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The Potential of Circulating Free DNA of Methylated Leptin Gene as a Biomarker for Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and hyperglycemia, with a steadily increasing global prevalence. One of the risk factors for T2DM is Gestational Diabetes Mellitus (GDM), which refers to the body's inability to regulate glucose during pregnancy and significantly increases the risk of developing T2DM in both the mother and her offspring. Among the various metabolic changes observed in GDM, leptin has been widely studied for its role in energy metabolism and insulin resistance. Leptin levels generally increase in obesity and GDM conditions, and one influencing factor is epigenetic mechanisms, including DNA methylation. Increased methylation in the leptin gene promoter region has been associated with decreased leptin expression, indicating a potential pathway linking GDM to T2DM susceptibility in offspring. Given the importance of epigenetic regulation, analysis of leptin DNA methylation patterns—particularly through non-invasive detection of placental circulating free DNA (cfDNA)—has the potential to serve as a biomarker for detecting T2DM risk from prenatal stages. This approach not only offers early detection but also opens opportunities for more targeted preventive measures to reduce T2DM incidence in the future.

KEYWORDS *biomarkers; cfDNA; diabetes diagnosis; epigenetics; methylated leptin*

INTRODUCTION

In the area International Diabetes Federation (IDF) Western Pacific Region, an estimated 215 million people are living with diabetes, and this number is projected to increase to 254 million people by 2050, out of a total of 589 million cases globally. In Indonesia alone, in 2024 there will be an adult population of 185.2 million people, with a prevalence of diabetes of 11.3% or equivalent to 20.4 million cases (International Diabetes Federation, 2025). This high number places Indonesia as one of the countries with a significant burden of diabetes in the region, so more comprehensive prevention, early detection, and treatment efforts are needed.

Diabetes Mellitus Gestational (GDM) is one of the important factors that contribute to the development of Type 2 Diabetes Mellitus (T2DM). Women with a history GDM and being overweight increases the risk GDM in the next pregnancy (Giuliani et al., 2022). In addition, women with a history of GDM have a higher risk of developing diabetes later in life, while exposure to high blood sugar during pregnancy can affect the child's metabolic development depending on the time and severity GDM maternal (Xiang, 2023). Further, the evidence suggests that leptin higher levels of pregnancy are associated with an increased risk of developing GDM (Ye et al., 2022).

Among the various metabolic changes observed in GDM, rate change leptin It has been widely studied for its role in insulin resistance. Leptin has a close relationship with insulin

resistance, especially in obese conditions. Body Mass Index (BMI) and insulin resistance are the main factors that affect the level of insulin resistance. leptin (Nomair et al., 2014). In individuals with obesity, leptin often increases, which leads to a condition known as resistance leptin. Although adipose cells produce leptin in large numbers, the body becomes less responsive to its signals. As a result, the brain does not sense enough signals leptin to reduce appetite or increase energy expenditure, which contributes to weight gain and insulin resistance (Kumar et al., 2020). Even though leptin has the potential to affect energy metabolism, its effects on obese mothers are more complex due to the presence of resistance leptin (Zielinska-Pukos et al., 2024). Mothers with GDM Have a basic concentration leptin but its increase during pregnancy is less, indicating the presence of resistance leptin (Xiao et al., 2020). As a result, the rate leptin that increase cannot be fully attributed to GDM, as it can be caused by a variety of other factors. However, in the context of GDM, increased rate leptin consistently observed. Therefore, more precise biological markers are needed to measure levels of leptin in mothers with GDM or obesity.

Emerging evidence suggests that the metabolic changes observed in GDM, including dysregulation of leptin and insulin resistance, can be affected by epigenetic mechanisms such as DNA methylation. DNA methylation plays an important role in the development of diabetes by modulating gene expression. In Diabetes Mellitus, variations in DNA methylation patterns can affect the expression of key genes that regulate metabolism, including genes associated with insulin resistance and glucose regulation (Fu et al., 2023; He et al., 2022; Suksmarini et al., 2018). DNA methylation often reflects stable and long-lasting changes in gene expression influenced by environmental and lifestyle factors (De Mendoza et al., 2022; Dhar et al., 2021). In particular, the metabolic condition of the mother, including excessive weight gain, GDM, and other metabolic disorders before and during pregnancy, have been shown to alter the DNA methylation profile in the placenta. These modifications can have long-term consequences, as the epigenetic traces formed in the womb can persist after birth and potentially affect susceptibility to metabolic diseases in offspring (Lesseur, Armstrong, Murphy, et al., 2014).

Given the role of epigenetic modification in pregnancy-related metabolic disorders, interest in developing non-invasive methods to monitor these changes is increasing. One promising approach is the analysis of circulating free DNA or Cell-free DNA (cfDNA), which acts as biomarker potential to assess genetic and epigenetic changes in the fetus and mother. cfDNA It is usually released into the bloodstream through the process of cell death and active secretion mechanisms (Qi et al., 2024; Sharma et al., 2022). Use cfDNA In genetic analysis, the embryo offers a significantly safer and less intrusive method than commonly used invasive techniques (Layek et al., 2024). Extraction of DNA from the maternal environment in which the embryo develops eliminates the risk of physical damage to the embryo, which is a common problem in traditional biopsy procedures (Gordevičius et al., 2020). Therefore, the use of cfDNA in embryo genetic analysis provides a safer and less invasive alternative to traditional biopsy methods, eliminating the risk of physical harm to the embryo by extracting DNA from the maternal environment.

The increasing prevalence of T2DM and its relationship to GDM emphasizes the importance of understanding the metabolic and epigenetic factors that contribute to disease susceptibility. Leptin as a regulator of energy metabolism and insulin resistance has been an important focus in various GDM-related studies. The latest findings suggest that leptin levels

are not only affected by metabolic changes, but also by epigenetic modifications such as DNA methylation. Thus, understanding the interaction between metabolic and epigenetic factors in pregnancy conditions is an important foundation to explain the mechanisms underlying the risk of diabetes in the future, both in pregnant women and their offspring.

Building upon this understanding, the present review aims to comprehensively evaluate the existing evidence on the potential of circulating free DNA (cfDNA) of the methylated leptin gene as a biomarker for Type 2 Diabetes Mellitus. Specifically, this systematic literature review seeks to: (1) synthesize current knowledge on leptin levels and gene expression in women with GDM, impaired glucose tolerance, and obesity; (2) examine the patterns and extent of leptin gene methylation in placental tissue and cfDNA across different maternal metabolic conditions; (3) assess the feasibility and potential clinical utility of cfDNA-based methylation analysis as a non-invasive diagnostic tool; and (4) identify gaps in current knowledge and propose directions for future research.

The theoretical significance of this review lies in advancing our understanding of the epigenetic mechanisms linking maternal metabolic status during pregnancy to long-term diabetes risk in both mothers and offspring. From a practical perspective, establishing cfDNA methylation patterns of the leptin gene as a reliable biomarker could revolutionize prenatal screening approaches, enable early identification of at-risk individuals and facilitating timely preventive interventions. This could ultimately reduce the intergenerational transmission of metabolic dysfunction and contribute to breaking the cycle of diabetes across generations.

The expected benefits of this review include: (1) providing healthcare professionals with evidence-based insights into novel biomarkers for diabetes risk assessment; (2) informing clinical decision-making regarding prenatal screening protocols; (3) supporting the development of personalized preventive strategies for women with GDM and their offspring; (4) guiding future research priorities in the field of epigenetic biomarkers for metabolic diseases; and (5) contributing to public health efforts aimed at reducing the growing burden of T2DM globally. By synthesizing existing evidence on this promising biomarker, this review aims to facilitate the translation of epigenetic research findings into clinical practice, ultimately improving maternal and child health outcomes.

METHOD

The methodology used in this review is the Systematic Literature Review (SLR) which aims to identify and synthesize research results regarding the relationship between leptin, methylated leptin genes, and diabetes mellitus, especially gestational diabetes mellitus (GDM). This systematic approach ensures a comprehensive and unbiased assessment of available evidence while minimizing selection bias and enhancing the reproducibility of findings.

A total of 107 relevant scientific articles were collected from three main databases, namely Google Scholar, PubMed, and ScienceDirect. The search was conducted between October 2024 and January 2025 to capture the most current evidence available. The search was conducted using a combination of keywords: "leptin", "cfDNA", "methylation", "diabetes" and "gestational diabetes mellitus" using Boolean operators (AND, OR). The complete search string used was: ("leptin" OR "leptin gene") AND ("cfDNA" OR "cell-free DNA" OR "circulating DNA") AND ("methylation" OR "DNA methylation" OR "epigenetic") AND ("diabetes" OR "gestational diabetes mellitus" OR "GDM" OR "type 2 diabetes" OR "T2DM").

Additional searches were performed by screening the reference lists of selected articles to identify any relevant studies not captured in the initial database search (backward citation tracking).

The articles included in this review meet the following inclusion criteria:

1. It is an experimental or observational study in humans,
2. Reporting leptin levels or leptin gene methylation,
3. Using maternal or placental samples (cfDNA or gDNA),
4. Published in English or Indonesian,
5. Published in the period 2010-2025. This timeframe was selected to capture studies utilizing contemporary molecular techniques for DNA methylation analysis and cfDNA detection, which have advanced significantly in the past 15 years.

Exclusion criteria include a) Articles in the form of reviews, case reports, editorials, or conference abstracts without primary data, as these do not provide original empirical evidence suitable for synthesis in a systematic review. b) Research on animals or cell cultures, to ensure the findings are directly applicable to human clinical contexts. c) Studies that did not involve leptin analysis or DNA methylation. Additionally, articles with incomplete methodological descriptions or those that did not report sufficient data for quality assessment were excluded.

After the screening process based on titles, abstracts, and full content, 8 final articles were obtained that met all inclusion criteria and were analyzed in this study. The study selection was conducted in three phases: (1) Initial screening based on titles to remove obviously irrelevant articles (n=107 reduced to n=45); (2) Abstract screening to assess relevance to research questions (n=45 reduced to n=15); and (3) Full-text review to verify that studies met all inclusion criteria and contained sufficient data for analysis (n=15 reduced to n=8). Two independent reviewers conducted the screening process, with disagreements resolved through discussion or consultation with a third reviewer when necessary to ensure inter-rater reliability.

Data extraction was performed systematically using a standardized form developed specifically for this review. The following information was extracted from each included study: (1) study characteristics (author, year, country, study design); (2) participant characteristics (sample size, maternal metabolic status, gestational age); (3) sample type (cfDNA, genomic DNA from placental tissue); (4) measurement methods (ELISA, qPCR, immunohistochemistry, pyrosequencing, etc.); (5) leptin levels or expression data; (6) DNA methylation percentages or patterns; and (7) main findings and statistical results.

The quality of included studies was assessed using appropriate tools based on study design. For observational studies, we applied a modified Newcastle-Ottawa Scale considering: (1) representativeness of the study population; (2) comparability of groups; (3) assessment of exposure or outcome; and (4) adequacy of follow-up (where applicable). Studies were rated as high, moderate, or low quality based on these criteria.

The data of the synthesis results are presented in Table 1 and Table 2. A narrative synthesis approach was employed given the heterogeneity in study designs, methodologies, and outcome measures across included studies, which precluded meta-analysis. The synthesis focused on identifying patterns and trends across studies regarding: (1) leptin levels/expression in different maternal metabolic conditions; (2) leptin gene methylation patterns across gestational periods; (3) associations between maternal metabolic status and epigenetic

modifications; and (4) the potential utility of cfDNA-based methylation analysis as a biomarker.

Results were organized thematically, with separate analyses for: (a) leptin levels and gene expression patterns; (b) DNA methylation patterns in different maternal conditions; and (c) methodological considerations for cfDNA analysis. Where possible, comparisons were made across different measurement methods and sample types to assess consistency of findings. The strength of evidence for each key finding was evaluated based on the number of studies, sample sizes, methodological quality, and consistency of results across studies.

This systematic review acknowledges several limitations, including: (1) the relatively small number of studies meeting inclusion criteria (n=8), which may limit the generalizability of findings; (2) heterogeneity in measurement methods and sample types across studies, making direct comparisons challenging; (3) potential publication bias, as studies with positive findings may be more likely to be published; (4) language restriction to English and Indonesian, which may have excluded relevant studies in other languages; and (5) the observational nature of most included studies, which limits causal inferences regarding the relationship between leptin methylation and diabetes risk.

RESULTS AND DISCUSSION

Leptin and Its Role in Diabetes

Leptin is a hormone primarily produced by adipose cells that regulate energy balance by signaling to the brain about the body's energy reserves. As an endocrine organ, adipose tissue produces leptin, and in obesity there is resistance leptin that can be modulated by liraglutide through receptor sensitivity improvements leptin adipose (Lyu et al., 2022). Power leptin Higher levels reduce hunger, while lower levels increase food-seeking behavior. Secretion leptin follows a daily rhythm and is generally higher in women. This hormone plays an important role in maintaining weight and homeostasis energy, even though most individuals with obesity show resistance leptin (Ramos-Lobo & Donato, 2017). Under resistance conditions leptinability leptin to regulate glucose metabolism and insulin function becomes impaired. Leptin It usually increases insulin sensitivity by reducing glucose production in the liver. However, when the signal leptin impaired, glucose production in the liver increases, which worsens insulin resistance and hyperglycemia in individuals with T2DM (Guzzardi et al., 2022). Therefore, resistance leptin closely related to insulin resistance in T2DM, up to the rate leptin high in the body obesity plays a role in resistance leptin and will affect insulin resistance.

Moreover leptin affect glucose metabolism through activation of signaling pathways such as JAK2/STAT3, PI3Kand AMPK, which plays a role in regulating glucose production in the liver as well as glucose uptake by peripheral tissues (Huang et al., 2022; Pereira et al., 2021). Leptin also interacts with receptors leptin in the hypothalamus and peripheral tissues, indicating the presence of cross-talk Between the Lines leptin and insulin in keeping homeostasis Energy and glucose (Z. Liu et al., 2023).

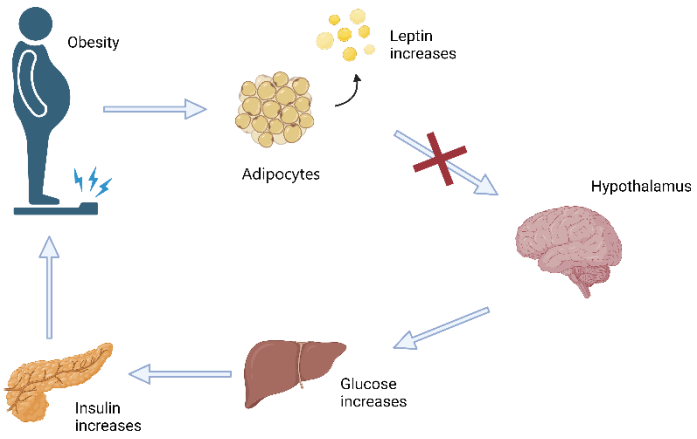

Figure 1. In individuals with obesity

Leptin levels often increase due to increased adipose tissue. However, this increase in leptin levels does not always function effectively, leading to leptin resistance. As a result, despite high leptin levels, the ability of this hormone to regulate appetite and energy expenditure is inhibited. This inefficiency can contribute to high blood glucose levels, which in turn increases insulin levels and can lead to insulin resistance. As a result, increased appetite and weight gain often occur, exacerbating obesity.

In addition to its role in appetite regulation, leptin serves as a key marker of obesity and other metabolic disorders (Aaty et al., 2020; Wang et al., 2019; Xiao et al., 2020). But, until leptin can fluctuate significantly from day to day depending on food intake, meal timing, stress, and physical activity (Gaeini et al., 2021). In pregnant women, both at normal weight and obese, leptin higher on the fetal side than on the maternal side, indicating that leptin plays an important role in fetal growth. In addition, there was no significant difference in levels leptin between the normal weight and obesity groups (Nogues et al., 2019). Therefore, studies show no significant differences in leptin between normal weight and obese pregnant individuals, highlighting the complex interplay between pregnancy physiology and metabolic adaptation.

Elevated leptin levels can affect glucose regulation and insulin resistance, which in turn, affects fetal growth. During normal pregnancy, the leptin usually increases as part of physiological adaptations to support fetal development (Obeidat et al., 2021; Walsh et al., 2014). However, in pregnancy with GDM, increased rate leptin observed showed altered metabolic and hormonal regulatory mechanisms, confirming the need for biomarker more precise to assess the function leptin in maternal health (Ye et al., 2022). Given the limitations of measurement leptin Conventionally, more research is needed to establish reliable markers that can accurately reflect metabolic status and predict long-term health outcomes in both mothers and offspring.

cfDNA Methylated Leptin Gene as a Non-Invasive Biomarker

The placenta and fetus develop from the same cell, so the DNA methylation patterns in the placenta can mirror the baby's methylation patterns during pregnancy. Placenta sampling is an invasive procedure known as a sampling Chorionic Villus Sampling (CVS). Although this procedure is generally considered safe, the estimated risk of miscarriage after CVS It is about

0.5% to 1% for a single pregnancy and about 2% for a double pregnancy. Additionally, the procedure can cause discomfort or cramps similar to menstrual pain for several hours afterwards (Woods, 2023). Therefore, alternative methods are needed to minimize these risks.

cfDNA is a fragment of nucleic acid that is released into the bloodstream through apoptosis Of necrosis, or active secretion (Li et al., 2024). During pregnancy, the rate cfDNA increased due to additional contributions from placental DNA (Tang et al., 2024). Power cfDNA increased in pregnant women with GDM has been associated with increased oxidative stress (Yin et al., 2022) and systemic inflammation (Cardelli et al., 2024), both of which are hallmarks of pathophysiology GDM (Zhou et al., 2024).

Potential cfDNA is beyond metabolic disorders to their application in prenatal detection. NIPT has taken advantage of cfDNA to identify aneuploids fetus (Pratella et al., 2022), single-gene disorders (Young et al., 2020), and even epigenetic modification (Y. Liu et al., 2022). This approach offers a minimally invasive, highly accurate, and safe alternative to traditional invasive techniques such as amniocentesis or recruitment CVS. With progress in sequencing next generation (Next Generation Sequencing/NGS) (Y. Liu et al., 2022)analysis cfDNA expanding its scope, paving the way for early detection of genetic and epigenetic abnormalities in the fetus, which can improve prenatal care and reduce the risk of pregnancy-related complications.

It also improves our understanding of the normal dynamics of the network (Lehmann-Werman et al., 2016). This is supported by studies that have identified tissue-specific cell death using circulating DNA methylation patterns, providing evidence of possible islet cell death in adolescents with obesity and diabetes (Syed et al., 2020). Methylation can be identified at the beginning of the first trimester of pregnancy using analytical techniques cfDNA (Salvianti et al., 2015; Xiao et al., 2020). Placental fraction in GDM Significantly increased compared to normal pregnancies in the first trimester, this trend persisted into the second and third trimester, although it did not reach statistical significance (Del Vecchio et al., 2021). These findings suggest that methylation patterns can be detected early in pregnancy.

Then, analysis of cfDNA, specifically placental fractions and their methylation patterns, presents a promising non-invasive approach for early detection of fetal and maternal health conditions, including GDM. Given that cfDNA levels increase during pregnancy and are affected by metabolic status, their assessment can provide valuable insights into pregnancy-related complications. The increased cfDNA observed in GDM, even in the early trimester, emphasizes its potential as an early biomarker for metabolic dysfunction. Although more research is needed to establish its clinical usefulness, these findings highlight the evolving role of cfDNA methylation analysis in prenatal screening, offering a safer alternative to invasive procedures such as CVS and improving early intervention strategies for better maternal and fetal health outcomes.

Leptin levels in GDM and obese maternal exhibit variability, ranging from low to high, but generally tends to increase. This variation can be caused by differences in measurement methods. Although there were some lower values in the data, the data analysis showed no significant difference, so it can be concluded that the leptin higher in mothers with GDM and obesity compared with those with normal metabolic status (Table 1). In addition to differences in gene expression, epigenetic modifications such as DNA methylation can provide further insight into the regulatory mechanisms involved. Methylation levels in placental tissue samples

from mothers with GDM increase, indicating potential regulatory changes associated with hyperglycemia. Similarly, placental tissue from women with impaired glucose tolerance (Impaired Glucose Tolerance/IGT) and obesity showed higher levels of methylation compared to tissues from pregnant women with normal metabolic conditions (Table 2). It should be noted, a progressive trend is seen, with methylation levels gradually increasing from obese mothers to those with IGT, and reaching the highest levels in mothers with GDM. This pattern highlights the potential epigenetic influence of maternal metabolic status on placental function and fetal development (Bouchard et al., 2010; Lesseur, Armstrong, Paquette, et al., 2014; Nogues et al., 2019; Sletner et al., 2021).

Table 1. Leptin Level/Expression Data in Journals

Experimental Design	Leptin Levels/Expression	Methylation Rate	Method	Sample	Gestational Age	Citation
Normal *vs.* *GDM Maternal*	High	-	ELISA	cfDNA	First Trimester	*(Xiao et al., 2020)*
Normal *vs.* *GDM Maternal*	Low (not significantly different)	-	ELISA	cfDNA	Third Trimester	*(Xiao et al., 2020)*
Normal *vs.* *GDM Maternal*	High	-	Luminex xMAP	gDNA	Third Trimester	*(Sletner et al., 2021)*
Normal *vs.* *GDM Maternal*	High	-	Multiplex assay	cfDNA	Third Trimester	*(Vasilakos et al., 2022)*
Normal *vs.* *GDM Maternal*	High	-	Immunoassay sandwich multiplex	cfDNA	First Trimester	*(Ye et al., 2022)*
Normal *vs.* *IGT Maternal*	Low	-	qPCR	cfDNA	Third Trimester	*(Bouchard et al., 2010)*
Normal *vs.* *GDM Maternal*	High	-	Immunohistochemistry	gDNA	Third Trimester	*(Pérez-Pérez et al., 2015)*
Normal *vs.* *Obesity Maternal*	Low	-	qPCR	gDNA	Third Trimester	*(Nogues et al., 2019)*

(GDM: Gestational Diabetes Mellitus, DM: Diabetes Mellitus, IGT: Impaired Glucose Tolerance)

Table 2. Leptin Methylation Data in the Journal

Experimental Design	*Leptin Levels/Expression*	Methylation Rate	Method	Sample	*Gestational Age*	Citation

Normal *vs.* *IGT* *Maternal*	**-**	**High**	**Sequenom EpiTYPER system**	**gDNA**	**Third Trimester**	***(Bouchard et al., 2010)***
Normal vs. GDM Maternal	**-**	**High**	**Pirosequencing bisulfite**	**gDNA**	**Third Trimester**	***(Lesseur, Armstrong, Paquette, et al., 2014)***
Normal *vs.* *Obesity* *Maternal*	**-**	**High**	**Pirosequencing bisulfite**	**gDNA**	**Third Trimester**	***(Nogues et al., 2019)***
Normal *vs.* *GDM* *Maternal*	**-**	**High**	**Pirosequencing bisulfite**	**gDNA**	**Third Trimester**	***(Sletner et al., 2021)***

(GDM: Gestational Diabetes Mellitus, DM: Diabetes Mellitus, IGT: Impaired Glucose Tolerance)

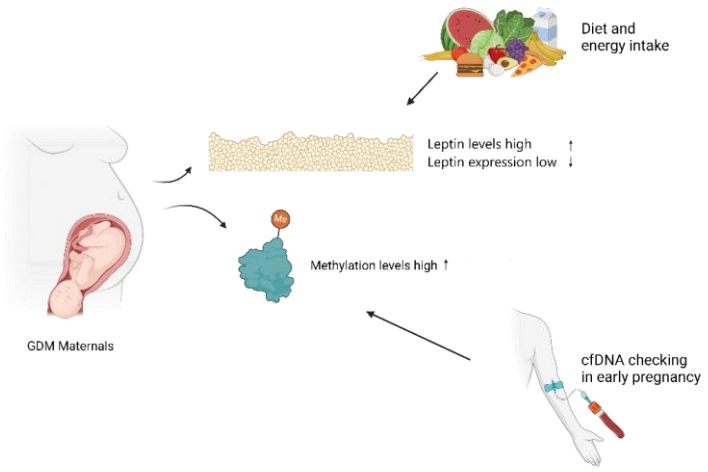

Figure 2. In GDM, maternal leptin levels

Figure 2. In GDM, maternal leptin levels are high and leptin expression is low. However, these fluctuations in leptin levels are not only caused by GDM but can also be affected by factors such as diet and energy intake. In pregnancy with GDM, methylation levels are consistently increased. Monitoring levels of methylated cfDNA associated with leptin early in pregnancy can help identify individuals at risk of developing T2DM.

These findings suggest that leptin levels in mothers with GDM and obesity show variation but are generally increased, with differences in measurement methodologies influencing the observed outcomes. While qPCR provides a more precise assessment of leptin gene expression, immunohistochemistry offers additional insights into protein localization. In addition, epigenetic modifications, specifically leptin DNA methylation, appear to be closely related to maternal metabolic status, with higher levels of methylation seen in placental tissue from mothers with GDM, IGT, and obesity. The progressive increase in methylation from obesity to GDM highlights the potential role of epigenetic regulation in metabolic adaptation

during pregnancy. These findings emphasize the importance of DNA methylation as a potential biomarker for assessing metabolic health during pregnancy and predicting future risks of metabolic disorders such as T2DM.

CONCLUSION

The increasing prevalence of T2DM and its strong association with GDM highlights the urgent need for early detection and prevention strategies. Leptin, a key regulator of energy metabolism and insulin resistance, has been widely studied in metabolic disorders, but its role goes beyond simple hormonal fluctuations. Recent findings suggest that epigenetic modifications, specifically DNA methylation in the leptin gene, can significantly affect its function. Methylation patterns in the leptin gene have been observed in placental tissue from mothers with GDM and obesity, demonstrating an adaptive response to metabolic stress. A progressive increase in methylation levels from obesity to GDM reinforces the potential role of epigenetics in metabolic adaptation during pregnancy. These insights provide a compelling reason to explore methylated cfDNA leptin as a new, non-invasive biomarker for assessing metabolic health during pregnancy and predicting future risks of T2DM in mothers and offspring. In addition, the use of cfDNA as a biomarker offers a promising approach to early detection of metabolic disorders, reducing the need for invasive procedures such as CVS. The ability to detect epigenetic changes in cfDNA opens new avenues for personalized treatment and early intervention, which ultimately improves maternal and fetal health outcomes. Further research is needed to validate the clinical usefulness of leptin gene methylation as a biomarker and to establish a standard methodology for its measurement. By integrating epigenetic markers into diagnostic frameworks, the medical community can move toward more precise, predictive, and personalized health strategies for diabetes management and prevention.

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