Recent updates on microRNA as potential biomarkers for diagnosis of gestational diabetes mellitus

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ABSTRACT

Background/Aims: Screening for gestational diabetes mellitus (GDM) are currently done at 24 - 28 weeks of conception, missing out on the most vulnerable period of organogenesis and thus preventing clinicians from starting treatments until the late second or third trimester. MicroRNAs (miR) are small non-coding RNA molecules that could aid in detecting or predicting GDM through establishing a novel non-invasive prenatal testing (NIPT) tool. The objective of this study was to summarize the most recent updates on plasma microRNAs as GDM diagnostic biomarkers. **Methods:** Between April and June 2021, a PubMed literature search was undertaken to review recent articles on human plasma miR associated with GDM. Animal studies and papers that are written in languages other than English were excluded. Only plasma miRNAs were used to avoid coagulation biases. **Results:** A total of 31 miRNAs were found significantly upregulated in the plasma samples of patients with GDM. It was found mainly during the 2nd or 3rd trimester except for miR-223 and miR-23a that were upregulated at 9 – 11 weeks of gestation. **Conclusion:** Though extensive prospective cohort studies are required, miR-223 and miR-23a should be considered the most promising to develop a successful NIPT tool because they were found to be upregulated earliest, during the first trimester.

Keywords: microRNA, diabetes, pregnancy, prenatal testing.

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1 INTRODUCTION

Gestational diabetes mellitus (GDM), a medical condition with increasing incidence worldwide, affects 7% of all pregnancies (1). Moreover, implementing the new International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria compared to prior GDM criteria resulted in a 75% increase in the number of women with GDM who had evidence of heterogeneity (2). GDM is defined as any degree of glucose intolerance that begins or is first recognized during pregnancy and is associated with many complications, including infant macrosomia, hypoglycemia, and an increased risk of type 2 diabetes (T2D) development in both the mother and the infant (3, 4). GDM can be divided into two types: A1GDM and A2GDM.

The A1GDM can be managed without medications and responds to nutritional therapy, while A2GDM must be handled with medications to achieve adequate glycemic control (4). At present, screening of GDM is usually done in a single step through 75 g OGTT at 24 - 28 weeks of gestation, which means that treatments cannot begin until the late second or third trimester, hence carries a high risk of maternal and fetal morbidity and mortality (1, 5). As a result, early screening in the first or early second trimester of pregnancy is crucial to promptly establish an adequate therapy that normalizes blood glucose levels, reducing GDM adverse pregnancy outcomes (1).

MicroRNAs (miR) are short non-coding RNAs with 18–22 nucleotides that regulate post-transcriptional gene expression by binding to the translation section and causing mRNA degradation or translational inhibition (6). Approximately 10–12 miR expression is linked with GDM. MicroRNA-9, -27a, -33a, -92a, -30d, -137, -362-5p and -502-5p were increased in placental tissues, while miR-132, -29a, and -222 expression was significantly downregulated in serum samples of patients with GDM (7-9). The miRNA-16-5p, -17-5p, -19a-3p, -19b-3p and-20a-5p expression were seen to be increased in plasma samples of women during 16–19 weeks of pregnancy (10). In blood samples of patients with GDM, miR-33a-5p was also found to increase, and blood glucose levels were positively associated. A well-established model for assessing islet beta-cell functionality is INS-1 cells, proliferation, and insulin production of which were significantly reduced or increased by overexpression or inhibition of miR-33a-5p, respectively (11).

It should be emphasized that miR are released from intact cells or platelets during RNA extraction, so the use of plasma rather than serum to prevent coagulation biases is preferred (12). Therefore, the objectives of this study were to find out the recent updates of plasma miR as GDM diagnostic biomarkers, the expression of those miR throughout pregnancy, and the specific correlation between those miR and known GDM risk factors.

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2 MATERIALS AND METHODS

2.1. Data Sources and Search Strategies

A PubMed literature search was conducted between April and June 2021. Different types of research were studied for this project, including original articles, systematic reviews, meta-analyses, reviews, and randomized controlled trials. Animal studies and articles written in a language other than English were excluded. We have filtered the search to recently published articles, between 2015 and 2021. The search was not limited to a particular population or ethnicity or a specific category of age. The following keywords in various combinations were employed to search the literature: "gestational diabetes", "diabetes mellitus", "type 2 diabetes", "diabetes in pregnancy", "glucose tolerance test", "early pregnancy screening", "pregnancy biomarker", "microRNA". Furthermore, the reference lists of the chosen publications and related reviews were manually searched.

2.2. Data Extraction and Analysis

One hundred twenty-one articles initially appeared from the PubMed search. Three reviewers analyzed the search results by reading the titles, abstracts, and objectives. Disagreements were solved through discussions, and the overall records identified by common acceptance were then narrowed down based on inclusion criteria. The articles selected were the ones with the objective of using miR as a diagnostic tool for diabetes in pregnancy. The data extracted included the GDM diagnostic criteria, adjustments made, pregnancy outcomes, study design, country, and the number of study participants.

3 DISCUSSIONS

3.1 Biogenesis of miRNAs:

miRs are non-coding RNA molecules with an approximate length of 19–22 nucleotides. miRs are primarily transcribed by RNA polymerase II, resulting in a primary miRNA (called pri-miRNA) with a length of 500–3000 nucleotides. A microprocessor complex then cleaves the pri-miRNA into a premature miR (called pre-miRNA), which is around 70-80 nucleotides long. Furthermore, pre-miRNA is transported into the cytoplasm by exportin 5, a nuclear export transporter that processes a 22-nucleotide "miRNA duplex" by engaging with the RNase III endonuclease dicer protein and a co-factor which is an RNA binding protein having double-strands with responsiveness to transactivation. After binding to an argonaute and glycine tryptophan repeat-containing protein, the miRNA duplex is integrated into an "RNA-induced silencing complex," where it attaches to partial or full-complementary sequences in the 3' untranslated region (3'-UTR) or 5'-UTR of the target mRNA, and thus participates in the regulatory gene expression procedure (7, 12). The process of miR biogenesis is displayed as a schematic in Figure 1.

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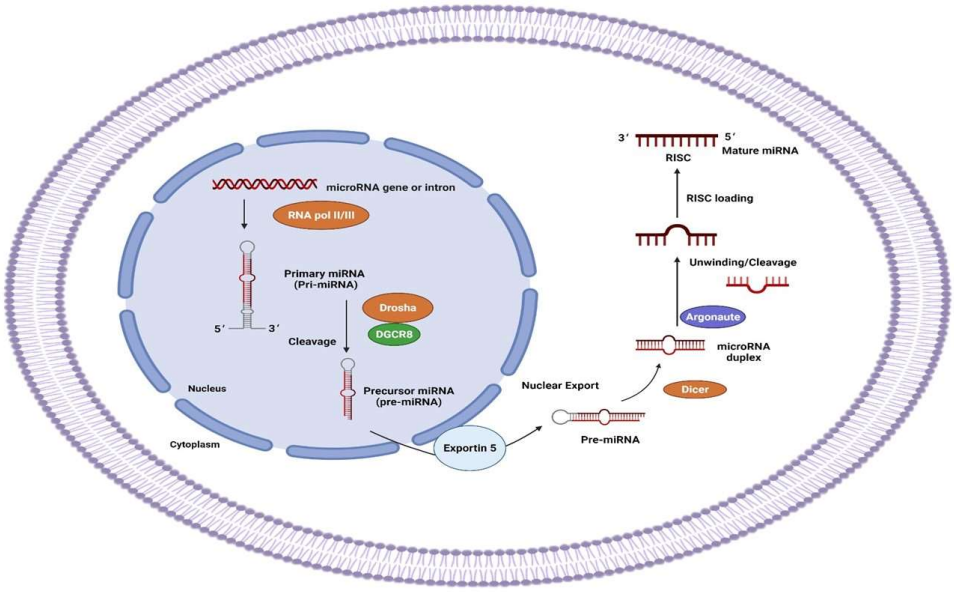

Figure 1. Schematic presentation of microRNA biogenesis

3.2 miR as markers of diabetes:

MicroRNAs play an essential role in regulating the physiology of insulin-producing β-cells. They contribute not only to the differentiation of these cells but also to developing their distinct secretory properties. Furthermore, miR assists β-cells in adapting to increasing insulin demand, and specific microRNAs, when produced in excess, can cause cellular dysfunction, and hasten the development of certain kinds of diabetes mellitus (13). Several researchers have looked at the expression of circulating microRNAs (plasma/serum) in diabetes to see if they may be used as early indicators for this group of chronic metabolic illnesses and their role in the pathogenetic pathways (14). Previously, the expression of circulating microRNAs in type 1 and type 2 diabetes was discussed in some articles. Still, only a limited number of studies investigated plasma microRNAs as a candidate biomarker of GDM.

3.3 Expression of miR in gestational diabetes:

Zhu et al used the next-generation sequencing technique to examine microRNA expression in the plasma of 10 patients with GDM and 10 controls without GDM at the 16th–19th week of pregnancy (15). As a result, they discovered upregulated microRNA signature in GDM patients comprised of five microRNAs, namely miR-16-5p, miR-17-5p, miR-19a-3p, miR-19b-3p, and miR-20a-5p. The microRNAs discovered by them have been linked to the different signaling pathways implicated in insulin secretion, for instance, the MAPK, insulin, TGF, and mTOR signaling pathways.

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In 2017 Cao et al. attempted to confirm the findings of the pilot study of Zhu et al. in a larger sample size of 85 GDM and 72 non-GDM women by analyzing the same microRNAs (miR-16-5p, miR-17-5p, miR-19a-3p, miR-19b-3p, and miR-20a-5p) that were found to be differentially expressed. They found no significant differences in miR-19a-3p and miR-19b-3p expression between GDM and non-GDM patients. Still, during 16th–19th, 20th–24th, and 24th–28th weeks of gestation, miR-16-5p, miR-17-5p, and miR-20a-5p were all progressively upregulated in plasma samples of women with GDM (10).

In a study of pregnancy complications, plasma samples from 36 GDM patients and 80 non GDM controls were collected during 7th–23rd week of gestation. They studied the expression of 10 microRNAs (miR-126-3p, miR-155-5p, miR-21-3p, miR-146b-5p, miR-210-3p, miR-222-3p, miR-223-3p, miR-517-5p, miR-518a-3p, and miR-29a-3p), which were chosen for their important roles in pregnancy and its complications and or association with T2D (16). They discovered that high plasma levels of miR-155-5p and miR-21-3p were associated with GDM, while levels of another two microRNAs (miR-21-3p and miR-210-3p) were specifically linked with GDM patients who were overweight/obese. Lastly, women suffering from GDM and carrying male fetuses were found to have high levels of six specific microRNAs such as miR-155-5p, miR-21-3p, miR-146b-5p miR-223-3p, and miR-517-5p.

Similarly, Tagoma et al found upregulation of the following miRNAs let-7e-5p, let-7g-5p, miR-101-3p, miR-146a, miR-18a-5p, miR-222-3p miR-23b-3p miR-30b-5p miR-30c-5p miR-30d-5p miR-342-3p miR-423-5p miR-92a-3p in GDM patients with highest increase in miR-195-5p levels in plasma (17). Moreover, Yoffe et al found upregulated levels of miR-223 & miR-23a in GDM patients during the first trimester of pregnancy, which might guide to develop of a non-invasive prenatal testing (NIPT) method during the first or early second trimester of pregnancy to manage GDM effectively and to prevent related risks on morphogenesis and organogenesis (1). Finally, Balci et al also reported increased miR-7-5p levels during the late second trimester of pregnancy in women with GDM (18). The key findings of recent literature on miR as GDM biomarker have been summarized in table 1.

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Table 1. Recent findings of plasma microRNA as GDM biomarker

Study	Sample	Method of miR extraction	Upregulated microRNA
Zhu et al, 2015	10 GDM patients and 10 controls; 16th–19th weeks of pregnancy	Ion Torrent qRT-PCR	miR-16-5p, miR-17-5p, miR-19a-3p, miR-19b-3p, miR-20a-5p
Cao et al, 2017	85 GDM patients and 72 controls; 16th–20th, 20th–24th, and 24th–28th weeks	qRT-PCR	miR-16-5p, miR-17-5p, miR-20a-5p
Wander et al, 2017	36 GDM patients and 80 controls; 7th–23rd week	qRT-PCR	miR-155-5p, miR-21-3p, miR-210-3p, miR-155-5p, miR-146b-5p, miR-223-3p, miR-517-5p, miR-29a-3p
Sebastiani et al, 2017b	21 GDM patients, 11 non-GDM control subjects; 28th–33rd weeks	Plasma Stem-loop RT PCR	miR-330-3p
Tagoma et al, 2018	13 GDM patients, 9 non-GDM controls; 23 - 31 weeks of pregnancy	qRT-PCR	let-7e-5p, let-7g-5p miR-101-3p miR-146a-miR-18a-5p miR-195-5p (mainly) miR-222-3p miR-23b-3p miR-30b-5p miR-30c-5p miR-30d-5p miR-342-3p miR-423-5p miR-92a-3p
Yoffe et-al, 2019	23 GDM patients, 20 non-GDM control; 9 – 11 weeks	qRT-PCR	miR-223, miR-23a
Balci et al, 2020	30 GDM patients, 30 non-GDM healthy controls; 24th– 28th weeks	qRT-PCR using 96.96 Dynamic Array IFCs	miR-7-5p

3.4 Expression of miR in other gestational conditions:

It is to be noted here that miR-16-5p is highly expressed and enriched in erythrocytes, so its expression level may vary during sample hemolysis. This characteristic makes measuring circulating miR-16-5p expression levels to monitor the progression of GDM potentially misleading, hence cannot be used as a reliable biomarker, though having a potentially pivotal role in the pathophysiology of GDM. The involvement of the miR-17-miR-20b microRNA family in

smooth muscle cell proliferation has previously been reported, potentially indicating a specific part for these microRNAs in diabetic vascular complications. Moreover, a prior study linked miR-17 and miR-20b to preeclampsia that impairs perinatal outcomes and is substantially correlated with GDM in terms of severity of glucose intolerance and its risk factors. Due to the reported overlaps of various pathologic conditions during pregnancy, it is not unlikely that diverse pregnancy complications, like preeclampsia, may share similar circulating microRNA changes (12).

The development of novel analytical methodologies is a step forward. For example, next-generation sequencing (NGS) is a novel tool for the identification as well as absolute quantification of RNA. Significantly, new cDNA library preparation chemistries will enable researchers to work with as small as 1ng of plasma (12). It was observed in several pieces of research that preanalytical conditions such as methods of blood sampling and lack of standardized collection as well as processing techniques of blood samples may have a significant impact on the stability of microRNA, resulting in their variances in expression.

4 CLINICAL IMPLICATIONS

Regarding GDM, limited information is available on the expression of circulating plasma microRNAs. The findings of the preceding studies point to some potential candidates for circulating biomarkers for GDM. Developing a NIPT tool, based on miRNA to detect or predict GDM during the embryonic and early fetal period of pregnancy should be considered as an additional measure to prevent associated congenital anomalies.

5 FUTURE DIRECTION

Additional research is needed to understand the physiological and pathological patterns of expression of these molecules during pregnancy. Furthermore, to establish miR as an early GDM biomarker, standard operating procedures (SOPs) for collecting plasma, sample processing to extract RNA, quality control evaluation, measuring circulating microRNAs, and analyzing their expression profile must be characterized.

6 CONCLUSIONS

GDM reduces pancreatic-cell expansion during pregnancy, and screening for GDM is usually done at 24–28 weeks of gestation. This poses a risk to fetal health, and clinicians miss an opportunity to intervene and manage GDM in a timely manner. According to the available research, some specific miRNAs can be used as a potential biomarker for early diagnosis, as discussed above. Even though there are numerous preanalytical and analytical challenges for clinical use of plasma miRNAs,

standardization of these methods may aid in reproducibility among studies. Large prospective cohort studies are needed to determine the diagnostic and or prognostic potential of plasma miR.

8 REFERENCES

1. Yoffe L, Polsky A, Gilam A, Raff C, Mecacci F, Ognibene A, et al. Early diagnosis of gestational diabetes mellitus using circulating microRNAs. European Journal of Endocrinology. 2019;181(5):565-77.
2. Saeedi M, Cao Y, Fadl H, Gustafson H, Simmons D. Increasing prevalence of gestational diabetes mellitus when implementing the IADPSG criteria: A systematic review and meta-analysis. Diabetes Research and Clinical Practice. 2021;172:108642.
3. Doughty KN, Taylor SN. Barriers and benefits to breastfeeding with gestational diabetes. Seminars in Perinatology. 2021;45(2):151385.
4. Rodriguez Q, Mahdy H. Gestational diabetes. 2019.
5. Sert UY, Ozgu-Erdinc AS. Gestational Diabetes Mellitus Screening and Diagnosis. Advances in Experimental Medicine and Biology: Springer International Publishing; 2020. p. 231-55.
6. Siddika T, Heinemann IU. Bringing MicroRNAs to Light: Methods for MicroRNA Quantification and Visualization in Live Cells. Front Bioeng Biotechnol. 2021;8:619583-.
7. Zhang T-N, Wang W, Huang X-M, Gao S-Y. Non-Coding RNAs and Extracellular Vehicles: Their Role in the Pathogenesis of Gestational Diabetes Mellitus. Front Endocrinol (Lausanne). 2021;12:664287-.
8. Filardi T, Catanzaro G, Mardente S, Zicari A, Santangelo C, Lenzi A, et al. Non-Coding RNA: Role in Gestational Diabetes Pathophysiology and Complications. Int J Mol Sci. 2020;21(11):4020.
9. Poirier C, Desgagné V, Guérin R, Bouchard L. MicroRNAs in Pregnancy and Gestational Diabetes Mellitus: Emerging Role in Maternal Metabolic Regulation. Current Diabetes Reports. 2017;17(5).
10. Cao Y-L, Jia Y-J, Xing B-H, Shi D-D, Dong X-J. Plasma microRNA-16-5p, -17-5p and -20a-5p: Novel diagnostic biomarkers for gestational diabetes mellitus. Journal of Obstetrics and Gynaecology Research. 2017;43(6):974-81.
11. Feng Y, Qu X, Chen Y, Feng Q, Zhang Y, Hu J, et al. MicroRNA-33a-5p sponges to inhibit pancreatic β-cell function in gestational diabetes mellitus LncRNA DANCR. Reprod Biol Endocrinol. 2020;18(1):61-.
12. Guarino E, Delli Poggi C, Grieco GE, Cenci V, Ceccarelli E, Crisci I, et al. Circulating MicroRNAs as Biomarkers of Gestational Diabetes Mellitus: Updates and Perspectives. Int J Endocrinol. 2018;2018:6380463-.
13. Guay C, Regazzi R. New emerging tasks for microRNAs in the control of β-cell activities. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2016;1861(12):2121-9.
14. Sebastiani G, Nigi L, Grieco GE, Mancarella F, Ventriglia G, Dotta F. Circulating microRNAs and diabetes mellitus: a novel tool for disease prediction, diagnosis, and staging? Journal of Endocrinological Investigation. 2017;40(6):591-610.

15. Zhu Y, Tian F, Li H, Zhou Y, Lu J, Ge Q. Profiling maternal plasma microRNA expression in early pregnancy to predict gestational diabetes mellitus. International Journal of Gynecology & Obstetrics. 2015;130(1):49-53.
16. Wander PL, Boyko EJ, Hevner K, Parikh VJ, Tadesse MG, Sorensen TK, et al. Circulating early- and mid-pregnancy microRNAs and risk of gestational diabetes. Diabetes research and clinical practice. 2017;132:1-9.
17. Tagoma A, Alnek K, Kirss A, Uibo R, Haller-Kikkatalo K. MicroRNA profiling of second trimester maternal plasma shows upregulation of miR-195-5p in patients with gestational diabetes. Gene. 2018;672:137-42.
18. Balci S, Gorur A, Derici Yıldırım D, Cayan F, Tamer L. Expression level of miRNAS in patients with gestational diabetes. Turkish Journal of Biochemistry. 2020;45(6):825-31.
19. Sebastiani G, Guarino E, Grieco GE, Formichi C, Delli Poggi C, Ceccarelli E, et al. Circulating microRNA (miRNA) Expression Profiling in Plasma of Patients with Gestational Diabetes Mellitus Reveals Upregulation of miRNA miR-330-3p. Front Endocrinol (Lausanne). 2017;8:345-.