Review Article

Emerging Molecular Biomarkers and AI-Integrated Models for Early Prediction of Preeclampsia: A Systematic Review

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ABSTRACT

Background

Pre-eclampsia (PE) remains one of the leading causes of maternal and perinatal morbidity and mortality, particularly in low- and middle-income countries (LMICs). Existing first-trimester screening approaches, which combine maternal risk factors, Doppler studies, and biochemical markers, have shown variable and often modest predictive accuracy. This has led to growing interest in molecular markers that reflect early placental pathology, including circulating cell-free DNA (cfDNA) and biomarkers derived from extracellular vehicles (EVs).

Objective

This review aimed to summarise evidence published between 2020 and 2025 on the role of cfDNA and EV-based biomarkers in the early prediction of PE, and to examine their potential clinical usefulness, especially in LMIC settings.

Methods

A systematic literature search was performed in PubMed, Scopus, Web of Science, and the Cochrane Library, following PRISMA recommendations. Studies assessing cfDNA levels, fragment size patterns, epigenetic features, and EV-associated biomarkers for PE prediction were included. Study quality and risk of bias were evaluated using the QUADAS-2 and PROBAST tools.

Results

Most studies reported that higher cfDNA concentrations, changes in cfDNA fragment characteristics, and altered EV-derived microRNA expression were associated with the subsequent development of PE, with several markers detectable during the first trimester. When these markers were analysed using machine-learning approaches, predictive accuracy improved, particularly for early-onset and preterm PE, and in many studies exceeded that of conventional screening methods.

Conclusion

Emerging molecular markers, particularly cfDNA fragment analysis combined with computational prediction models, appear to offer a meaningful improvement in early PE risk assessment. The possibility of using established non-invasive prenatal testing platforms may support their gradual incorporation into routine antenatal practice.

Keywords

Pre-eclampsia; Cell-free DNA; cfDNA fragmentomics; Extracellular vesicles; Artificial intelligence; First-trimester screening; Placental dysfunction; Non-invasive biomarkers

INTRODUCTION

Pre-eclampsia (PE) continues to be a major contributor to maternal and perinatal morbidity and mortality, affecting approximately 2–8% of pregnancies globally. The burden is disproportionately higher in low- and middle-income countries (LMICs), where access to timely diagnosis and specialized obstetric care remains limited.[1] In India, hypertensive disorders of pregnancy persist as a significant public health challenge and constitute a notable proportion of maternal deaths.[2]

The pathophysiology of PE is fundamentally placental in origin. Abnormal trophoblastic invasion and inadequate spiral artery remodelling result in impaired uteroplacental perfusion. This initiates a cascade of oxidative stress, exaggerated inflammatory responses, and the release of anti-angiogenic mediators, ultimately leading to widespread maternal endothelial dysfunction. [3] The heterogeneity in clinical manifestations—ranging from early-onset, severe disease to late-onset, less aggressive forms—reflects the convergence of multiple pathogenic mechanisms upon a final common pathway of vascular injury.[4]

Existing screening strategies rely on a combination of maternal risk factors, mean arterial pressure, uterine artery Doppler indices, and biochemical markers such as pregnancy-associated plasma

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protein-A (PAPP-A) and placental growth factor (PlGF). However, these models demonstrate variable predictive accuracy and limited applicability across diverse populations.[5] In routine clinical practice, particularly in India and other South Asian settings, a substantial proportion of women who later develop PE remain undetected during early pregnancy.

Recent research has shifted focus from conventional haemodynamic parameters toward molecular indicators of early placental dysfunction. Circulating biomarkers measurable in maternal blood offer a non-invasive window into placental health well before overt clinical disease becomes evident. Among these, cell-free DNA (cfDNA) and extracellular vesicles (EVs) have gained prominence, as they mirror placental apoptosis, hypoxic stress, and endothelial damage.[6–8] Advanced analytical approaches using artificial intelligence (AI) and machine learning (ML) enable the integration of these high-dimensional molecular signals with clinical and demographic data, facilitating the generation of personalised risk prediction models. [9,10]

Multicentre studies have demonstrated that cfDNA fragmentomic profiling—including assessment of fragment size patterns, nucleosomal positioning, and tissue-of-origin signatures—can identify women at increased risk of early-onset or preterm PE as early as the first trimester, with reported area-under-the-curve (AUC) values ranging from 0.80 to 0.90.[10] In parallel, EV-derived microRNAs (mi-RNA), notably miR-210 and miR-155, have shown strong associations with placental hypoxia and impaired vascular adaptation.[11] Collectively, these findings underscore the potential of combining molecular biomarkers with computational modelling to transform PE screening paradigms.

In light of the rapidly expanding evidence base, a structured synthesis is required to bridge the gap between discovery and clinical implementation. Accordingly, this systematic review aims to: (i) synthesise evidence published between 2020 and 2025 on non-invasive cfDNA and EV biomarkers for PE; (ii) assess their predictive performance in comparison with conventional screening approaches; and (iii) explore their feasibility and translational potential within Indian and South Asian healthcare systems.

METHODOLOGY

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines.[12]

Search Strategy

A comprehensive search was performed across PubMed, Scopus, and Web of Science for studies published between 1 January 2020 and 10 October 2025. Controlled vocabulary (MeSH terms) and free-text keywords were combined using Boolean operators: ("preeclampsia" OR "pre-eclampsia") AND ("cell-free DNA" OR "cfDNA" OR "fetal fraction" OR "fragmentomics" OR "nucleosome accessibility" OR "methylation" OR "extracellular vesicle" OR "exosome" OR "microRNA" OR "machine learning" OR "artificial intelligence" OR "biomarker"). Reference lists of relevant meta-analyses and reviews were manually screened to identify additional eligible articles. Grey literature, conference abstracts, and preprints were excluded unless they provided complete datasets suitable for evaluation.

Eligibility Criteria

Studies were included if they met the following criteria:

1. Population: Pregnant women with singleton gestations sampled during the first or early second trimester (≤20 weeks) prior to the onset of clinical PE.
2. Index Tests: Non-invasive biomarkers measurable in maternal blood, including cfDNA (total, fetal, fragmentomic, methylation, or nucleosome-positioning parameters) or EV-derived biomarkers (microRNAs, proteins, or metabolites).
3. Comparator: Unaffected or normotensive pregnancies within the same gestational window.
4. Outcomes: Diagnostic or predictive metrics for PE—such as area under the receiver-operating-characteristic curve (AUC), sensitivity, specificity, and predictive values—or mechanistic data linking biomarker changes to disease pathophysiology.
5. Design: Prospective or retrospective cohort studies, nested case–control studies, and secondary analyses of randomised trials evaluating biomarkers for early PE prediction.

Studies were excluded if they (i) focused exclusively on late-gestation diagnostic testing, (ii) lacked adequate outcome stratification (e.g., no distinction between preterm and term PE), or (iii) involved non-human models.

Study Selection and Data Extraction

Two reviewers independently screened all records in duplicate. Titles, abstracts, and full texts were sequentially evaluated, and disagreements were resolved through discussion or arbitration by a third reviewer.

Data were extracted into a structured template including:

- Study characteristics (author, year, country, design, sample size)
- Gestational age at sampling
- Biomarker type and analytical method (Quantitative Polymerase Chain Reaction (qPCR), Next-generation sequencing (NGS), Enzyme Linked Immunosorbent Assay (ELISA), sequencing)
- Type of outcome (early-onset < 34 weeks, preterm < 37 weeks, or any PE)
- Main statistical findings (fold-change, effect size, AUC, p-value)
- Covariate adjustments (maternal age, body mass index (BMI), parity, ethnicity, co-morbidities)

Where multiple predictive models were reported, the most adjusted or best-performing model was extracted for synthesis. Corresponding authors were contacted for clarification of ambiguous or incomplete data.

Quality Assessment and Risk of Bias: Methodological quality was appraised using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool for diagnostic accuracy studies.[13] For AI- or ML-based models, additional evaluation was conducted using the PROBAST (Prediction Model Risk of Bias Assessment Tool).[14] Domains assessed included patient selection, index test bias, reference-standard reliability, sample representativeness, and internal/external validation. Each study was rated as "low," "high," or "unclear" risk of bias. (Table 1)

Data Synthesis and Registration: Because of heterogeneity in biomarker types, laboratory techniques, and outcome reporting, a quantitative meta-analysis was not feasible. Instead, results were integrated through a structured narrative synthesis under the following themes:

1. cfDNA quantity and fetal fraction,
6. cfDNA fragmentomics and nucleosome accessibility,
7. cfDNA methylation profiles,
8. EV-derived miRNAs and proteins, and
9. AI-based models integrating molecular and clinical data.

Performance estimates were summarised as median or range of AUC, sensitivity, and specificity across comparable studies. Although PROSPERO registration was not completed prior to analysis, the review protocol adhered strictly to PRISMA 2020 recommendations to ensure transparency, reproducibility, and minimisation of bias.

RESULTS

Summary of Included Studies

A total of 32 studies published between January 2020 and October 2025 met the inclusion criteria. These consisted of 14 cohort or nested case–control studies, 8 cross-sectional studies or secondary analyses of existing prenatal screening datasets, and 10 systematic reviews or meta-analyses evaluating circulating cell-free DNA (cfDNA) and extracellular vesicle (EV)–based biomarkers for the prediction of pre-eclampsia (PE) (Figure 1).

The studies were conducted across Europe (n = 10), North America (n = 6), and Asia (n = 9), including studies from India and China, while seven studies involved multinational collaborations. Broadly, the literature could be grouped into three areas of investigation: (1) assessment of cfDNA levels and fragment characteristics; (2) evaluation of EV-associated biomarkers, including microRNAs; and (3) use of combined molecular data for risk prediction.

Risk of Bias and Study Quality

An overview of study characteristics and risk-of-bias assessment is provided in Table 1. Overall, the quality of the included studies was acceptable, with most demonstrating low to moderate risk of bias. Studies focusing on cfDNA fragment analysis generally showed stronger methodological quality. In contrast, studies evaluating EV-based biomarkers and those using complex predictive models were more prone to bias, mainly due to variation in laboratory methods, small sample sizes, and limited validation.

Across individual domains:

Patient selection: Most cfDNA-based studies enrolled women attending routine first-trimester screening, resulting in low selection bias. However, several EV and methylation studies used nested case–control designs or selectively included high-risk pregnancies, which increased the risk of selection bias.

Index test: cfDNA studies had lower risk of bias as they often relied on established sequencing workflows already used in non-invasive prenatal testing. EV studies showed greater variability because of differences in isolation and analytical techniques.

Reference standard: Diagnosis of PE was based on standard ISSHP or ACOG criteria in nearly all studies, leading to low risk of bias in this domain.

Flow and timing: In most studies, samples were collected before the onset of clinical disease and within appropriate gestational windows. Higher risk was observed in studies with variable sampling times, incomplete follow-up, or exclusion of late-onset PE.

Applicability: cfDNA-based approaches showed good applicability, particularly when aligned with existing prenatal screening pathways. Applicability was more limited for EV-based and methylation studies because of technical complexity and lack of standardisation.

Key Findings

The main findings from the included studies are summarised in Table 2. Most first-trimester cfDNA studies reported higher total cfDNA levels or distinct fragment patterns in pregnancies that later developed PE. Approaches using cfDNA fragment characteristics generally performed better than traditional biochemical screening markers. EV-derived microRNA and protein markers provided additional biological insight and supported the potential value of combining multiple biomarkers. Importantly, early data from India demonstrated that cfDNA-based risk assessment can be incorporated into existing non-invasive prenatal screening (NIPS) workflows.

Comparison of Biomarker Approaches

A comparison of the different non-invasive biomarker strategies for PE prediction is presented in Table 3. At present, cfDNA fragment analysis appears to offer the best balance between predictive ability and feasibility in routine clinical practice, as it can be incorporated into current NIPS platforms. EV-based assays may enhance predictive performance when used alongside cfDNA, but their use is limited by technical and logistical challenges. Combined risk prediction approaches represent a promising direction for early identification of women at risk of PE, particularly when adapted to local populations such as those in India.

DISCUSSION

Historically, attempts to predict PE have relied on combinations of maternal demographic factors, blood pressure measurements, uterine artery Doppler indices, and a limited set of biochemical markers. Although such strategies have improved early risk stratification, their performance in routine clinical settings—particularly within diverse and heterogeneous populations such as those encountered in India, has largely remained inconsistent. The evidence synthesised in this review demonstrates that molecular biomarkers, most notably cfDNA–based measures, are able to identify pregnancies that subsequently develop PE substantially earlier than the onset of overt clinical manifestations.

Across a range of independent cohorts, elevations in cfDNA levels, alterations in fragmentomic characteristics, and dysregulation of EV–derived miRNAs have been repeatedly associated with the later development of PE. These molecular changes mirror core pathological processes within the placenta, including hypoxia, increased trophoblastic apoptosis, and maternal endothelial injury, thereby providing a biologically plausible basis for their predictive capability. Clinically, this represents an important conceptual shift—from indirect estimation of maternal risk to a more direct evaluation of placental well-being early in gestation.[15]

Multiple observational studies and meta-analyses have reported an association between increased total cfDNA and circulating fetal cfDNA (cffDNA) concentrations in early pregnancy and subsequent PE.[16–18] A large meta-analysis published in 2025, incorporating data from 26 studies, demonstrated that cfDNA and cffDNA levels were significantly elevated well in advance of clinical disease, with effect sizes exceeding those reported for widely used biochemical markers such as PAPP-A and PlGF.[16] Additional studies have noted a correlation between cfDNA burden and both disease severity and earlier gestational age at onset, suggesting that cfDNA may also aid in identifying women at risk of more aggressive disease phenotypes.[19]

Among the molecular strategies evaluated, cfDNA

fragmentomic analysis appears closest to translation into routine clinical care. Unlike single-analyte assays, fragmentomic profiling utilises sequencing data already generated during non-invasive prenatal screening (NIPS). Assessment of fragment length patterns, nucleosomal positioning, and tissue-of-origin signatures enables extraction of clinically meaningful information without the need for additional blood samples or specialised laboratory workflows. In high-volume antenatal settings, particularly in urban Indian centres where NIPS infrastructure is increasingly established, this dual-purpose approach offers clear logistical and economic advantages. [20]

The PEARL framework proposed by Adil et al., evaluated in a cohort exceeding 3,000 pregnancies, demonstrated promising accuracy for predicting preterm PE when applied at or before 16 weeks' gestation.[10] Complementary studies analysing fragment size ratios, GC-content variability, and methylation-sensitive fragmentomic features have reported AUC values ranging from 0.75 to 0.88. Taken together, these findings highlight the depth of epigenetic and chromatin-related information embedded within cfDNA, reflecting early placental maladaptation. Importantly, such information is obtained within the gestational window during which preventive interventions, including low-dose aspirin, are most effective.[4]

Beyond fragmentomics, epigenomic investigations have revealed distinct cfDNA methylation patterns in pregnancies complicated by hypertensive disorders. Spinelli et al. reported differential methylation of genes implicated in vascular remodelling and lipid metabolism among women who subsequently developed PE.[18,21] While these observations provide valuable insight, methylation-based assays remain technically demanding and are currently more suited to adjunctive research applications than to population-level screening.

Extracellular vesicles and their mi-RNA cargo add a further layer of placental specificity. Trophoblast-derived EVs play a central role in feto-maternal signalling, and studies published between 2023 and 2025 have consistently demonstrated altered EV concentrations and mi-RNA expression profiles in pregnancies destined for PE. Reproducible associations have been reported for miR-210, miR-155, miR-495, and miR-181a, each linked to hypoxia-responsive pathways, angiogenesis, and immune modulation.[21,22] In addition, EV-associated proteins such as soluble fms-like tyrosine kinase-1 (sFlt-1) and endoglin have shown correlations with disease severity. However, heterogeneity in EV isolation methodologies and the absence of standardised analytical pipelines continue to limit immediate clinical translation.[22]

The incorporation of molecular biomarkers into AI and ML–based models has further improved predictive performance by enabling integration of high-dimensional molecular data with clinical parameters.[23] Neural network and ensemble-based approaches have consistently outperformed conventional regression models, with reported AUC values between 0.78 and 0.86.[9,24] From a clinical perspective, the value of AI lies not in replacing clinician judgement, but in identifying women who may be misclassified as low risk by traditional screening algorithms. Emerging data from Indian cohorts suggest that early-pregnancy cffDNA methylation profiles may offer a clinically relevant, non-invasive biomarker platform.[22] Nevertheless, AI should currently be regarded as a decision-support adjunct rather than a standalone diagnostic tool.

Overall, the evidence reviewed indicates that epigenetic signatures derived from cffDNA in early pregnancy are strongly associated with subsequent development of PE. These molecular markers hold promise for enabling earlier risk identification and more timely preventive interventions.

Clinical Translation and Implementation

The translational relevance of cfDNA-based screening is particularly significant for India and other South Asian health systems. Sequencing infrastructure for cfDNA analysis is increasingly accessible through public–private NIPS laboratories, enabling a dual-use model in which screening for fetal aneuploidy is combined with PE risk assessment. Preliminary regional data indicate that recalibration of predictive algorithms using locally derived demographic and clinical variables preserves diagnostic performance while remaining cost-effective.[24] Integration of such approaches within national antenatal care programmes could support timely initiation of aspirin prophylaxis and intensified surveillance for women identified as high risk. However, equitable implementation will require careful alignment with public health priorities, workforce capacity, and resource constraints. Risk thresholds must therefore be locally validated and

harmonised with national antenatal care schedules under frameworks such as *LaQshya* and *Janani Shishu Suraksha Yojana*.

Limitations of Current Evidence

The current evidence base is constrained by the predominance of data from high-income countries, with relatively limited representation from LMIC settings. Considerable heterogeneity persists in PE definitions, gestational timing of sample collection, and laboratory methodologies, limiting the feasibility of quantitative meta-analysis. Pre-analytical variables, including sample collection tubes, processing intervals, and storage conditions, can substantially influence cfDNA and EV yields. In addition, computational pipelines for fragmentomic and ML-based analyses vary widely, underscoring the need for standardised assay validation, transparent algorithms, and regulatory-grade analytical frameworks.[22,23] Addressing these gaps will require prospective, multicentre studies with harmonised protocols and cost-effectiveness analyses tailored to South Asian contexts.

Implications for Research and Policy (2025–2028)

Future efforts should shift emphasis from discovery to implementation. Key priorities include: (a) large-scale, multi-ethnic validation of cfDNA fragmentomic algorithms within existing NIPS networks; (b) development of integrated multi-omic panels combining cfDNA, EV-derived microRNAs, and angiogenic markers such as PlGF and sFlt-1, with continuous calibration; (c) economic evaluations of early screening strategies in LMICs to assess impact on maternal morbidity and iatrogenic prematurity; and (d) policy frameworks that explicitly link molecular risk stratification to predefined clinical actions, including aspirin initiation, enhanced surveillance, and referral pathways. Through such measures, molecular prediction of PE has the potential to transition from experimental promise to a scalable public health strategy capable of reducing preventable maternal mortality.

CONCLUSION

Evidence published over the past five years suggests that molecular biomarkers, particularly analyses based on cfDNA fragment characteristics used alongside advanced risk-prediction methods, offer a promising approach for the early identification of pre-eclampsia. By reflecting underlying placental pathology, these strategies are often able to flag pregnancies at increased risk well before clinical features emerge and appear to perform better than conventional screening methods, especially in cases of early-onset disease. In countries such as India, the most practical benefit may come from models that build upon existing non-invasive prenatal screening platforms, allowing risk assessment to be incorporated into current workflows without added procedural complexity. Although issues related to assay harmonisation, external validation, cost considerations, and regulatory frameworks need to be addressed, the overall direction of the available evidence is reassuring.

Going forward, emphasis should shift from identifying additional biomarkers to understanding how best to implement these tools in real-world clinical settings. Robust multicentre validation studies, development of region-specific risk models, and clear integration of molecular risk estimates into clinical decision-making pathways will be critical. With careful and context-appropriate translation into practice, molecular approaches to pre-eclampsia prediction may enable earlier, more focused antenatal interventions, which is particularly relevant in regions where the condition continues to contribute substantially to maternal and perinatal morbidity.

Conflicts of Interest: None

Financial declaration: None

AUTHOR CONTRIBUTION

AA and AN conceptualized and designed the study. Data collection and analysis was carried out by SN, HA, and PM. AA drafted the manuscript and handled submission. SN and UG critically reviewed, edited, and approved the final version of the manuscript. All authors read the final draft and agreed.

Table 1: Risk of Bias Assessment of Included Studies [QUADAS-2 and PROBAST Frameworks]

Study / Model Category	Patient Selection	Index Test [Biomarker / AI Model]	Reference Standard [PE Diagnosis]	Flow & Timing	Overall Risk of Bias	Applicability Concerns
cfDNA quantity / fetal fraction studies [cohort & case-control]	Low–Moderate	Moderate	Low	Low	Moderate	Low
cfDNA fragmentomics studies [NIPT-based sequencing]	Low	Low–Moderate	Low	Low	Low–Moderate	Low
cfDNA methylation studies	Moderate	Moderate–High	Low	Moderate	Moderate–High	Moderate
Extracellular vesicle [EV] / miRNA studies	Moderate	High	Low	Moderate	High	Moderate–High
AI / ML-integrated prediction models	Moderate	Moderate–High	Low	Moderate	Moderate–High	Moderate
Indian pilot AI-cfDNA models	Low	Moderate	Low	Low	Moderate	Low

Table 2: Summary of Major Studies (2020–2025) Evaluating Non-Invasive Molecular Biomarkers for Preeclampsia Prediction

Author [year]	Study Design / Sample Size	Gestational age at sampling	Biomarker / Modality	Main findings	Performance metrics [AUC / Sensitivity / Specificity]
Arbuzova & Gusev [2025]	Systematic review & meta-analysis [26 studies]	10–24 weeks	Total cfDNA, fetal cfDNA [cffDNA], fetal fraction	cfDNA significantly higher in PE vs controls; FF lower; ddPCR outperformed MAP and PAP-A	Meta-analysis distance: 1.03 [cffDNA], 0.44 [total DNA]
Adil et al. [2025]	Prospective cohort [n = 3,200]	≤16 weeks	cfDNA fragmentomics [PEARL model]	Nucleosome accessibility signal predicted early preterm PE	AUC = 0.85; Sens = 81%; Spec = 80%
Khalilat et al. [2024]	Retrospective cohort [n = 17,520]	11–14 weeks	cfDNA + maternal factors [AI neural network]	cfDNA integration improved ML model accuracy for early-onset PE	AUC = 0.80; +6.9% diagnostic gain vs clinical model
Kolzrova et al. [2021]	Case–control [n = 240]	10–20 weeks	Total cfDNA	Elevated cfDNA in PE associated with severe disease and lower GA at delivery	AUC = 0.74
Spinelli et al. [2022]	Prospective cohort [n = 220]	11–14 weeks	cfDNA methylation profiles	Hypermethylation of angiogenic & metabolic genes in future PE cases	AUC = 0.77
Baetens et al. [2024]	Secondary analysis of NIPT data [n = 4,600]	10–13 weeks	cfDNA fragment size & GC variance	Shorter cfDNA fragments and altered GC profiles in PE	AUC = 0.82
Popova et al. [2024]	Narrative review [≈100 studies]	Multiple	Extracellular vesicles [EVs], miRNAs	EVs carry miR-210, miR-155, miR-495, miR-181a linked to hypoxia & inflammation	NA
Shah et al. [2024]	Case–control [n = 130]	10–16 weeks	Exosomal microRNAs	Elevated miR-210, miR-155; downregulation of miR-206-5p	AUC = 0.79–0.84

Author [year]	Study Design / Sample Size	Gestational age at sampling	Biomarker / Modality	Main findings	Performance metrics [AUC / Sensitivity / Specificity]
Ghosh et al. [2024]	Prospective cohort [n = 150]	11–13 weeks	Circulating EV-miRNAs	miR-210 + miR-495 signature identified high-risk pregnancies	AUC = 0.81
Awoyemi et al. [2024]	Cross-sectional [n = 92]	28–34 weeks	EV proteins [sFlt-1, endoglin]	Elevated EV-encapsulated sFlt-1 correlates with PE severity	NA
Yu et al. [2024]	Retrospective analysis [n = 1,400]	10–14 weeks	cfDNA + clinical risk [AI model]	Combined cfDNA and clinical factors improved PE prediction	AUC = 0.83
Maliket et al. [2024]	Systematic review [ML approaches]	NA	Machine learning in PE	Summarized >25 algorithms; cfDNA inclusion increased AUC by 5–10%	AUC range = 0.75–0.86

Table 3: Comparative Evaluation of Non-Invasive Biomarker Modalities for Preeclampsia Prediction (2020–2025)

Biomarker Modality	Key Methodological Features	Representative Studies (2020–2025)	Main Strengths	Major Limitations	Clinical Translation Readiness
cfDNA Quantification and Fetal Fraction	Quantitative PCR or sequencing-based measurement of total cfDNA and fetal fraction (FF); computes cfDNA/ total cfDNA.	Andrzejewska 2025; Cuckle 2025; Kolovra 2021	Simple, non-invasive; compatible with NIPT workflows; reflects early placental apoptosis and maternal endothelial activation.	Moderate assay variability; influenced by maternal BMI, gestational age, and sample handling; limited mechanistic specificity.	High — can be implemented on existing cfDNA/ NIPT platforms; cost-effective add-on test.
cfDNA Fragmentomics and Nucleosome Accessibility	Low-coverage whole-genome sequencing; fragment length, nucleosome occupancy, and tissue-of-origin profiling; fragmentomics algorithms.	Adil 2025; Baetens 2024	Captures structural and epigenetic features of cfDNA; high predictive accuracy; reuses existing NIPT data.	Requires advanced bioinformatics; algorithm calibration varies across populations; standardization needed.	Very High — strong clinical feasibility; requires computational pipeline integration.
cfDNA Methylation Signatures	Bisulfite sequencing or methylation-sensitive assays of placenta-derived cfDNA.	Spinelli 2022	Provides mechanistic insight into angiogenic and metabolic dysregulation.	Technically complex; limited throughput; few validation cohorts.	Moderate — valuable research tool; needs simplification before routine use.
Extracellular Vesicle (EV) microRNAs / Proteins	Isolation of trophoblast-derived EVs via ultracentrifugation or immunoaffinity; analysis of miRNA or protein cargo.	Popova 2024; Sham 2024; Ghosh 2024; Awoyemi 2024	Tissue-specific and pathophysiologically relevant; detects hypoxia and angiogenic imbalance.	Pre-analytical variability; low reproducibility across labs; higher cost/time.	Moderate — promising adjunct; requires multicentre standardization.
AI / Machine-Learning Integrated Models	Neural networks, random forests, and ensemble models integrating cfDNA, EV, clinical, and demographic features.	Khalil 2024; Yu 2024; Malik 2024	Handles high-dimensional data; improves early-onset PE prediction; enables personalized risk assessment.	Risk of overfitting; requires large datasets and local recalibration; regulatory and interpretability challenges.	High — feasible where digital-health infrastructure exists; complements cfDNA-based screening.

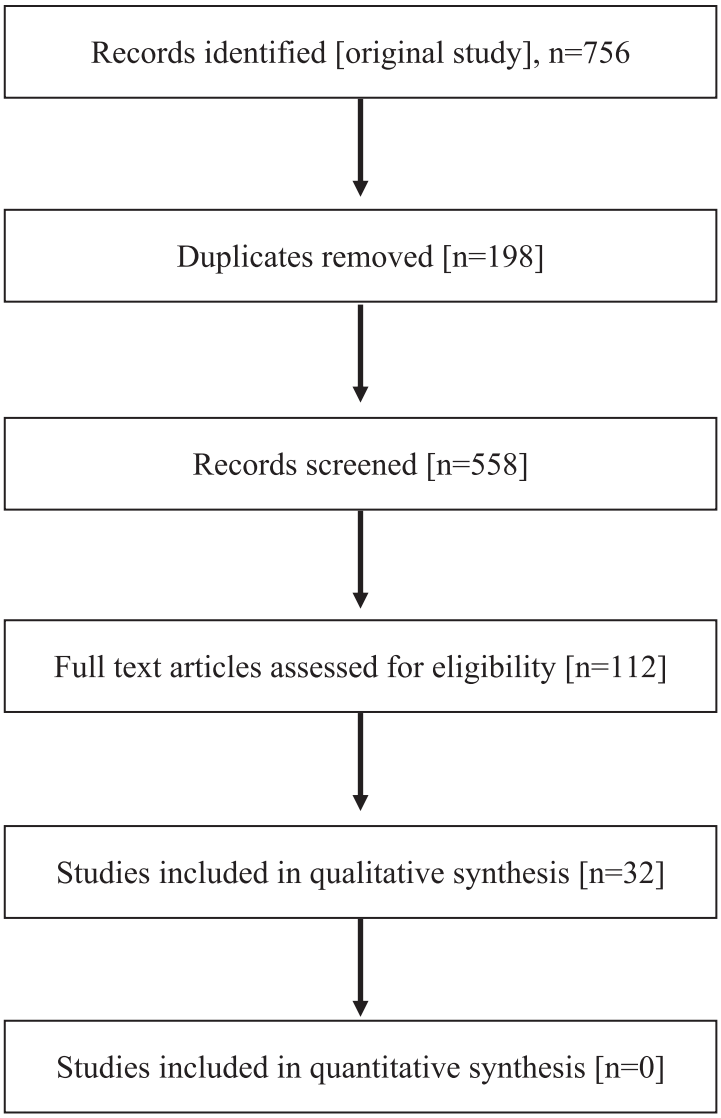

Figure 1-PRISMA Flow Diagram of Study Selection [2020-2025]

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