RESEARCH PAPER

EPIGENETIC CHANGES IN PREECLAMPSIA: A SYSTEMATIC REVIEW OF DNA METHYLATION AND ITS POTENTIAL AS AN EARLY PREDICTOR

[1] Hatsari Marintan Porman Surtana Siahaan, [2] Johny Marpaung, [3] Sarma Nursani Lumbanraja

[1,2,3] Department Obstetric and Gynaecology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

Corresponding Author :

Sarma Nursani Lumbanraja. Department Obstetric and Gynaecology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia. Email : sarma.nursani@usu.ac.id. Whatsapp : +628126536472

ABSTRACT

Introduction: Preeclampsia is a severe pregnancy complication with significant maternal-fetal risks, yet early and accurate prediction remains a clinical challenge due to a lack of reliable biomarkers. Epigenetic modifications, particularly DNA methylation, are emerging as a promising field for understanding and potentially predicting this complex disorder by revealing subtle biological changes that precede its clinical onset. This systematic review evaluates the existing evidence on DNA methylation changes in preeclampsia and assesses their potential as early predictors.

Methods: Following PRISMA 2020 guidelines, a systematic search was conducted across PubMed, Semantic Scholar, Springer, and Google Scholar. Studies were included if they were human cohort or case-control studies investigating DNA methylation in biological samples as an early predictor for preeclampsia. After screening 255 records, eight studies met the full inclusion criteria and were synthesized.

Results: The included studies identified tissue-specific differentially methylated regions (DMRs) in maternal blood, cell-free DNA, cord blood, and placenta. Key genes implicated in preeclampsia pathophysiology, including sFLT-1, TRAF3IP2-AS1/TRAF3IP2, and AVP, showed altered methylation patterns. However, the clinical utility for early prediction was limited; predictive models demonstrated high specificity (88-97%) but low sensitivity (22-33.3%). Furthermore, findings were significantly influenced by confounding factors such as maternal race and body mass index (BMI), and most markers lacked replication in large, diverse cohorts.

Conclusion: DNA methylation is a biologically relevant feature of preeclampsia but is not yet sufficiently robust for clinical application as a predictive tool. The translation of these findings is hindered by low predictive sensitivity and a lack of validation. Future research must prioritize large-scale, longitudinal studies in multi-ethnic populations to validate markers and develop integrated prediction models.

Keywords: Preeclampsia, DNA Methylation, Epigenetics, Biomarkers, Early Prediction

How to cite this article: Siahaan HMPS, Marpaung J, Lumbanraja SN. Epigenetic Changes in Preeclampsia: A Systematic Review of DNA Methylation and Its Potential as an Early Predictor. Int J Drug Deliv Technol. 2026;16(53s): 753-767. DOI: 10.25258/ijddt.16.53s.124

Source of support: Nil.

Conflict of interest: None.

INTRODUCTION

Preeclampsia stands as a formidable complication of pregnancy, characterized by new-onset hypertension and proteinuria, posing significant risks to both maternal and fetal health. The complexity of its pathophysiology has long been a challenge for clinicians, making early and accurate prediction difficult. The absence of

reliable early-stage biomarkers means that diagnosis often occurs only after the clinical syndrome has manifested, limiting opportunities for preventive interventions. This clinical challenge underscores the urgent need for novel approaches to identify at-risk individuals early in gestation, thereby enabling timely monitoring and management to mitigate adverse outcomes. The exploration of molecular markers has thus become a critical frontier in obstetric research, aiming to uncover the subtle biological changes that precede the onset of this devastating condition (Nagarajan et al., 2016).

In recent years, the field of epigenetics has emerged as a highly promising avenue for understanding the multifactorial nature of complex diseases like preeclampsia. Epigenetic modifications, such as DNA methylation, do not alter the genetic sequence itself but regulate gene expression in response to environmental and physiological cues. DNA methylation, the addition of a methyl group to a cytosine-phosphate-guanine (CpG) site, is a key mechanism for controlling gene activity. Aberrant methylation patterns have been implicated in a wide range of human diseases, suggesting that these changes could serve as sensitive indicators of disease processes. Given the profound physiological changes that occur during pregnancy, investigating the role of DNA methylation in preeclampsia may provide crucial insights into its origins and progression (Liu et al., 2023).

A growing body of research has specifically investigated the link between differential DNA methylation and preeclampsia. These studies have analyzed various biological samples, including maternal blood, plasma, cord blood, and placental tissue, to identify epigenetic signatures associated with the disorder. Several studies have successfully identified differentially methylated regions (DMRs) and specific CpG sites in genes thought to be involved in pathways relevant to preeclampsia, such as immune regulation, cardiovascular function, and stress response. For instance, genes like sFLT-1, TRAF3IP2-AS1, AVP, and NR3C1 have been highlighted as having altered methylation patterns in women who develop preeclampsia, pointing toward a tangible epigenetic component in the disease's development (Baetens et al., 2024; Sharp et al., 2019).

Despite these promising discoveries, the findings across studies have often been inconsistent, and the clinical utility of these methylation markers remains limited. A significant challenge is that many predictive models, while demonstrating high specificity, suffer from low sensitivity, making them unreliable for widespread screening. Furthermore, the majority of these findings originate from small, homogeneous cohorts, and their replication and validation in larger, more diverse populations are critically lacking. This gap in the research limits the generalizability of the results and hinders the translation of these potential biomarkers into clinical practice (Baetens et al., 2024).

Moreover, the interpretation of DNA methylation data is complicated by several confounding factors. Studies have consistently reported that variables such as maternal race, body mass index (BMI), and the gestational age at which samples are collected significantly influence methylation patterns. For example, some methylation changes are only detectable in specific trimesters, and certain epigenetic signatures appear to be race-specific, indicating that a one-size-fits-all predictive model may not be feasible. These population-specific considerations highlight the need for a comprehensive and systematic evaluation of the existing literature to untangle these complex interactions and assess the true potential of methylation markers (Heinsberg et al., 2021; Ray et al., 2020).

To address these limitations and synthesize the current state of knowledge, this study was conducted as a systematic review. By adhering strictly to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines, we aimed to provide a methodologically rigorous and unbiased assessment of the available evidence. A systematic review is the ideal methodology to aggregate findings from disparate studies,

critically appraise their quality, and identify consistent patterns and persistent knowledge gaps. This approach allows for a comprehensive overview that can guide future research efforts and inform the potential development of clinically relevant predictive tools (Sharp et al., 2019).

The primary objective of this systematic review is to evaluate the existing evidence on epigenetic changes in preeclampsia, with a specific focus on DNA methylation and its potential as an early predictor of the condition. To achieve this, we sought to identify specific genes and genomic regions that exhibit significant methylation changes in preeclamptic pregnancies compared to normotensive controls. We also aimed to assess the predictive performance of proposed methylation markers, examining their reported sensitivity and specificity, and to understand how factors such as sample type, timing of measurement, and population characteristics influence these findings.

By systematically searching multiple databases and applying stringent eligibility criteria, this review consolidates research focusing on pregnant women with preeclampsia, DNA methylation analysis, and the outcome of early prediction. We specifically included prospective cohort, retrospective cohort, and case-control studies that analyzed human biological samples to ensure clinical relevance. Through this comprehensive analysis, this review aims to clarify the current landscape of DNA methylation markers in preeclampsia, highlight the most promising candidates for future investigation, and provide a clear roadmap for the research required to translate these epigenetic discoveries into effective tools for the early detection and management of preeclampsia (Liu et al., 2023).

METHODS

Protocol

The study strictly adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines to ensure methodological rigor and accuracy. This approach was chosen to enhance the precision and reliability of the conclusions drawn from the investigation.

Criteria for Eligibility

This systematic review aims to evaluate epigenetic changes in preeclampsia: DNA methylation and its potential as an early predictor

Screening

We screened in papers that met these criteria:

- **Study Population**: Does the study include pregnant women diagnosed with preeclampsia AND include an appropriate control group of normal pregnancies?
- **DNA Methylation Analysis**: Does the study examine DNA methylation patterns (not solely other epigenetic modifications) comparing preeclamptic and normal pregnancies?
- **Biological Samples**: Does the study analyze methylation patterns in maternal blood, placental tissue, or other relevant biological samples from human subjects?
- **Study Timing**: Does the study investigate methylation patterns before or during early stages of preeclampsia (not exclusively after diagnosis)?
- **Study Design**: Is the study design either a prospective cohort, retrospective cohort, or case-control study (not a case report or case series)?
- **Species**: Is the study conducted in humans (not in animals or using only in vitro experiments)?

We considered all screening questions together and made a holistic judgement about whether to screen in each paper.

Data extraction

We asked a large language model to extract each data column below from each paper. We gave the model the extraction instructions shown below for each column.

- **Study Design Type**:
  Identify the specific type of study design used. Look in the methods section for explicit description of the study design. Possible types include:
- Cohort study

- Case-control study
- Cross-sectional study
- Longitudinal study
- Prospective or retrospective analysis

If multiple design elements are present, list all relevant types. If the design is not clearly stated, write ”Design not clearly specified” and note the page or section where design details were sought.

- **Epigenetic Analysis Method**:
  Specify the exact method used for DNA methylation analysis. Look in methods section for technical details. Extract:
- Type of methylation analysis (e.g., genome-wide, targeted)
- Specific technique (e.g., bisulfite sequencing, methylation-specific PCR, microarray)
- Specific regions examined (e.g., whole genome, specific chromosomes, gene-specific)

If multiple methods were used, list all. If method details are incomplete, note ”Incomplete method descrip- tion” and specify what information is missing.

- **Participant Population Characteristics**:
  Extract demographic and clinical details about study participants:
- Total number of participants
- Gestational age range
- Maternal age range
- Inclusion/exclusion criteria for preeclampsia diagnosis
- Proportion of participants with preeclampsia vs control group

Use exact numbers and percentages where possible. If any information is missing, clearly indicate ”Not reported” for that specific characteristic.

- **Key Epigenetic Findings**:
  Extract the primary epigenetic findings related to preeclampsia:
- Specific genes or genomic regions with significant methylation changes
- Magnitude of methylation differences (e.g., fold change, statistical significance)
- Timing of methylation changes (early vs late pregnancy)
- Potential functional implications of observed methylation changes

Prioritize statistically significant findings. If findings are complex, summarize the most important results. Use exact p-values and effect sizes when reported.

- **Predictive Potential of Methylation Markers**:
  Describe the study's assessment of methylation as a predictive tool:
- Predictive accuracy metrics (sensitivity, specificity, positive/negative predictive values)
- Timing of potential prediction (first trimester, specific gestational age)
- Any developed prediction models or risk scores
- Statistical validation methods used

If multiple predictive metrics are reported, list all. If prediction potential is not fully explored, note the limitations in the study's approach.

Search Strategy

The keywords used for this research based **PICO :**

Element	Keyword 1	Keyword 2	Keyword 3	Keyword 4
Population (P)	Individual with Preeclampsia	Pregnant women diagnosed with preeclampsia	Woman with preeclamptic condition	Individual diagnosed with Preeclampsia
Intervention (I)	DNA Methylation	Epigenetic Changes	Differentially Methylated Regions (DMRs)	Epigenetic Modifications
Comparison (C)	Control Group	Healthy Pregnancies	Normal Pregnancies	Uncomplicated Pregnancies

Outcome (O)	Early Predictor	Early Detection	Predictive Value	Risk Assessment

The Boolean MeSH keywords inputted on databases for this research are: *("Individual with Preeclampsia" OR "Pregnant women diagnosed with preeclampsia" OR "Woman with preeclamptic condition" OR "Individual diagnosed with Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications") AND ("Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies") AND ("Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")*

Data retrieval

Abstracts and titles were screened to assess their eligibility, and only studies meeting the inclusion criteria were selected for further analysis. Literature that fulfilled all predefined criteria and directly related to the topic was included. Studies that did not meet these criteria were excluded. Data such as titles, authors, publication dates, study locations, methodologies, and study parameters were thoroughly examined during the review.

Quality Assessment and Data Synthesis

Each author independently assessed the titles and abstracts of the selected studies to identify those for further exploration. Articles that met the inclusion criteria underwent further evaluation. Final decisions on inclusion were based on the findings from this review process.

Table 1. Article Search Strategy

Database	**Keywords**	**Hits**
Pubmed	*("Individual with Preeclampsia" OR "Pregnant women diagnosed with preeclampsia" OR "Woman with preeclamptic condition" OR "Individual diagnosed with Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications") AND ("Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies") AND ("Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")*	1
Semantic Scholar	*("Individual with Preeclampsia" OR "Pregnant women diagnosed with preeclampsia" OR "Woman with preeclamptic condition" OR "Individual diagnosed with Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications") AND ("Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies") AND ("Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")*	250
Springer	*("Individual with Preeclampsia" OR "Pregnant women diagnosed with preeclampsia" OR "Woman with preeclamptic condition" OR "Individual diagnosed with Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications" AND "Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies" AND "Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")*	11
Google Scholar	*("Individual with Preeclampsia" OR "Pregnant women diagnosed with preeclampsia" OR "Woman with preeclamptic condition" OR "Individual diagnosed with Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications") AND ("Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies") AND ("Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")*	5

Preeclampsia") AND ("DNA Methylation" OR "Epigenetic Changes" OR "Differentially Methylated Regions (DMRs)" OR "Epigenetic Modifications") AND ("Control Group" OR "Healthy Pregnancies" OR "Normal Pregnancies" OR "Uncomplicated Pregnancies") AND ("Early Predictor" OR "Early Detection" OR "Predictive Value" OR "Risk Assessment")

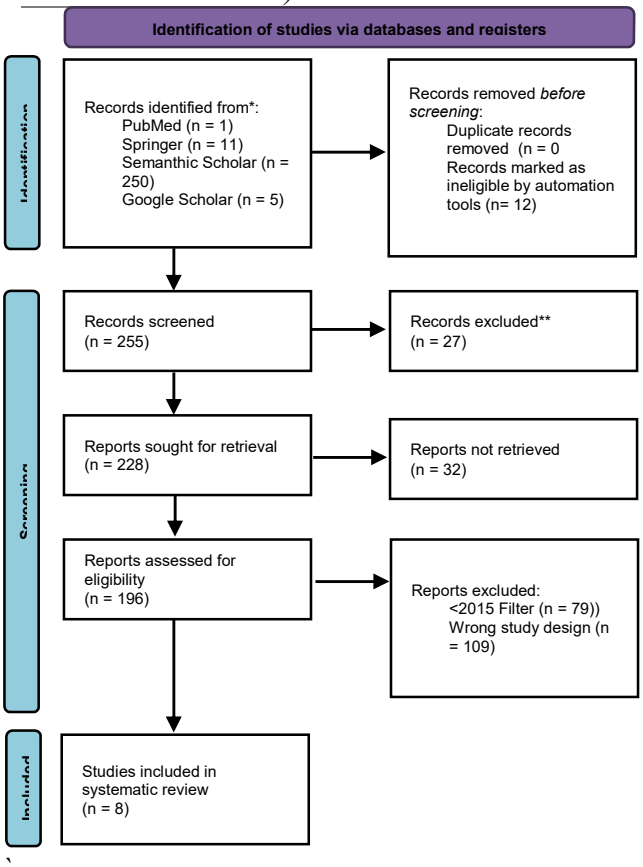

Figure 1. Article search flowchart

RESULTS

Characteristics of Included Studies

Study	Sample Type	Population Characteristics	Primary Findings
Baetens et al., 2024	Maternal plasma (cell-free DNA)	56 preeclampsia cases, 183 controls; gestational age 11-38 weeks; detailed cohort structure; maternal age not reported	Early and late pregnancy differentially methylated regions (DMRs) in cell-free DNA; sFLT-1 (soluble fms-like tyrosine kinase-1) methylation; high specificity, low sensitivity prediction models
Liu et al., 2023	Maternal blood	Discovery: 28 cases, 28 controls; Replication: 64 cases, 50 controls; all trimesters; age less than 14 or greater than 40 excluded	16 cytosine-phosphate-guanine (CpG) sites with suggestive significance; DMRs in TRAF3IP2-AS1/TRAF3IP2 genes; validation in one sample, not replicated
Ray et al., 2020	Maternal blood	28 cases, 28 controls; mean age 23.94; trimesters 1-3; race-stratified	Race- and trimester-specific white blood cell (WBC) proportion differences; no direct gene-level methylation findings
Heinsberg et al., 2021	Maternal blood	28 cases, 28 controls; trimester-specific samples; age not reported	No association between methylation age and preeclampsia; race and body

			mass index (BMI) effects on methylation age
Heinsberg et al., 2020	Maternal blood	28 cases, 28 controls; age 14-40; trimesters 1-3	No association between methylation age acceleration and preeclampsia; race and BMI effects
Knihtilä et al., 2023	Cord blood	No mention found	Cord blood methylation changes in neonates of preeclamptic mothers; no specifics
Sharp et al., "Disorders of Pregnancy"	Cord blood	Hypertensive disorders of pregnancy: 5242 (476 cases); preeclampsia: 2219 (135 cases); age, gestational age not reported	26 CpG sites associated with preeclampsia; modest methylation differences; AVP (arginine vasopressin) gene implicated
Nagarajan et al., 2016	Placenta (various), maternal/cord blood	No mention found	NR3C1 (glucocorticoid receptor) and HSD11 (11-hydroxysteroid dehydrogenase type 2) methylation associated with preeclampsia
			and adverse outcomes

Study Design

- Five studies used a longitudinal, case-control, or prospective design.
- Two studies were systematic reviews.
- One study was a meta-analysis of cohort studies.
- One study did not clearly specify its design.

Sample Type

- Five studies used maternal blood, including plasma or cell-free DNA.
- Two studies used cord blood.
- One study used placenta samples.
- One study did not mention the sample type.
- One systematic review included multiple sample types (placenta, maternal blood, cord blood).

Primary Findings

- Five studies reported differentially methylated regions or CpG methylation findings.
- Four studies reported specific gene-level methylation associations (sFLT-1, TRAF3IP2-AS1/TRAF3IP2, AVP, NR3C1, HSD11).
- Two studies reported on methylation age or age acceleration.
- Two studies did not report methylation-specific findings.
- Two studies reported race or BMI effects on methylation.
- One study reported on prediction models for preeclampsia.
- One study reported on white blood cell proportion differences.
- One study focused on first trimester risk models (not methylation-specific).
- One study reported cord blood methylation changes without further specifics.

DNA Methylation Patterns in Preeclampsia

Tissue-Specific Methylation Changes

Study	Tissue Type	Key Methylation Changes	Clinical Significance

Baetens et al., 2024	Maternal plasma (cell-free DNA)	DMRs in symptomatic and presymptomatic cases; sFLT-1 hypomethylation	Early detection; sFLT-1 hypomethylation may be associated with higher expression in preeclampsia
Liu et al., 2023	Maternal blood	DMRs in TRAF3IP2-AS1/TRAF3IP2 genes	Immunoregulatory pathway involvement
Ray et al., 2020	Maternal blood	White blood cell proportion differences (B cells, neutrophils)	Immune modulation in preeclampsia; race-specific
Heinsberg et al., 2021	Maternal blood	No significant gene-level changes	No clinical significance for preeclampsia
Heinsberg et al., 2020	Maternal blood	No significant gene-level changes	No clinical significance for preeclampsia
Kniht ilä et al., 2023	Cord blood	No mention found	No mention found
Sharp et al., "Disorders of Pregnancy"	Cord blood	26 CpG sites (e.g., AVP gene)	Developmental, neurological, cardiovascular implications
Nagarajan et al., 2016	Placenta, maternal/cord blood	NR3C1, HSD11 methylation	Biomarkers for stress, neurobehavior, blood pressure
Brunelli and Prefumo, 2015	No mention found	No mention found	No mention found

Tissue types analyzed:

- Maternal blood (including plasma, cell-free DNA, white blood cells): six studies
- Cord blood: three studies
- Placenta: one study

Key methylation changes:

- Five studies reported specific DMRs or gene methylation changes

Clinical significance:

- Two studies described early detection or biomarker potential
- Three studies described immune, developmental, or cardiovascular implications

Temporal Methylation Dynamics

Study	Trimester	Key Methylation Changes	Clinical Significance
Baetens et al., 2024	1st-3rd	Early DMRs at 12 weeks; late hypomethylation	Early prediction; dynamic methylation
Liu et al., 2023	1st-3rd	DMRs in each trimester; strongest	Potential for trimester-specific

		in 3rd	predictio n
Ray et al., 2020	1st-3rd	B cell/neutr ophil differences in 1st/2nd trimesters	Immune changes precede preecla mpsia
Heinsb erg et al., 2021/2 020	1st-3rd	No associatio n with preeclamp sia; race/BMI effects vary by trimester	No predictiv e value for preecla mpsia
Sharp et al., "Disor ders of Pregna ncy"	Birth, adoles cence	Cord blood changes persist to adolescen ce	Long-term develop mental impact
Others	No mentio n found	No mention found	No mention found

Trimester of sampling/analysis:

- Four studies analyzed methylation changes across all trimesters (1st–3rd)
- One study examined changes at birth and adolescence

Key methylation changes:

- Two studies reported DMRs detected, with some showing trimester-specific patterns
- One study found immune cell-specific methylation differences in early pregnancy
- One study reported no association with preeclampsia, but noted race/BMI effects by trimester
- One study found that cord blood methylation changes persisted into adolescence

Clinical significance:

- One study suggested early prediction of preeclampsia and dynamic methylation changes
- One study indicated potential for trimester-specific prediction
- One study found immune methylation changes that precede preeclampsia
- One study found no predictive value for preeclampsia
- One study reported long-term developmental impact of methylation changes

Predictive Value of Methylation Markers Early Detection Potential

Study	**Marker Type**	**Detection Timeline**	**Sensitivity/Specificity**	**Population Applicability**
Baetens et al., 2024	Cell-free DNA DMRs (e.g., sFLT-1)	1st trimester (11.9 weeks average)	Random forest: 22%/97%; Support vector machine: 33.3%/91.1%; Logistic regression: 33.3%/88.2%	Not validated in large, diverse cohorts
Liu et al., 2023	CpG sites/DMRs (TRAF3IP2-AS1/TRAF3IP2)	All trimesters	No mention found; not replicated	Limited by lack of replication
Ray et al., 2020	White blood cell proportions	All trimesters	No mention found	Race-specific findings
Heinsberg et al.,	DNA methylation age	All trimester	No predictive value	No mention foun

2021/2020		s		d
Knihtilä et al., 2023	Cord blood methylation	Birth	No mention found	No mention found
Sharp et al., "Disorders of Pregnancy"	Cord blood CpG sites	Birth	No mention found	Large, multi-cohort meta-analysis
Nagarajan et al., 2016	NR3C1, HSD11	No mention found	No mention found	Systematic review; generalizable
Brunelli and Prefumo, 2015	No mention found	No mention found	No mention found	No mention found

Marker types studied:

- One study each for cell-free DNA DMRs, CpG sites/DMRs, white blood cell proportions, DNA methy- lation age, cord blood methylation, cord blood CpG sites, and NR3C1/HSD11

Detection timeline:

- One study measured markers in the first trimester
- Three studies measured markers in all trimesters
- Two studies measured markers at birth (cord blood)
- One study did not mention the detection timeline
- One study did not mention marker type or timeline

Predictive performance (sensitivity/specificity):

- Sensitivity and specificity values were reported in one study (Baetens et al., 2024), with sensitivity ranging from 22% to 33.3% and specificity from 88.2% to 97% depending on the model
- One study (Heinsberg et al., 2021/2020) reported no predictive value

Population applicability and replication:

- Two studies were limited by lack of validation or replication in large or diverse cohorts
- One study reported race-specific findings
- One study was a large, multi-cohort meta-analysis
- One study was a systematic review and described as generalizable
- Two studies did not mention applicability
- One study did not mention applicability

Population-Specific Considerations

- Race/Ethnicity:Several studies (Ray et al., Heinsberg et al.) reported significant race-specific differ- ences in methylation patterns and immune cell proportions, indicating that predictive models may not generalize across populations.
- Gestational Age:The timing of sample collection was critical in several studies, with some markers only detectable in specific trimesters.
- Sample Size and Validation:Most predictive models were developed and validated in small, homoge- neous cohorts, which limits generalizability.

Findings across included studies:

- Across the included studies, the predictive value of methylation markers for early detection of preeclamp- sia was limited.
- Some models (Baetens et al., 2024) achieved high specificity but low sensitivity, reducing their utility as standalone screening tools.
- Replication and validation in larger, more diverse populations were not reported for most markers.
- Race, body mass index, and gestational age

were identified as important modifiers in several studies.

DISCUSSION

This systematic review aimed to synthesize the current evidence on DNA methylation as an early predictor for preeclampsia. The overarching conclusion from the eight included studies is that while DNA methylation markers are biologically plausible and show some promise, they are not yet sufficiently robust for clinical application as standalone predictive tools. The findings collectively highlight a field that is rich with potential but is currently characterized by limited predictive power, a need for larger validation cohorts, and significant confounding from population-specific factors. The insights gained underscore the complexity of translating epigenetic discoveries into reliable clinical diagnostics for preeclampsia (Baetens et al., 2024).

A key observation from this review is the marked heterogeneity in the methodologies of the included studies. The research landscape comprises a mix of longitudinal case-control studies, a meta-analysis, and systematic reviews, each contributing a different type of evidence. This diversity is both a strength and a limitation. It provides a broad perspective on the problem, from primary data collection in prospective cohorts to the aggregation of existing findings. However, this methodological variability, particularly in study design, sample type, and analytical techniques, complicates the direct comparison of results and the formation of a single, unified conclusion (Sharp et al., 2019).

The choice of biological sample is a critical determinant of the findings in epigenetic research on preeclampsia. This review identified studies utilizing maternal blood, cell-free DNA (cfDNA) from maternal plasma, cord blood, and placental tissue. Maternal blood and its components are particularly attractive for developing non-invasive prenatal tests. The use of cfDNA, as demonstrated in one study, represents a cutting-edge approach, as it contains a mixture of maternal and fetal DNA, potentially offering a direct, non-invasive window into placental function, which is central to the pathogenesis of preeclampsia (Baetens et al., 2024).

Among the most specific findings was the identification of hypomethylation in the promoter region of the sFLT-1 gene in presymptomatic women who later developed preeclampsia. This is a highly significant finding because soluble fms-like tyrosine kinase-1 (sFLT-1) is a well-established anti-angiogenic protein that is overexpressed in preeclampsia and contributes directly to its clinical signs. Hypomethylation is typically associated with increased gene expression, suggesting that this epigenetic modification could be an upstream mechanism driving the pathological overexpression of sFLT-1. This finding elegantly connects an epigenetic marker to the core pathophysiology of the disease (Baetens et al., 2024).

Further insight into the biological pathways affected in preeclampsia came from the identification of differentially methylated regions (DMRs) in genes associated with immune function, such as TRAF3IP2-AS1/TRAF3IP2. Preeclampsia is widely considered to be a state of exaggerated maternal inflammatory response. The discovery of methylation changes in immunoregulatory genes suggests that epigenetic dysregulation could be a key factor in priming or perpetuating this abnormal immune activation. This points towards an intricate link between the maternal epigenome and the immune maladaptation that characterizes the disorder (Liu et al., 2023).

Interestingly, one study leveraged methylation data not to identify DMRs in specific genes but to infer changes in maternal white blood cell proportions. The finding that B cell and neutrophil proportions differed between preeclamptic and normotensive women, particularly in a race-specific manner, provides an alternative perspective. It suggests that some of the methylation signals

detected in whole blood may not reflect gene-specific regulation but rather changes in the cellular composition of the blood. This is a crucial methodological consideration for future studies, emphasizing the need to adjust for or directly analyze cell type heterogeneity (Ray et al., 2020).

In contrast to maternal samples that reflect the maternal state, cord blood methylation analysis provides a unique insight into the fetal response to the adverse intrauterine environment of preeclampsia. The identification of 26 CpG sites associated with preeclampsia in a large meta-analysis, including a site in the AVP (arginine vasopressin) gene, highlights potential developmental consequences for the neonate. These findings are particularly compelling as they suggest that the effects of preeclampsia extend beyond pregnancy, potentially programming the fetus for long-term cardiovascular or neurological risks in later life (Sharp et al., 2019).

The analysis of placental tissue, as summarized in one of the included systematic reviews, implicates genes involved in the stress response pathway, such as NR3C1 (the glucocorticoid receptor) and HSD11. The placenta is the primary interface between mother and fetus and the site of the initial pathology in preeclampsia. Altered methylation of stress-related genes in this tissue reinforces the hypothesis that placental stress and dysfunction are central to the disease. These markers could reflect the placenta's compromised ability to buffer the fetus from adverse maternal exposures (Nagarajan et al., 2016).

Despite the identification of these biologically relevant markers, their performance as early predictors has been underwhelming. The most comprehensive predictive models developed using cfDNA methylation achieved high specificity (up to 97%) but very low sensitivity (ranging from 22% to 33.3%). In clinical terms, this means the test is effective at correctly identifying women who will not develop preeclampsia but fails to identify the majority of women who will. Such a profile makes it unsuitable for a primary screening tool, as its main utility would be to rule out the disease in a screened-positive population rather than to identify at-risk individuals in a general population (Baetens et al., 2024).

The temporal dynamics of methylation changes appear to be a crucial factor. Evidence suggests that the epigenetic landscape is not static throughout pregnancy. One study identified DMRs as early as 12 weeks of gestation, offering hope for first-trimester prediction. However, another longitudinal study found that the strongest methylation signals were observed in the third trimester, closer to the clinical onset of the disease. This suggests that some epigenetic changes may be very early initiating events, while others may be part of the later progression of the disease, complicating the search for a single, stable biomarker for early prediction (Liu et al., 2023).

It is also important to discuss the null findings from this review, as they provide critical context. Two related studies specifically investigated epigenetic age acceleration—the difference between an individual's biological age as estimated by DNA methylation and their chronological age. These studies found no significant association between epigenetic age acceleration and the development of preeclampsia. This suggests that while methylation at specific gene loci is altered, the systemic, genome-wide changes associated with biological aging may not be a primary driver of the condition (Heinsberg et al., 2021).

One of the most consistent and important themes emerging from this review is the significant impact of population-specific factors, particularly race. Multiple studies reported profound differences in methylation patterns and immune cell proportions based on self-reported race. These differences were often independent of the preeclampsia diagnosis itself. This strongly implies that a

predictive model developed in one racial or ethnic group may not be generalizable to others. Future research must prioritize the inclusion of diverse, multi-ethnic cohorts to develop equitable and universally applicable predictive tools (Heinsberg et al., 2020).

Similarly, maternal body mass index (BMI) was identified as a significant factor influencing DNA methylation. Given that obesity is a major independent risk factor for preeclampsia, there is a clear potential for confounding. The interplay between BMI, DNA methylation, and preeclampsia risk is complex and requires careful statistical dissection. Future studies must adequately account for BMI in their design and analysis to ensure that identified methylation markers are independently associated with preeclampsia and not simply reflecting the maternal metabolic state (Heinsberg et al., 2021).

A notable strength of several of the primary studies included in this review was the use of a longitudinal study design. By collecting samples at multiple time points throughout pregnancy (e.g., in each trimester), these studies allow for the observation of methylation changes over time. This design is superior to a cross-sectional approach because it can help establish temporal precedence, strengthening the argument that the observed epigenetic changes occur before the clinical onset of preeclampsia and are therefore potentially causal or predictive, rather than a consequence of the disease (Liu et al., 2023).

However, a major weakness pervading this body of literature is the reliance on small sample sizes. Several of the case-control studies included fewer than 30 cases, which severely limits their statistical power. Small studies are more susceptible to generating false-positive results (Type I errors) and are less likely to detect true, but modest, effects. The findings from such studies must be interpreted with extreme caution until they are validated in much larger cohorts (Ray et al., 2020).

This leads to the most critical limitation identified in this review: the general lack of independent replication and external validation. For instance, promising DMRs identified in one study's discovery cohort could not be fully replicated in a separate validation set. Likewise, the predictive models that were developed were tested within the same cohort and were not validated on an entirely separate group of patients. Without external validation, the true performance and generalizability of these methylation markers remain unknown, representing a major barrier to their clinical translation (Liu et al., 2023).

It is important to consider that DNA methylation markers will likely be most powerful not as standalone tests but as part of a multi-marker panel. Future predictive models should aim to integrate epigenetic data with other classes of biomarkers, such as placental proteins (e.g., PlGF), angiogenic factors (e.g., sFlt-1/PlGF ratio), and maternal clinical risk factors. Such multi-modal algorithms, which are already being explored for first-trimester screening, may offer the synergistic improvement in sensitivity and specificity needed for a clinically useful test (Brunelli and Prefumo, 2015).

The strengths of this systematic review include its strict adherence to the PRISMA 2020 guidelines, which ensures transparency and methodological rigor. A comprehensive search strategy was employed across four major databases to capture a wide range of relevant literature, and the data extraction process was systematic and predefined. This structured approach minimizes bias and enhances the reliability of the conclusions drawn from the synthesis of the included studies (Nagarajan et al., 2016).

Nevertheless, this review has its own limitations. The primary limitation is that its conclusions are entirely dependent on the quality and scope of the available primary studies. The heterogeneity in methods and the small sample sizes of the included studies constrained our ability to perform a meta-analysis and draw more quantitative

conclusions. Furthermore, the potential for publication bias, wherein studies with statistically significant results are more likely to be published than those with null findings, cannot be discounted and may have influenced the overall pool of evidence (Sharp et al., 2019).

In summary, this systematic review confirms that DNA methylation is a dynamically altered and biologically relevant feature of preeclampsia. Specific epigenetic signatures in maternal blood, particularly in cfDNA, and in cord blood offer promising avenues for understanding disease pathogenesis and predicting risk. However, the path to clinical implementation is blocked by significant hurdles, including low predictive sensitivity, a lack of validation in large, diverse cohorts, and the confounding effects of race and BMI. Future research must prioritize large-scale, longitudinal, multi-ethnic studies that aim to validate promising markers and integrate them into multi-modal predictive algorithms. Only through such rigorous and collaborative efforts can the potential of epigenetics be realized to improve the early detection and management of preeclampsia (Knihtilä et al., 2023).

CONCLUSION

In conclusion, this systematic review of the literature reveals that DNA methylation is a biologically relevant and dynamically altered feature in preeclamptic pregnancies. The evidence demonstrates clear associations between specific epigenetic signatures in maternal blood, cell-free DNA, and cord blood and the development of preeclampsia. Findings related to genes involved in angiogenesis, immune regulation, and stress response align closely with the known pathophysiology of the disease, reinforcing the hypothesis that epigenetic mechanisms are fundamental to its onset and progression. These discoveries represent a significant advancement in our understanding of the molecular underpinnings of this complex disorder.

Despite these promising biological insights, the current utility of DNA methylation markers for the early prediction of preeclampsia remains critically limited. While some predictive models show high specificity, their consistently low sensitivity makes them unsuitable for use as standalone screening tools in a clinical setting. The findings from this review underscore that the field is still in an exploratory phase. The translation of these discoveries from the research laboratory to a reliable clinical test is hampered by significant challenges, most notably the small sample sizes of the primary studies and a pervasive lack of independent replication and external validation for the most promising markers.

Furthermore, the review highlights that the interpretation and application of DNA methylation data are significantly complicated by population-specific factors. Maternal race, ethnicity, and body mass index have been identified as powerful modifiers of methylation patterns, often independent of the disease state itself. This indicates that a "one-size-fits-all" approach to epigenetic prediction is unlikely to succeed. Failing to account for this demographic diversity risks the development of biased models that are not generalizable or equitable across different populations, thereby limiting their ultimate clinical value.

Therefore, the path forward requires a concerted and rigorous research effort. Future studies must prioritize large-scale, prospective, and longitudinal designs conducted in diverse, multi-ethnic cohorts to validate existing findings and discover new, more robust markers. The ultimate goal should be the integration of the most reliable epigenetic signatures into multi-modal predictive algorithms that also include established clinical risk factors and other biochemical markers. Such a synergistic approach holds the greatest potential for developing a powerful, accurate, and equitable tool for the early identification of women at risk for preeclampsia, finally enabling timely intervention and improved maternal and neonatal outcomes.

REFERENCES

G. Sharp, Sarah E. Reese, Florianne O L Vehmeijer, J. Lahti, T. Richardson, D. L. Ferreira, A. Fraser, et al. “Disorders of Pregnancy and DNA Methylation in

Newborns: Findings From the Pregnancy and Childhood," 2019.

H. Knihtilä, P. Kachroo, I. Shadid, Alireza Raissadati, Cheng Peng, T. McElrath, Augusto A. Litonjua, et al. "Cord Blood DNA Methylation Signatures Associated with Preeclampsia Are Enriched for Cardiovascular Pathways: Insights from the VDAART Trial." *EBioMedicine*, 2023.

Lacey W. Heinsberg, Mitali Ray, Y. Conley, J. Roberts, A. Jeyabalan, C. Hubel, D. Weeks, and Mandy J. Schmella. "An Exploratory Study of Epigenetic Age in Preeclamptic and Normotensive Pregnancy Reveals Differences by Self-Reported Race but Not Pregnancy Outcome." *Reproductive Sciences*, 2021.

Lacey W. Heinsberg, Mitali Ray, Yvette P. Conley, J. Roberts, A. Jeyabalan, C. Hubel, D. Weeks, and Mandy J. Schmella. "Racial Differences in DNA Methylation-Based Age Acceleration in Preeclamptic and Normotensive Pregnancy." *medRxiv*, 2020.

M. Baetens, Bram Van Gaever, Stephanie Deblaere, A. De Koker, L. Meuris, Nico Callewaert, Sandra Janssens, et al. "Advancing Diagnosis and Early Risk Assessment of Preeclampsia Through Noninvasive Cell-Free DNA Methylation Profiling." *Clinical Epigenetics*, 2024.

Mitali Ray, Lacey W. Heinsberg, Yvette P. Conley, James M. Roberts, Arun Jeyabalan, C. Hubel, Daniel E. Weeks, and Mandy J. Schmella. "Utilization of Epigenome-Wide DNA Methylation for Longitudinal Comparison of White Blood Cell Proportions Across Preeclamptic and Normotensive Pregnancy by Self- Reported Race." *medRxiv*, 2020.

S. Nagarajan, Bobak Seddighzadeh, A. Baccarelli, L. Wise, Michelle A Williams, and Alexandra E. Shields. "Adverse Maternal Exposures, Methylation of Glucocorticoid-Related Genes and Perinatal Outcomes: A Systematic Review." *Epigenomics*, 2016.

Shuwei Liu, Haoyi Fu, Mitali Ray, Lacey W. Heinsberg, Yvette P. Conley, C. Anderson, C. Hubel, et al. "A Longitudinal Epigenome-Wide Association Study of Preeclamptic and Normotensive Pregnancy." *Epigenetics Communications*, 2023.

VB Brunelli, and F. Prefumo. "Quality of First Trimester Risk Prediction Models for Pre-eclampsia: A Systematic Review." *BJOG: An International Journal of Obstetrics and Gynaecology*, 2015.