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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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MIR-29A, MIR-222 AND MIR-132 IN THE BLOOD PLASMA OF PREGNANT WOMEN AS PREDICTORS OF GESTATIONAL DIABETES

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Abstract.

Background: Gestational Diabetes Mellitus (GDM) is a significant medical problem worldwide and the cause of many complications for the mother and the foetus, in terms of pregnancy management and outcome. Early assessment of the risk of diabetes in pregnant women with hyperglycaemia is particularly important, as it allows timely preventive measures to help avoid potential complications

Aim: Our aim was to identify microRNAs that could enable the assessment of diabetes risk in pregnant women with hyperglycaemia.

Methods: The study analyzed the expression of the following microRNAs: miR-132, miR-29a, miR-222, miR-93and miR-17-5p, from the blood samples of pregnant women with hyperglycaemia, with gestational diabetes mellitus, with type 1 or type 2 diabetes and healthy pregnant between 24 and 28 weeks of their pregnancy. The miR-17-5p was used as a reference.

Results: A significant difference in the miR-222 and miR-29a expression level was found in plasma samples. Compared to the control group Among pregnant patients with hyperglycaemia miR-222 and miR-29a in some sample exhibit increased levels while others show reduced levels—suggesting its potential for subgroup differentiation within this population. MiR-93 remains uniformly low in in Diabetes, GDM, Hyperglycaemia groups compared to the control group. A significant difference in the miR-93 expression level was found in plasma samples. miR-132 is also upregulated in GDM and diabetic patients, with the highest levels observed in the diabetic group, compared to the control group. In contrast, its expression fluctuates among pregnant women with hyperglycaemia.

Conclusion: The wide variability in the expression levels of miR-29a, miR-222 and miR-132 suggests that they may serve as useful predictive biomarkers for evaluating diabetes risk in hyperglycemic pregnant women. Further studies involving longitudinal follow-up of hyperglycaemic pregnant women are needed to determine the predictive value of these microRNAs.

Key words. Gestational diabetes, miR-17, miR-222, miR-29a, miR-132, biomarker, hyperglycaemia.

Introduction.

Gestational diabetes mellitus (GDM) is a form of diabetes that develops during pregnancy. Global incidence of GDM has increased significantly in recent years [1,2]. Several pre-pregnancy conditions, including female obesity, overweight, sedentary lifestyle, advanced maternal age, metabolic syndrome, dietary patterns, history of preeclampsia, ethnicity, and socioeconomic or geographic influences have been identified as potential risk factors for GDM [3]. Last decades maternal age is increasing, and obesity rates among women of reproductive age continue to rise—factors that are expected to further elevate the global incidence of gestational diabetes mellitus (GDM) [4].

Both pre-existing diabetes and GDM can lead to numerous pregnancy-related complications, affecting not only the course and outcome of pregnancy but also the long-term health of both the mother and the offspring. Accumulating evidence has indicated that maternal hyperglycaemia and gestational diabetes contribute significantly to adverse maternal and foetal outcomes during pregnancy and in the postpartum period [5-8].

Maternal diabetes, whether pre-existing or developed during pregnancy, has also been associated with an increased risk of metabolic syndrome and cardiovascular disease in the offsprings. Moreover, longitudinal studies suggest that maternal hyperglycaemia may predispose the child to cardiovascular disease within the first four decades of life [6]. Maternal complications of diabetes include a higher likelihood of ceaserean delivery, often indicated due to macrosomia or abnormal foetal position. Additionally, preeclampsia and eclampsia remain common complications in diabetic pregnancies [9].

Gestational diabetes mellitus (GDM) is typically diagnosed between the 12th and 26th weeks of pregnancy, when a glucose screening test is performed [4,10,11].

Over the past few decades, numerous studies have revealed important connections between microRNAs and diabetes mellitus, providing a foundation for exploring similar associations in gestational diabetes mellitus (GDM) [12].

MicroRNAs are small, non-coding, single-stranded RNA molecules approximately 22 nucleotides in length. They are found in plants, animals, and viruses, as well as in human tissues and body fluids. MicroRNAs regulate gene expression primarily through RNA silencing and post-transcriptional control of messenger RNA (mRNA) stability and translation, thereby influencing protein synthesis. To date, more than 2,500 microRNAs have been identified in the human genome. microRNAs are also secreted into the extracellular space and subsequently circulate within the bloodstream [13,14]. Because of their wide distribution in body fluids such as serum, plasma, urine, sweat, and saliva, circulating microRNAs represent accessible and promising biomarkers. In recent years, microRNAs have attracted growing attention as potential diagnostic and therapeutic tools due to their demonstrated association with GDM [15]. Consensus has not yet been reached on which specific microRNAs show consistent alterations in this condition. Therefore, further research and comprehensive analysis are warranted to identify and validate these molecular signatures [16].

Before initiating our research, we conducted an extensive literature review of approximately one hundred scientific

publications, including original studies and review articles indexed in leading international peer-reviewed databases. This review enabled us to identify and select a set of microRNAs of particular relevance for expression analysis in maternal blood samples from pregnant women with hyperglycaemia.

Materials and Methods.

A total of 100 participants, including patients and healthy volunteers, were enrolled in this study. All participants provided written informed consent prior to inclusion. The study was approved by the Institutional Ethics Committee of the Aleksandre Natishvili Institute of Morphology, Ivane Javakhishvili Tbilisi State University.

Patients were recruited from the following medical centers: Pineo Medical Ecosystem, Georgian-German Center for Reproductive Medicine, and Diakor– Center for Diabetes, Endocrine and Cardiopulmonary Diseases.

Participants were classified into four groups based on glucose metabolism status according to the World Health Organization (WHO, 2013) criteria. The healthy control group comprised pregnant women with normal fasting plasma glucose levels (<5.1 mmol/L) and no history of diabetes or hyperglycemia during pregnancy; an oral glucose tolerance test was not performed in this group. The hyperglycemia group included pregnant women with elevated fasting plasma glucose levels that remained below the WHO diagnostic thresholds for gestational diabetes mellitus (GDM). Gestational diabetes mellitus was diagnosed using a 75-g oral glucose tolerance test performed at 24–28 weeks of gestation, in accordance with WHO criteria. The diabetic group consisted of pregnant women with pre-existing (pregestational) diabetes mellitus, including type 1 and type 2 diabetes, diagnosed prior to pregnancy according to WHO criteria.

Blood samples for all groups were collected during 24–28 weeks of gestation.

Potential confounding maternal factors known to influence glucose metabolism and circulating microRNA expression were considered in the study design. Participants across all study groups were non-smokers. The majority of women had overweight status, with body mass index (BMI) values ranging from 27 to 29 kg/m², and no statistically significant differences in BMI were observed between the groups. In addition, participants were characterized as being prone to hypertension; however, no cases of chronic hypertension requiring pharmacological treatment were present at the time of sample collection.

Given the comparable distribution of these variables among the groups, additional statistical adjustment for BMI, smoking status, or hypertension was not applied.

Blood samples were collected and analyzed from the following groups:

- Pregnant women with hyperglycemia (n = 45).
- Pregnant women with gestational diabetes mellitus (GDM) (n = 25).
- Pregnant women with type 1 or type 2 diabetes (n = 5).
- Healthy pregnant women (n = 25).

The group of pregnant women with hyperglycemia included 45 patients (45%) with a mean age of 28.0 ± 3.6 years (age range: 24–32 years).

The group of patients with type 1 or type 2 diabetes consisted of 5 participants (5%), with a mean age of 29.0 ± 4.6 years (age range: 28–33 years).

The group of pregnant women with gestational diabetes mellitus included 25 patients (25%), with a mean age of 33.0 ± 1.7 years (age range: 30–34 years).

The group of healthy pregnant women included 25 participants (25%) who had no history of diabetes or any chronic disease. Their mean age was 23.0 ± 4.1 years (age range: 19–24 years).

Sample Collection and Plasma Separation:

Peripheral blood samples were collected in 5 mL BD Vacutainer tubes (EDTA–K_2; BD, New Jersey, USA). To obtain plasma, the samples were centrifuged at 3000 rpm for 15 minutes, after which approximately 1 mL of the supernatant was transferred into 1.5 mL microtubes (Eppendorf) and stored at −80 °C until further analysis.

RNA Extraction and Quantification:

Total RNA, including small RNA fractions, was isolated from plasma samples using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The concentration of extracted nucleic acids was measured using a Qubit 4 Fluorometer (Thermo Fisher Scientific, USA). Samples with total RNA concentrations ranging from 1 to 10 ng/μL were included in the analysis.

MicroRNA (miRNA) Expression Analysis:

MicroRNA expression was assessed using the TaqMan™ Advanced miRNA Assay System (Applied Biosystems, USA). Complementary DNA (cDNA) synthesis was performed with the TaqMan™ Advanced miRNA cDNA Synthesis Kit, and quantitative real-time polymerase chain reaction (qPCR) was carried out using the TaqMan™ Fast Advanced Master Mix, in accordance with the manufacturer's instructions.

The study analyzed the expression of the following microRNAs: miR-132, miR-29a, miR-222, miR-93 and miR-17-5p. The stably expressed miR-17-5p was used as an internal control for normalization to minimize inter-sample variation.

miR-17-5p was selected as the endogenous normalization control due to its reported stable expression during pregnancy and under conditions of impaired glucose metabolism. It is well recognized that no universally accepted endogenous reference miRNA exists for circulating microRNA analyses, particularly in pregnancy. Therefore, the choice of miR-17-5p was guided by both published evidence and validation within our own dataset.

Previous reference gene selection studies have demonstrated that members of the miR-17 family exhibit low expression variability and can be identified as stable candidate reference miRNAs using established stability algorithms such as geNorm and NormFinder [17,18].

In the present study, endogenous control stability was further evaluated using DataAssist v3.01 (Applied Biosystems) software, which incorporates stability assessment algorithms for candidate reference miRNAs. Among the tested candidates, miR-17-5p demonstrated the highest stability score across all study groups, supporting its suitability as a normalization control. ΔΔCq values were calculated using this validated reference miRNA.

Studied microRNAs and their Roles in Metabolism and Disease Pathways are shown in Table 1.

Statistical analysis. The Mann-Whitney U test was used to determine the statistical significance for comparisons data of $2^{-\Delta CT}$ and fold change. For multiple comparison nonparametric Kruskal-Wallis test followed by the Dunn's post hoc tests were used (ANOVA). Statistical analyses were carried out using SPSS (version 23.0; IBM Corp.) and GraphPad Prism (version 8.0; Dotmatics).

Results.

MiR-93 remains uniformly low in in Diabetes, GDM, Hyperglycaemia groups compared to the control group. A significant difference in the miR-93 expression level was found in plasma samples. The $^{2-}\Delta\Delta Cq$ mean for miR-93a in Diabetes, GDM, Hyperglycaemia and Control group was 0.07 (±0.54), 0.86 (±0.12), 2.6(±0.29), 4.5(±0.5), respectively. miR-93 was consistently low in the diabetes group (0.07) and higher

Table 1. *Studied MicroRNAs and their Roles in Metabolism and Disease Pathways.*

microRNA	Major Metabolic Roles	Main Pathogenic Pathways
miR-132	Glucose/lipid metabolism, neuroendocrine regulation	Inflammation, angiogenesis, neurological disease
miR-93-5p	Adipogenesis, insulin signaling	Cancer, hypoxia, endothelial dysfunction
miR-29a	insulin resistance	Anti-fibrotic, immunomodulation
miR-222	IRS regulation, lipid metabolism	Atherosclerosis, cancer, apoptosis resistance
miR-17-5p	Lipid/glucose metabolism, energy balance	Oncogenesis, immune modulation, angiogenesis

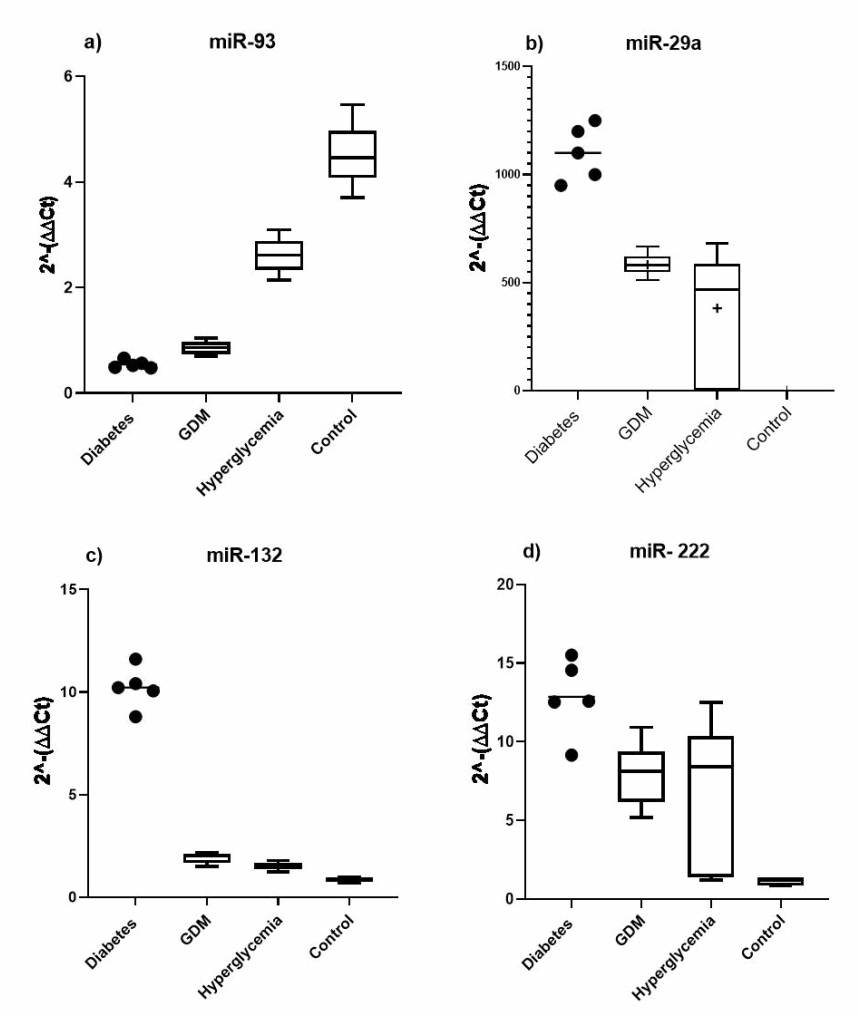

Figure 1. *expression levels of miR-93; miR-29a; miR-132 and miR-222, found in plasma samples.*
1a) *miR-93 was consistently low in the diabetes group (0.07) and higher in controls (4.5), individual control data points are elevated and diabetes group values are diminished.*
1b) *miR-29a in Diabetes, GDM group elevated, Among pregnant patients with hyperglycaemia, its expression differs from sample to sample, some exhibit increased levels while others show reduced levels.*
1c) *MiR-132 is upregulated in diabetic, GDM patients, with the highest levels observed in the diabetic group, compared to the control group. Its expression fluctuates among pregnant women with hyperglycaemia.*
1d) *MiR-222 is upregulated in diabetic and GDM patients, but its expression fluctuates among pregnant women with hyperglycaemia.*

in controls (4.5), which is consistent with Figure 1a, where individual control data points are elevated and diabetes group values are diminished. The differences between groups were statistically significant with P<0.001 (Figure 1a).

A significant difference in the miR-29a expression level was found in plasma samples. The $^{2^-}\Delta\Delta$Cq mean for miR-29a in Diabetes, GDM, Hyperglycaemia and Control group was 1100 (±127.5), 583.7 (±46.41), 381.5 (±257.9), 1.092 (±0.21), respectively. The differences between groups were statistically significant with P<0.001 (Figure 1b). Among pregnant patients with hyperglycaemia, its expression differs from sample to sample—some exhibit increased levels while others show reduced levels—The heterogeneous expression of miR-29a observed in the hyperglycemic group, characterized by increased levels in some individuals and decreased levels in others, indicates substantial biological variability within this group. This pattern likely reflects underlying metabolic heterogeneity rather than a uniform molecular signature. Given the cross-sectional design of the study and the absence of longitudinal follow-up, causal or predictive interpretations cannot be made. Accordingly, these findings should be interpreted with caution and warrant further investigation in larger, longitudinally designed cohorts.

miR-29a expression was markedly elevated in the diabetes group (1100 ± 127.5) compared to controls (1.092 ± 0.21), representing an approximately 1000-fold difference. To exclude PCR artifacts, assay performance was evaluated, including amplification efficiency, melt curve analysis, and technical replicates, confirming that the observed fold change reflects true differential expression.

MiR-132 is also upregulated in diabetic and GDM patients, with the highest levels observed in the diabetic group, compared to the control group. In contrast, its expression fluctuates among pregnant women with hyperglycaemia. A significant difference in the miR-132 expression level was found in plasma samples. The $^{2^-}\Delta\Delta$Cq mean for mir-132 in Diabetes, GDM, Hyperglycaemia and Control group was 10.2 (±1.0), 1.93(±0.23), 1.52 (±0.17), 0.853 (±0.084), respectively. The differences between groups were statistically significant with P<0.001 (Figure 1c).

A significant difference in the miR-222 expression level was found in plasma samples. The $^{2^-}\Delta\Delta$Cq mean for miR-222 in Diabetes, GDM, Hyperglycaemia and Control group was 12.87(±2.438) 7.92(±1.9), 6.11(±4.52), 1.09 (±0.21), respectively. MiR-222 is upregulated in diabetic and GDM patients, but its expression fluctuates among pregnant women with hyperglycaemia. The differences between groups were statistically significant with P<0.001 (Figure 1d).

Discussion.

The aim of this study was to evaluate the microRNA expression profile in pregnant women with hyperglycemia in order to identify potential liquid biopsy biomarkers for predicting gestational diabetes (GDM). The biomarker potential of specific microRNAs in gestational diabetes has been discussed for many years. Although miR-93, miR-29a, miR-222, and miR-132 have all been associated with GDM, their expression patterns in pregnant women with hyperglycemia remain unclear. Clarifying these profiles may help stratify the risk of developing GDM in this population.

Our findings partially align with the results reported by Gillett et al. The authors analyzed microRNA profiles in extracellular vesicles (EVs) isolated from serum samples collected early in pregnancy. Their objective was to determine whether specific EV-derived miRNAs could serve as early biomarkers for predicting GDM before clinical signs appear. They compared first-trimester serum samples from women who later developed GDM with samples from women who remained normoglycemic throughout pregnancy [19].

The study identified several microRNAs—miR-132-3p, miR-29a-3p, miR-122-5p, miR-136-5p, miR-182-3p, miR-210-3p, and miR-29b—that were differentially expressed early in pregnancy between women who subsequently developed GDM and those who did not [19].

These findings are consistent with our data, particularly regarding miR-132 and miR-29a, which also showed altered expression in our study

It is noteworthy that these microRNAs are directly involved in the pathogenesis of diabetes. For instance, miR-132-3p participates in the regulation of glucose metabolism, while miR-29a-3p is implicated in insulin resistance in skeletal muscle cells.

Tiziana Filardi and colleagues investigated the expression of miR-222-3p and miR-409-3p in blood plasma and exosomes during the third trimester of pregnancy. The researchers demonstrated that miR-222-3p and miR-409-3p, either individually or in combination with other biomarkers, may enhance the accuracy of gestational diabetes mellitus (GDM) diagnosis and contribute to a better understanding of its pathophysiology and related complications. Activation of miR-222-3p and miR-409-3p was confirmed. Expression levels of miR-222-3p and miR-409-3p showed a positive correlation with fasting plasma glucose (FPG) [20].

Our findings are partially consistent with previous studies. Based on our results, which also include data on miR-222, we observed that miR-222 levels are elevated not only in patients with diabetes and GDM but also in those with hyperglycemia. Therefore, it may have limited usefulness for stratifying patients with gestational diabetes.

An important and insightful observation has been made by researchers showing that microRNAs circulating in maternal plasma during the first trimester of pregnancy are associated with fasting glucose levels later in gestation. Cecilia Légaré and colleagues investigated the relationship between specific microRNAs present in maternal plasma during the first trimester and fasting glucose levels measured at the end of the second trimester. The aim of the study was to elucidate the link between microRNA expression and glucose regulation throughout pregnancy. The results demonstrated that several circulating microRNAs in early pregnancy—miR-16-5p, miR-29a-3p, miR-134-5p, and miR-103a-3p—were significantly associated with fasting glucose levels in late pregnancy. These microRNAs are known to play key roles in glucose metabolism, insulin signaling, and placental development) [21].

Regarding the results of our study, the expression levels of miR-29a are consistent with the findings reported by Legaré and colleagues. In our analysis, miR-29a is upregulated in both prediabetic and diabetic pregnant women. Among pregnant

patients with hyperglycemia, its expression varies from sample to sample—some show elevated levels, while others display reduced levels. This variability suggests that miR-29a may serve as a potential marker for identifying the risk of developing gestational diabetes.

The study conducted by Ling He, Xiaoli Wang, and Xiangyi Chen aimed to identify specific microRNAs that are differentially expressed in gestational diabetes mellitus (GDM) and to elucidate their roles in the pathophysiology of the disease, particularly in relation to insulin resistance, β-cell dysfunction, inflammation, and placental abnormalities. Several microRNAs were found to be significantly dysregulated in GDM, among them miR-29a: Downregulated in GDM; plays an essential role in insulin-stimulated glucose metabolism, and its reduced expression may contribute to insulin resistance [22].

In contrast to the findings of the aforementioned study, our data show that microRNA-29a expression levels vary among pregnant women with hyperglycaemia, highlighting its potential predictive value for the stratification of gestational diabetes. The discrepancy between our findings and those reported by He et al., who observed downregulation of miR-29a in GDM, may be attributable to methodological and population-related differences. These include ethnic background of the study cohorts, differences in biological material analyzed (plasma in the present study versus serum in previous reports), and variation in gestational age at sample collection. The landscape of miRNA expression in gestational diabetes mellitus (GDM) is highly heterogeneous, reflecting the complexity of its underlying pathophysiology across different populations and ethnic backgrounds, also at different stages of pregnancy. For instance, miR-29a-3p and miR-330-3p have been reported to be upregulated in Mexican women with GDM, while miR-9-5p was shown to target genes involved in glycolytic pathways and insulin secretion [21]. In contrast, Zhao et al. (2011) demonstrated significant downregulation of miR-132, miR-29a, and miR-222 in serum samples obtained from women with GDM (n = 24) during early second-trimester gestation (16–19 weeks), compared with healthy pregnant controls (n = 24) [23].

Our study data correlates with the results of Martinez-Ibarra's study. In a study by Martínez-Ibarra et al., serum levels of microRNAs associated with GDM Compared with non-diabetic subjects. higher levels of miR-29a, miR-222 were observed in the serum of patients with gestational diabetes [24].

In the samples we collected, the expression of miR-29a in the blood of pregnant women with hyperglycemia showed considerable variability, with some cases exhibiting high levels and others showing reduced expression. Similarly, the expression of miR-222 was elevated in both diabetic and hyperglycaemic pregnant women.

The key contribution of our study is the identification of this expression variability within the hyperglycemic group. Such variability further supports the potential of these microRNAs to serve as early predictors of gestational diabetes.

Conclusion.

In conclusion, we identified the expression profiles of miR-29a, miR-222, miR-93, and miR-132 in women with gestational diabetes and hyperglycaemia. Among these, miR-29a and miR-222 appears to be a promising predictive biomarker for assessing diabetes risk in hyperglycaemic pregnant women. MiR-132 also shows potential as a biomarker and warrants further investigation in larger study populations.

These results underscore the important contribution of these microRNAs to the development of gestational diabetes. Such insights may be valuable for improving early detection and management of GDM and the postpartum period.

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Conflict of interest.

The author declares no conflicts of interest related to this work.

Abbreviations.

GDM: Gestational Diabetes Mellitus; RNA: Ribonucleic Acid; MicroRNA: Micro Ribonucleic Acid; Camp: Cyclic Adenosine Monophosphate; CREB: cAMP Response Element-Binding Protein; p300: Protein 300; SIRT1: Sirtuin 1-deacetylase involved in metabolism, aging, inflammation, insulin sensitivity, and stress responses; ACHe: Acetylcholinesterase; The enzyme that breaks down the neurotransmitter acetylcholine at synapses; IRS: Insulin Receptor Substrate; PI3K: Phosphoinositide 3-kinase; BMI: Body Mass Index.

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