 2003/02/20.
37. Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. ObstetGynecol. 2003;102: 181-92.
38. SmidM,Galbiati S, Lojacono A, Valsecchi L, Platto C, Cavoretto P, et al. Correlation of fetal DNA levels in maternal
39. Stein W, Müller S,Gutensohn K, Emons G, Legler T. Cell-free fetal DNA and adverse outcome in low risk pregnancies. Eur J ObstetGynecolReprodBiol. 2013;166: 10-3.
40. Tubbergen P, Lachmeijer AM, Althuisius SM, Vlak ME, van Geijn HP, Dekker GA. Change in paternity: a risk factor for preeclampsia in multiparous women? J ReprodImmunol. 1999; 45: 81–8.
41. Vogel JP, Souza JP, Mori R, Morisaki N, Lumbiganon P, Laopaiboon M, et al. Maternal complications and perinatal mortality: findings of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014;121(Suppl 1):76–88.
42. Ward K, Taylor RN. Genetic factors in the etiology of preeclampsia. In Taylor RN, Roberts JM, Cunningham FG (eds): Chesley's Hypertensive Disorders in Pregnancy, 4th ed. Amsterdam, Academic Press, 2015.
43. Zhong XY, Holzgreve W, Hahn S. The levels of circulatory cell free fetal DNA in maternal plasma are elevated prior to the onset of preeclampsia. Hypertens Pregnancy.2002;21:77-83.