



Association between Levels of Total Cell-Free DNA and Development of Preeclampsia—A Literature Review

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Abstract

Objectives The aim of the study is to synthesize the evidence and evaluate the total cell-free deoxyribonucleic (cfDNA) associated with the prediction of preeclampsia (PE). Total cfDNA is constituted by both cell-free fetal DNA (cffDNA) originated mainly from the placenta, and maternal cfDNA derived from maternal leukocytes.

Methods A systematic review was conducted by searching PubMed and Medline. Literature reporting levels of total cfDNA in the development of PE was included. Studies that only reported cffDNA, but no cfDNA concentrations were not included in this review.

Results Eight studies were included. Seven reported values of cfDNA in PE patients, regardless of early or late onset PE, six of which demonstrated a significant increase of cfDNA in patients who subsequently developed PE. Seven studies evaluated cfDNA levels in the first trimester, six of which showed significant increase of cfDNA concentrations in women who later developed PE. Five studies investigated cfDNA levels in the second trimester, all presenting increased total cfDNA levels in the PE group compared with normal controls.

Conclusion Total cfDNA may play a role as a biochemical marker of PE, compared with fetal cfDNA. Large prospective studies with homogeneous populations and standardized methodology are needed to further confirm its predictive value.

Keywords

- ▶ cell-free DNA
- ▶ preeclampsia
- ▶ cfDNA
- ▶ pregnancy

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Preeclampsia (PE) is a pregnancy complication characterized by the onset of hypertension and proteinuria after 20 weeks of gestation.¹ PE affects approximately 5 to 9% of pregnancies worldwide and accounts for 10 to 15% of maternal death globally.^{2,3} Its etiology is multifactorial and prophylactic intervention before the clinical onset of the disease is the key to reduce maternal and fetal morbidity and mortality.⁴ According to recent research conducted in this field, biochemical markers such as placental growth factor have been found to perform well in early identifying women at high risk of developing PE.⁵ Biochemical markers reflective of placental insufficiency have become effective tools in identifying women at risk of adverse pregnancy outcomes.

Recent studies suggest that genetic markers may have a role on PE prediction.^{6–9} Total cell-free deoxyribonucleic (cfDNA) was described in 1997, and it is constituted by both cell-free fetal DNA (cffDNA) derived mainly from the placenta, and maternal cfDNA originates from maternal leukocytes.¹⁰ Since then, the possibility of using genetic markers to screen for fetal diseases has been explored. cfDNA was described as a marker of ischemia by Miranda et al,¹¹ when a gradual and strong relationship between cfDNA levels and the severity of PE was observed, with the highest levels corresponding to those patients with HELLP syndrome. Thus, cfDNA levels, both fetal and total, may represent a biomarker probably related to the aforementioned placental ischemia.¹¹

Based on this data, we searched for evidence investigating the association between the levels of total cfDNA and PE. This study was designed to systematically review the literature and investigate the association between cfDNA levels and the association with the subsequent development of PE.

Study Design

Data Sources and Search Strategy

A protocol with defined objectives, criteria for study selection, and quality assessment of included literature was developed. Covidence online software (from <https://www.covidence.org>, on October 31, 2018) was used to screen titles and abstracts. We conducted a literature search of research indexed in PubMed and Medline from 1997 to 2019 using a various combination of keywords: total cfDNA, cffDNA, maternal cfDNA, PE, and adverse pregnancy outcome.

Study Selection

The inclusion criteria were as follows: (1) studies that reported cfDNA levels with or without cffDNA levels in maternal plasma/serum in PE patients; (2) studies that collected maternal blood samples before the clinical onset of PE; (3) studies that included a comparison group of normal pregnancy; and (4) studies that reported the association between cfDNA levels and PE. Studies that only reported cffDNA but no cfDNA concentrations were excluded. Additionally, reviews, editorials, and letters to editors were excluded.

Assessment of Quality

The quality of the included articles was assessed using the Grading of Recommendations Assessment, Development,

and Evaluation (GRADE) system, which has been adopted by the Society for Maternal–Fetal Medicine.¹²

Data Extraction and Synthesis

The baseline characteristics of the eight studies are summarized in ► **Table 1**. This includes cfDNA analyzing methodology, marker utilized, gestational age (GA) when samples were collected, case and control sample sizes, median and interquartile ranges of cfDNA levels, and *p*-value for each subgroup. The studies included in this systematic review were published between 2004 and 2017. Two of the studies were conducted in South Korea, one in the United States, and five were done in Europe. Of the eight studies included in this systematic review, two were retrospective case–control studies and six were prospective case–control. In total, there were 377 cases of PE and 2,525 controls. Cases were defined as patients who developed PE and compared with healthy patients. Results were reported as mean and standard deviation, being *p* < 0.05 statistically significant.

Results

The literature and article selection process are presented in ► **Fig. 1**. A total of 206 articles were identified in PubMed and Medline through title and abstract screening. Once 99 duplicates were removed, 107 studies remained. The papers were reviewed by title and abstract by two independent investigators (Y.W. and W.C.). Based on the study selection criteria, eight papers were eligible for a full-text review.^{13–20} We listed all the excluded studies by category in Appendix 1. Each study was reviewed in detail and assessed for quality. All included literature was determined to be low quality received “C” due to the observational nature of the investigations (► **Table 2**).

Demographic variables were listed in ► **Table 3**. No studies showed significant difference between maternal age and PE. Three out of eight studies found a correlation between increased body mass index (BMI) and PE.^{13,14,19} One study indicated lower percentage of smoking in the PE group when compared with normal controls (*p* = 0.028).¹³

Different methodologies were used to analyze cfDNA. Poon et al¹⁷ used chromosome sequencing in the first trimester and Rolnik et al¹⁹ used targeted chromosome sequencing polymorphism-dependent method for samples collected in first and second trimesters. There were no significant differences found in the study conducted by Poon et al¹⁷ when the analyses were adjusted for maternal weight, race, and smoking.

The prospective case–control study conducted by Rolnik et al¹⁹ assessed cases of “early-PE” (who delivered before 34 weeks) and “late-PE” (who delivered after 34 weeks), which were screened for total cfDNA and cffDNA levels in the first and second trimesters. Despite the fact that high median total cfDNA levels were observed in first trimester screening in the early-PE group (*p* < 0.05), the multiple of the median (MoM) values were not significant after multivariate analysis was performed. In the late-PE group, no significant differences were found for total cfDNA or cffDNA levels for either absolute or MoM values at first and second trimester screening (► **Table 1**).

Table 1 Characteristics, sample size, and *p*-values of the eight included articles

Authors	Gestational age (wk)	cfDNA levels			Controls	<i>p</i> -Value
		PE				
		All PE	Early onset PE	Late onset PE		
Papantoniou et al ¹³	11–13	9,402 ^c	–	–	2,698 ^c	0.000
Kim et al ¹⁴	6–14	7,169.6 ^b (4,895.2–12,384.1)	–	–	5,188.1 ^b (2,042.6–7,682.5)	<0.05
	15–23	11,262.4 ^b (9,416.3–16,781.1)	–	–	8,505.4 ^b (5,794.7–11,479.2)	<0.05
Farina et al ¹⁵	20.4 ± 3.45	439 ± 299 ^a	–	–	284 ± 138 ^a	–
Silver et al ¹⁶	9–12	3.52	–	–	3.74	0.96
		(0.11–25.3)			(0.12–21.14)	
Poon et al ¹⁷	11–13	138.3 ^a	–	–	113.86 ^a	<0.0125
		(98.80–200.07)			(98.61–154.84)	
Salvianti et al ¹⁸	6–16	290.41 (113–313) ^a	–	–	282.07 (15–795) ^a	<0.05
	17–23	769.50 (102–1,073) ^a	–	–	310.93 (71–1,867) ^a	NS
	24–30	1,262 (311–2,330) ^a	–	–	295.60 (30–825) ^a	<0.05
	31–34	2,830.50 (393–4,163) ^a	–	–	626.72 (143–2,421) ^a	NS
	≥35	2,172.00 ^a	–	–	532.50 (54–2,245) ^a	NS
Rolnik et al ¹⁹	11–13	–	2,104 ^a (1,454–3,547)	2,178 ^a (1,123–2,847)	1,590 ^a (1,111–2,312)	<0.025
	20–24	–	–	2,140 ^a (1,067–2,934)	1,746 ^a (1,162–2,311)	NS
Kim et al ²⁰	7–14	26,289.1 ± 8,923.9 ^b			4,007.2 ± 537.1 ^b	<0.001
	15–28	7,962.3 ± 2,529.9 ^b			3,254.2 ± 287.7 ^b	0.01
	29–41	41,206.8 ± 6,488.2 ^b			8,771.2 ± 681.5 ^b	<0.001

Abbreviations: AUC, area under the curve; cfDNA, cell-free deoxyribonucleic acid; NS, no significance; n/a, not applicable (chromosome sequencing methodology does not use epigenetic markers); PE, preeclampsia; SD, standard deviation.

^aGE/mL.

^bCopies/mL.

^cEq/mL; no median or SD was mentioned, just boxplot and AUC curve.

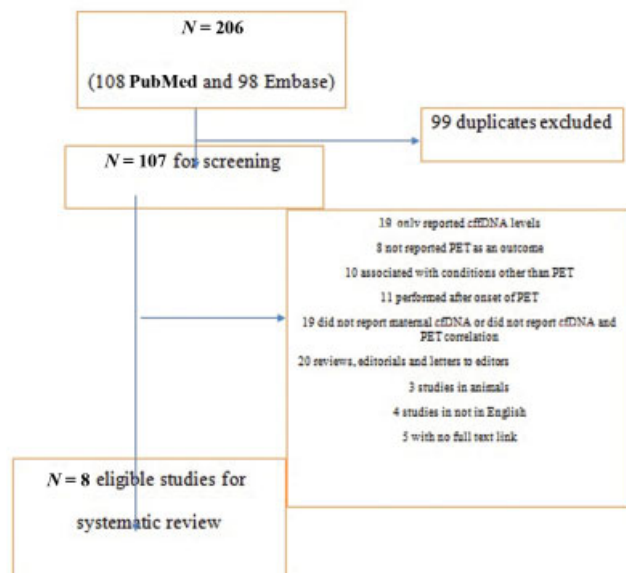


Fig. 1 Flowchart of study review and selection (PRISMA).

Six studies analyzed cfDNA levels by real-time polymerase chain reaction (PCR), using different epigenetic markers to identify the maternal and fetal fractions.^{13–16,18,20} RASSF1A was used in five of six studies as a marker of cfDNA.^{13,14,16,18,20} Five of six studies found significantly higher median levels of cfDNA in the first trimester of patients who developed PE when compared with healthy controls.^{13–15,18,20} Silver et al¹⁶ conducted a prospective multicenter double-blinded randomized case-control trial and found no significant correlation between cfDNA levels of women who developed PE and controls (*p* = 0.96).

When evaluating total cfDNA levels in maternal circulation in the second trimester, five studies presented increased total cfDNA levels in PE group compared with healthy controls.^{14,15,18–20} Salvianti et al¹⁸ demonstrated a significant increase of total cfDNA concentrations in maternal plasma in the late second trimester, from 24 to 30 weeks; however, they did not find the same results at 17 to 23 weeks.

Four of the eight included studies showed a longitudinal design evaluating cfDNA and cffDNA levels among different gestational ages.^{14,18–20} Arrows were used to represent the

Table 2 The individual study methodology

Authors	Laboratory methodology (epigenetic marker)	Design	Cases (number)	Control (number)	p-Value	Grade
Papantoniou et al ¹³	qRT-PCR (RASSF1A)	RCC	24	48	0.000	C
Kim et al ¹⁴	Real-time PCR (HYP2)	PCC	34	84	<0.05	C
Farina et al ¹⁵	Real-time PCR (β -Globin)	PCC	8	40	>0.05	C
Silver et al ¹⁶	Real-time PCR (α -actin)	PCCR	175	175	>0.05	C
Poon et al ¹⁷	Chromosome-selective sequencing (n/a)	RCC	46	1,805	<0.0125	C
Salvianti et al ¹⁸	qPCR (RASSF1A)	PCC	21	73	<0.05 ^a	C
Rolnik et al ¹⁹	Targeted sequencing polymorphism-dependent method (n/a)	PCC	40	200	<0.025 ^b	C
Kim et al ²⁰	Real time PCR (RASSF1A)	PCC	29	229	<0.01	C

Abbreviations: PCC, prospective case-control study; PCR, polymerase chain reaction; PCCR, prospective case-control study with retrospective analysis, double-blinded; PE, preeclampsia; RCC, retrospective case-control study.

^aSalvianti et al showed p-value < 0.05 at 6 to 16 wk and 24 to 30 wk.

^bRolnik et al showed p-value <0.025 only for early onset PE vs. control.

Table 3 Maternal demographic characteristics

Authors	Maternal age (y)		BMI (kg/m ²)		Nulliparous (%)		Smoking (%)		Family history of mother with PE (%)	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	controls
Papantoniou et al ¹³	32.9 (28.8–37.3)	32.2 (28.8–37.3)	27.9 ^a (23.4–32.9)	24.8 (22.5–26.2)	91.6 ^b	60.4	50 ^c	73	0	0
Kim et al ¹⁴	34.1 (32.7–36.0)	33 (31.0–35.3)	22.3 ^d (20.3–23.6)	20.2 (18.7–21.7)	73.5	54.8	0	0	n/a	n/a
Farina et al ¹⁵	30.5 ± 5.03	33.5 ± 2.49	–	–	–	–	–	–	–	–
Silver et al ¹⁶	22.5 ± 4.4	22.7 ± 4.4	28.3 ± 6.9 ^d	25.0 ± 5.2 ^d	–	–	16	23.4	–	–
Poon et al ¹⁷	29.7 (24.8–33.6)	31.9 (27.8–35.4)	–	–	–	–	5.8	2.2	–	–
Salvianti et al ¹⁸	34	32	21	22.04	–	–	–	–	23	–
Rolnik et al ¹⁹	(11–13 wk) 29.3 (23.5–34.6)	31.6 (28.8–35.4)	29.8 ^d (24.4–35.2)	24.1 (21.5–27.9)	70 ^d	40.5 40.5	5	6 6	15	5.5 5.5
	(20–24 wk) 32 (29.6–33.3)		31.5 ^d (25.2–34.4)	25.7 (22.6–29.1)	50		10	5		
Kim et al ²⁰	34.3 ± 3.9	32.8 ± 3.7	25.8 ± 4.2 ^a	20.6 ± 3.0 ^a	36.3	41.0	–	–	–	–

Abbreviations: BMI, body mass index; PCR, polymerase chain reaction; PE, preeclampsia; SD, standard deviation.

^ap = 0.0001.

^bp = 0.013.

^cp = 0.028.

^dStatistically significant difference between cases and controls.

Note: Maternal age and body mass index (BMI) were described with Median ± SD or mean.

variation trend (→ **Table 4**). We found that total cfDNA was detected earlier in pregnancy when compared with cffDNA. Kim et al¹⁴ observed total cfDNA levels were increased as early as 6 to 14 weeks, as well as 15 to 23 weeks in patients who subsequently developed PE.

Kim et al¹⁴ used the different markers to evaluate levels of cfDNA and cffDNA in both the first and second trimesters. They found that median levels of total cfDNA when associated with marker HYP2 gene were significantly increased at 6 to 14 and 15 to 23 weeks' gestation for PE, as well as the MoM values were significantly different than the controls. However, the median levels and MoM values of cffDNA when associated with

markers DSCR3 and RASSF1A genes at 6 to 14 and 15 to 23 weeks were not significantly different in the PE group during the first or second trimester when compared with controls.

Discussion

This literature review identified eight studies that investigated the association between cfDNA levels and PE. Most studies showed similar results, demonstrating that median levels of total cfDNA were higher in patients who subsequently developed PE.^{13–15,17–20} Our results point out that

Table 4 Time points of levels of cfDNA and cffDNA in four studies focusing on different gestational ages

Authors	Gestational age (wk)	cfDNA level	cffDNA level	Epigenetic marker
Kim et al ¹⁴	6–14	↑	NS	<i>HYP2</i> , <i>DSCR3</i> and <i>RASSF1A</i>
	15–23	↑	NS	
Salviati et al ¹⁸	6–16	↑	NS	<i>RASSF1A</i>
	17–23	NS	NS	
	24–30	↑	↑	
	31–34	NS	NS	
	≥35	NS	NS	
Rolnik et al ¹⁹	11–13	↑	↓	^a
	20–24	NS	↓	
Kim et al ²⁰	7–14	↑	NS	<i>RASSF1A</i>
	15–28	↑	↑	
	29–41	↑	↑	

Abbreviations: cfDNA, cell-free deoxyribonucleic acid; cffDNA, cell-free fetal deoxyribonucleic acid; NS, no significance.

^aRolnik et al²⁴ determined cfDNA and cffDNA levels using targeted sequencing polymorphism-dependent method. Hence, there is no marker used in this study.

there could be a correlation between high levels of total cfDNA in the first trimester and subsequently developed early PE (before 34 weeks)^{13,17–20} and late PE (after 34 weeks).^{14,20} One study showed correlation between high levels of total cfDNA and development of severe PE.¹⁵ One study showed no significant difference of total cfDNA concentrations between PE patients and healthy controls.¹⁶ We believe that inconsistent results could be explained by different testing methods used to extract cfDNA.

Several maternal demographic factors associated with PE were identified. Silver et al¹⁶ showed a mild correlation between high BMI and levels of total cfDNA, and this was statistically significant even when adjusted by race. However, they did not find a significant association between cfDNA and development of PE. Smoking is known to impair placentation and stimulate trophoblastic death, therefore increasing levels of cfDNA.¹⁷ Two studies in our review found statistically higher median levels among Afro-Caribbean women when compared with Caucasian.^{17,19} The reason behind this fact is unclear.

This review identified several advantages of screening with cfDNA over cffDNA. First, cfDNA measurements are comparatively homogeneous, primarily tested by sequencing,^{21,22} as opposed to the measurements of cffDNA which are highly heterogeneous (including various methods and diverse genes representing cffDNA levels).^{7,9,14,19,23,24} Second, unlike cffDNA testing which needs to be determined by additional methods, such as real-time PCR or methylation modification, cfDNA levels can be measured simultaneously with noninvasive prenatal testing in the first trimester.^{25,26} Third, it is not gender-dependent, unlike the cffDNA testing that needs to focus on the Y-specific sequences, such as *DYS14* or *SRY*.^{6–8,10,27,28}

A sensitive and noninvasive test that could identify women with high risk of PE early in pregnancy would have a significant

impact in obstetric management. It would offer an opportunity to introduce prophylactic measures to selected patients, as well as increase clinical surveillance, and potentially reduce maternal–fetal morbidity and mortality. We believe that cfDNA could be a reliable noninvasive marker of PE when screened in the first trimester. However, more randomized controlled trials are needed to prove this association.

Limitations

The limitations of this review must be acknowledged. First, we were unable to perform a thorough analysis of the different results and meta-analysis as there was significant methodological heterogeneity between studies, with objectives comparing different fractions of DNA analyzed by either PCR or biochemical markers. Second, demographic confounding factors were not always adjusted for, and there were relatively small sample sizes in the PE groups. Further research within larger populations, controlled for demographic bias, with standardized methodology and similar gestational age gaps should be performed to draw more consistent conclusions.

Conclusion

In conclusion, this is a review concerning the potential association between total cfDNA and cffDNA in early identification of patients who are at high risk of subsequent PE. Data from current literature suggest that total cfDNA levels in maternal circulation could be used as a predictive marker for early detection of PE.

Author's Contributions

Y.W. and A.W. contributed equally for the development of this paper and should be considered first authors. Y.W., A.L., and M.W. developed the research question and protocol for this study. Y.W. wrote the first draft and extracted the data. A.W. critically reviewed, rewrote, and revised the manuscript. Y.W., A.W., and M.W. have primary responsibility for the final content. All authors provided feedback on the read and approved the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Appendix 1

1. *qStudies that only reported cffDNA levels without cell-free deoxyribonucleic levels were excluded.*

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