ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formatted version will be made available soon.

POLISH GYNECOLOGY
GINEKOLOGIA POLSKA
ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGICZNEGO
THE OFFICIAL JOURNAL OF THE POLISH GYNECOLOGICAL SOCIETY

ISSN: 0017-0011

e-ISSN: 2543-6767

Diagnostic potential of microRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia in the first trimester

Authors: Adrianna Kondracka, Bartosz Kondracki, Ilona Jaszczuk, Jakub Staniczek, Wojciech Kwasniewski, Agata Filip, Anna Kwasniewska

DOI: 10.5603/GP.a2023.0036

Article type: Research paper

Submitted: 2023-01-25

Accepted: 2023-03-11

Published online: 2023-04-05

Articles in "Ginekologia Polska" are listed in PubMed.

ORIGINAL PAPER / OBSTETRICS

Diagnostic potential of microRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia in the first trimester

Adrianna Kondracka[1], Bartosz Kondracki[2], Ilona Jaszczuk[3], Jakub Staniczek[4], Wojciech Kwasniewski[5], Agata Filip[3], Anna Kwasniewska[1]

[1]*Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, Poland*
[2]*Department of Cardiology, Medical University of Lublin, Poland*
[3]*Department of Cancer Genetics with Cytogenetic Laboratory, Medical University of Lublin, Poland*
[4]*Department of Gynecology, Obstetrics and Gynecologic Oncology, Medical University of Silesia, Katowice, Poland*
[5]*Department of Gynecology and Gynecologic Oncology, Medical University of Lublin, Poland*

Corresponding author:
Bartosz Kondracki
Department of Cardiology, Medical University of Lublin, Poland
e-mail: kondracki.bartosz@gmail.com

ABSTRACT

Objectives: MicroRNAs have been observed to play a major role in various physiological processes, for instance, programmed cell death, cell division, pregnancy development, and proliferation. With the help of profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems. The aim of the study was to evaluate the diagnostic potential of MicroRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia.

Material and methods: The study considered 53 patients who are in their first trimester of a singleton pregnancy. Participants have been divided into two study groups, one group with

normal pregnancy and another group having the risk of developing preeclampsia or who developed hypertension or preeclampsia during follow-up constitute the study group. In order to collect data associated with circulating miRNAs in serum, blood samples have been collected from the participants of the study.

Results: Based on the univariate regression model, increased expression of Mi 517 and 526 and parity status (primapara/multipara) has been obtained. The multivariate logistic analysis shows that independent risk factors for hypertension or preeclampsia are the presence of an R527 and being a primipara.

Conclusions: The study's findings have revealed that R517s and R526s act as major indicative biomarkers in the first trimester for the detection of hypertension and preeclampsia. The circulating C19MC MicroRNA was examined as a potential early indicator of preeclampsia and hypertension in pregnant individuals.

Key words: circulating microRNA; microRNAs; hypertension; biomarkers; pregnant women; pregnancy

INTRODUCTION

Up to 10% of pregnancies can become complicated by hypertensive disorders of pregnancy, which include preexisting and gestational hypertension, eclampsia, and preeclampsia [1]. These conditions are a substantial source of perinatal and maternal mortality and morbidity. Preeclampsia/eclampsia and preeclampsia along with persistent hypertension are all considered hypertensive diseases of pregnancy (HDP). After 20 weeks of pregnancy, pre-eclampsia, manifests as hypertension and proteinuria. It appears in between 2 and 10% of pregnancies [2]. Pre-eclampsia is a serious pregnancy complication, and its clinical symptoms start to appear in the second trimester. Preeclampsia is described as the co-occurrence of hypertension ‘proteinuria (> 0.3 g of protein in the 24-hour urine sample) and (blood pressure > 140/90 mmHg)’ in an initially normotensive woman after 20 weeks of pregnancy [3]. Pre-eclampsia is a significant factor in raising the risk of morbidity and mortality in fetuses or newborns and pregnant women due to the potential path leading to serious problems, such as proteinuria, convulsions (eclampsia), and hypertension. Preeclampsia may make future heart and blood vessel (cardiovascular) illnesses more likely. When a woman has experienced preeclampsia more than once, she is more vulnerable to developing cardiovascular problems in the future [4]. Pregnancy-

related hypertension and preeclampsia carry concerns for lower placental blood flow. The fetus can receive less oxygen and fewer nutrients in case the placenta doesn’t receive enough blood. Premature delivery, delayed growth (intrauterine growth restriction), and low birth weight can be consequential of the condition [5]. Before giving birth, the placenta in this syndrome separates from the uterus' inner wall. The risk of placental abruption is elevated by high blood pressure and preeclampsia. The life of both the mother and the unborn child may be in danger if there is a severe abruption that results in excessive bleeding. Slowed or reduced fetal growth may be a result of high blood pressure. The heart, brain, liver, eyes, kidneys, lungs, and other important organs might suffer damage from poorly controlled high blood pressure. It may even be fatal in extreme circumstances.

It is essential to identify a successful method to select out women who are likely to experience a severe case of preeclampsia for the prevention of this condition [3]. The prognostic indicators that are being considered include markers and biochemical aspects of maternal blood uterine activity, such as certain proteins, circulating microRNAs and circulating cell-free DNA, and ultrasonography [6]. The specific microRNAs from a group of non-coding RNAs that are relevant to this investigation are the main factors (ncRNAs). MicroRNAs (miRNAs) are single-stranded, 19–25 nucleotide-long RNA molecules. MicroRNAs are crucial in the post-transcriptional control of gene expression. In a process known as "silencing," microRNAs control the expression of more than 60% of all human genes.

MicroRNAs have been found to play a significant role in various physiological processes, such as programmed cell death, cell division, pregnancy development, and proliferation. It has been also found to play a role in a variety of pathological processes, including the development of myocardial infarctions, tumors, reactions to inflammation and infections, and the onset of chronic diseases [7]. In addition to that, for pre-eclampsia and other pregnancy-related disorders microRNAs have been identified as regulators of processes that occur throughout pregnancy. Pregnant women's serum microRNA profiles are different from those of non-pregnant women [8]. By contrasting the serum profile of microRNAs in non-pregnant and pregnant women, or between before and after labor, it is possible to distinguish microRNAs associated with pregnancy and the placenta [9]. Among pregnant women, the microRNA profile in the serum changes both qualitatively and quantitatively as the trophoblast develops and increases in volume and mass. According to recent findings, microRNAs may control ‘cell migration in pre-

eclampsia and apoptosis (programmed cell death)' [10]. Recent research on the pathogenesis of pregnancy complications linked to the presence of trophoblast (PIRCs, placental insufficiency-related complications) has revealed that microRNAs can be divided into four categories: placenta-associated, placenta-specific, circulating, uterine, and placenta-derived [11]. Through the profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems [12]. Thus, it is with the help of observing statistical differences in serum microRNAs expression levels among pregnant women with healthy pregnancies and women pregnant with pre-eclampsia, which enables selected, particular microRNAs to serve as safe clinical biomarkers of major complications at the time of the pregnancy. Studies have put forward the fact that up-regulation of miR-517-5p,miR-520h, and miR-518b are associated with the risk of later progression of preeclampsia [13]. It has also been identified in the screening of extracellular miR-517-5p during the first trimester a significant section of women develop subsequent preeclampsia. It has been mentioned in this context that the presence of elevated first-trimester plasma levels of miR-517-5p can be considered predictive of preeclampsia. In the study, it has been mentioned that miR-517-5p biomarker alone has been observed by the researchers as a predictive performance for preeclampsia [13]. The researchers also opined that C19MC microRNAs play a considerable role in the pathogenesis of complications associated with pregnancy. It has been reported in this context that in maternal circulation during the first trimester or early in the pregnancy C19MC microRNAs are dysregulated and it can play a role in stimulating preeclampsia and gestational hypertension [14].

Objectives

The objectives of the current study are to discuss the role of microRNAs and their biomarkers during pregnancy. It would also focus on identifying the role of microRNAs in pre-eclampsia development. The researcher would also evaluate the diagnostic potential of MicroRNAs Mi 517 and Mi 526 as biomarkers in the detection of hypertension and preeclampsia.

MATERIAL AND METHODS

Study design

Considering the need of the study, the researcher would be conducting a cohort study, wherein the researcher has considered two study groups, one group with normal pregnancy and another group having the risk of developing preeclampsia or who developed hypertension or preeclampsia during follow-up constitute the study group. This paper employs the STROBE guideline for reporting observational studies [15]. `

Participants

The study considered 53 patients who are in their first trimester, who were selected based on their availability and the stage of pregnancy of these patients. Herein, the study has considered only patients in their first trimester having a singleton pregnancy, which acted as an eligibility criterion for the selection of the participants. Among the chosen study group, 25 patients have been selected with normal pregnancies, who have been noted to not have risk factors associated with pre-eclampsia or who had not developed pre-eclampsia or hypertension at the time of follow-up. This section of the study group has been considered the control group for the study. Another 25 patients considered for the study comprised patients who had developed preeclampsia or hypertension at the time of follow-up or patients identified as having the risk factors of preeclampsia or hypertension during the follow-up. This section of the participants has been considered as the study group. The remaining three patients were excluded from the study analysis because of the absence of cDNA in the reverse transcription reaction, despite the various attempts. Four patients were further incorporated into the control group, which comprised a priori that they had no risk factors associated with developing preeclampsia or hypertension. Among these four patients, one patient had a stillbirth, and three patients developed gestational diabetes during pregnancy.

Sampling

For the purpose of determining the microRNA profile from participants of the study, sterile collection of 9.8 ml of peripheral venous blood (2 × 4.9 mL) has been performed into tubes with EDTA as an anticoagulant. Sampling has been performed at week 12 (first trimester).

Variables

Regarding the different variables associated with the study, the age of the patient has been identified as one of the variables. Herein, based on the age of the patients, the age group has been classified into two groups. One group comprised mothers below the age of 35 years and the other group comprised the advanced age of mothers who are of 35 years and above. Another variable is the risk of chromosomal aberrations, wherein, the study considered the risk of chromosomal aberrations: low risk > 1:1000, intermediate risk 1:300–1:1000; and high < 1:300. PAPP-A protein MoM is another variable considered for the study, for which, patients having PAPPA protein MoM less than 0.5–0.4 were identified to have elevated risk of developing preeclampsia.

Measurement

In order to collect data associated with circulating miRNAs in serum, blood samples have been collected from the participants of the study, which was the source of data for circulating miRNAs in serum. RNA isolation was the data source for collecting information associated with a fraction of short RNA fragments. RQ-PCR has been the data source for the miRNA expression profile, which provided information associated with 40 miRNAs that are associated with trophoblast. A group of miRNA molecules has been the data source for comparing statistically significant differences in expression levels between the control and study group.

Methodology for analyzing circulating miRNA's in serum

Blood samples have been collected from the participants under sterile conditions, followed by a 1-hour incubation period at room temperature. After collecting the blood samples, whole blood was centrifuged, for the purpose of separating separate serum from blood morphotic elements. Followed by collecting the serum which has been frozen at –80 degrees C. RNA isolation has been conducted with the help of a ready-made RNA isolation kit that is specifically for the fraction of short RNA fragments. With the help of light absorption at 260 nm in a spectrophotometer the concentration and purity of RNA have been checked. In order to reduce the risk of DNA contamination, for a duration of 30 minutes incubation with DNase at 37 degrees C has been performed. For determining the miRNA expression profile, the researcher has considered RQ-PCR based on Qiagen's dedicated kits. In this study, profiling of 40 miRNAs has been performed, the expression of which has been identified to be associated with the presence of trophoblastin in the pregnant population. After evaluating the results derived from

the sample patients who developed preeclampsia and comparing the results of the patients having physiologically normal pregnancies, a group of miRNA molecules has been selected, for which, the study has demonstrated statistically significant differences in expression levels. Furthermore, with the help of qRT-PCR the expression level of the selected miRNA molecules in each group of women have been rechecked.

Calculations according to the formula R= 2-ΔΔCT

For the purpose of determining the relative expression levels of individual miRNAs at the mRNA level, the ΔΔCt comparative method has been implemented in this study, much like the assessment of ABI-1 gene expression. In this context, in order to implement the ΔΔCt comparative method, the endogenous control miR-423-5p has been implemented. In this alignment, it has been assumed that the value; of $0.8 < R < 1.2$ is indicative of a normal amplification range (N). For the value of $R<0.8$ for the test group samples, it is indicative of a decrease in amplification (–). $R > 1.2$ for test group samples, it has been considered indicative of an increase in amplification (+). For $R = 0$ for test group samples, it implies no amplification (0) for the expression level of individual miRNAs in the group of patients.

Statistical analysis

Quantitative data are presented as a median with an interquartile range, while qualitative ones are presented as a number of cases with a percentage value. To compare quantitative variables Mann Whitney U test was used due to the nonnormal distribution of the data assessed by Shapiro-Wilk test. Only for the age variable T-student test was used due to its normal distribution. To compare qualitative variables, the Chi-square test was used for data with more than two categories, while dichotomic variables were analyzed with the use of the Fisher test. To assess usefulness of investigated miRNA as predictive factors, a general linear model (GLM) and logistic regression were applied. The predictive ability of the obtained model was analyzed by the ROC curve with cut off values derived from Youden index. Analysis was performed using RStudio software (Integrated Development for R. RStudio, PBC, Boston, MA, USA).

RESULTS

The total number of participants who remained for the study is 48. These 48 participants have been equally grouped for the study into two groups, that is, the control group comprised of 24 patients, and the study group comprised of 24 patients. The mentioned number of participants for both groups continued till the end of the study. All the participants in both groups were subjected to follow-up and the sampling was performed at week 12, which is their first trimester of pregnancy.

Descriptive data

The study comprised women aged in two categories, one group was below the age of 35 years and another group of women was above the above of 35 years. The patients considered for the study were in the first trimester and had a singleton pregnancy. In regard to the clinical conditions of the control group, the sample has not developed hypertension or pre-eclampsia during follow-up, and they were free from any risk factors for pre-eclampsia. In the case of the study group, the participants had developed hypertension or preeclampsia during follow-up or were having risk of developing preeclampsia. In the case of three patients in the control group, they had developed gestational diabetes.

Outcome data

Based on the observed findings from the present study, it has been noted that among all the factors of PAPP-A protein, hypothyroidism, diabetes mellitus, and gravidity, it is only the factor of gravidity among the patients, which has been noted to have a statistically significant association with developed hypertension or preeclampsia among the patients during their first trimester. The other mentioned aspects associated with the patients have been noted to have no statistically significant association with developed hypertension or preeclampsia. The aspect of parity also demonstrated a statistically significant association with hypertension or preeclampsia among the patients, wherein univariate analysis showed a significantly decreased risk associated with a plurality. Univariate analysis showed a significantly increased risk of the development of hypertension or preeclampsia associated with the presence of R517s. The univariate analysis also demonstrated a significantly increased risk associated with the presence of R526s.

Main results

It has been noted that there exist significant differences in miRNA 517ddct, miRNA 526ddct, and gestational age of delivery between the control and study group. These variables have been noted to be higher in the study group (Tab. 1).

Based on the collected samples from the participants of the study, the association of PAPP-A protein, hypothyroidism, diabetes mellitus, and gravidity with the development of hypertension or preeclampsia during their first trimester was analyzed. With the help of the analysis, it has been noted that, for none of the factors, apart from gravidity and parity the association with the development of hypertension or is statistically significant. On conducting univariate analysis, in context to parity, it has been noted that parity has a statistically significant association with hypertension or preeclampsia during the first trimester among patients, with reduced risk of developing the conditions with pluriparity odds ratio (OR) = 0.190; CI 0.04–0.81; p = 0.016]. This analysis demonstrated that gravidity significantly decreases the risk of developing hypertension or preeclampsia (OR = 0.050; CI 0.00–0.41; p = 0.001) (Tab. 2).

For R210s when the patients of both the control and the study group were compared it was noted that, for 14 patients in the control group the percentage derived is 58.33%, (–R210s), and for 7 patients it is 29.17% (+R210s), for the study group, among 8 patients it has been recorded to be 32.00% (–R210s), among 11 patients 44.00% (+R210s) based on which, it could be inferred that for R210s, no significant differences. For R517s as well, no significant differences were found between the control and the study group, wherein for 17 patients in the control group it has been recorded to be 70.83% (–R517s), for 1 patient 4.17% (+R517s) and when compared to study group it has been recorded to be 24.00% for 6 patients in the study group (–R517s), 40.00% for 10 patients in this group (+R517s), indicating no significant differences for R517s between the control and the study group, however, the percentage of patients having –R517s is significantly higher among the patients of the control group as compared to the study group, which is indicative of R517s have association with the development of hypertension or preeclampsia during the first trimester, which is statistically significant. The univariate analysis demonstrated a significantly increased risk of development of hypertension or preeclampsia associated with the presence of R517s (OR = 19.44; CI 2.20–957.22; p = 0.004). The univariate analysis showed also a significantly increased risk of developing hypertension or preeclampsia (OR = 9.28; CI 1.62–100.96; p = 0.004) associated with the presence of R526s.

Based on the univariate logistic regression model, increased expression of Mi 517 and 526 and parity status (primapara/multipara) has been obtained. The analysis shows that independent risk factors for hypertension or preeclampsia are the presence of an R527 and being a primipara (Tab. 4). The model characterizes by 82.4% sensitivity and 94.4% specificity (Fig. 1).

DISCUSSION

Based on the results from the current study, it has been observed that R517s and R526s act as major indicative biomarkers in the first trimester for the detection of hypertension and preeclampsia. Herein based on the univariate analysis it has been observed that the presence of R517s and R526s significantly increases the risk of hypertension and preeclampsia among the patients.

Limitations

The limited number of patients considered for the study can be regarded as one of the limitations of this study.

Interpretation

Based on the findings from the current study it can be seen to be aligned with the study conducted by Hromadnikovaet al. [13], wherein the researchers have stated that through the profiling of microRNAs in the serum of pregnant women, it is possible to link alterations in their concentration to the emergence of gestational problems. The researchers have stated in this context that observing statistical differences in serum microRNAs expression levels among pregnant women with healthy pregnancies and women pregnant with pre-eclampsia, enables selected, particular microRNAs to serve as safe clinical biomarkers of major complications at the time of the pregnancy. The previous findings have provided the observations that up-regulation of miR-517-5p,miR-520h, and miR-518b is related to the risk of later progression of preeclampsia. It has also been identified in the screening of extracellular miR-517-5p during the first trimester a significant section of women develop subsequent preeclampsia [14]. It has been concluded in that study that the presence of elevated first-trimester plasma levels of miR-517-5p can be considered as predictive of preeclampsia [13]. The findings of the current study can be

seen to be aligned with the mentioned findings wherein it has been noted that R517s act as a major indicative biomarker for the detection of hypertension and preeclampsia, wherein a similar finding has been reported that miR-517-5p biomarker alone has been observed by the researchers as a predictive performance for preeclampsia [13]. However, the finding of the present study on miRNA-210 can be seen to be contradicting with the study conducted by Jaszczuk et al. [16], wherein in the current study it has been noted that miRNA-210 is not statistically significant with the development of hypertension and preeclampsia. Currently, screening for preeclampsia is performed using biochemical tests, but the inclusion of new markers may lead to increased sensitivity and specificity of screening. The mRNA cannot be used to evaluate the entire population at present due to its expensive cost, but it may be applied to specific cases, such as high-risk. in addition, soon, we expect to reduce the cost of mRNA evaluation, after which it will be possible to use it in population screening. Therefore, research to reveal the mechanisms occurring is important to be able to use mRNA in further diagnostics.

CONCLUSIONS

In the conclusion, it can be stated that pregnant patients' use of biomarkers like C19MC MicroRNA would help define pregnancy monitoring and put therapies into place. The findings of the study revealed that independent risk factors for hypertension or preeclampsia are the presence of an R527s and being a primipara. In this work, the circulating C19MC MicroRNA was examined as a potential early indicator of preeclampsia and hypertension in pregnant individuals.

Ethics consideration

The study was approved by the Bioethics Board of Medical University of Lublin (KE-0254/107/2019) and complied with the Declaration of Helsinki and good clinical practice guidelines. All participants provided written informed consent.

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Kondracka A, Jaszczuk I, Koczkodaj D, et al. Blood serum microRNA profiles of pregnant women as biomarkers of pre-eclampsia evaluation. Journal of Pre-Clinical and Clinical Research. 2020; 14(4): 174–177, doi: 10.26444/jpccr/131597.

2. Schiesser M, Holzgreve W, Lapaire O, et al. Sirenomelia, the mermaid syndrome--detection in the first trimester. Prenat Diagn. 2003; 23(6): 493–495, doi: 10.1002/pd.624, indexed in Pubmed: 12813764.

3. Park HJ, Shim SS, Cha DH. Combined Screening for Early Detection of Pre-Eclampsia. Int J Mol Sci. 2015; 16(8): 17952–17974, doi: 10.3390/ijms160817952, indexed in Pubmed: 26247944.

4. Leslie MS, Briggs LA. Preeclampsia and the Risk of Future Vascular Disease and Mortality: A Review. J Midwifery Womens Health. 2016; 61(3): 315–324, doi: 10.1111/jmwh.12469, indexed in Pubmed: 27155218.

5. Duley L. Pre-eclampsia and the hypertensive disorders of pregnancy. Br Med Bull. 2003; 67: 161–176, doi: 10.1093/bmb/ldg005, indexed in Pubmed: 14711762.

6. Tan MY, Syngelaki A, Poon LC, et al. Screening for pre-eclampsia by maternal factors and biomarkers at 11-13 weeks' gestation. Ultrasound Obstet Gynecol. 2018; 52(2): 186–195, doi: 10.1002/uog.19112.

7. Vickers NJ. Animal Communication: When I'm Calling You, Will You Answer Too? Curr Biol. 2017; 27(14): R713–R715, doi: 10.1016/j.cub.2017.05.064, indexed in Pubmed: 28743020.

8. Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. PLoS One. 2008; 3(9): e3148, doi: 10.1371/journal.pone.0003148, indexed in Pubmed: 18773077.

9. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. Crit Rev Oncol Hematol. 2011; 80(2): 193–208, doi: 10.1016/j.critrevonc.2010.11.004, indexed in Pubmed: 21145252.

10. Zhao G, Zhou X, Chen S, et al. Differential expression of microRNAs in decidua-derived mesenchymal stem cells from patients with pre-eclampsia. J Biomed Sci. 2014; 21(1): 81, doi: 10.1186/s12929-014-0081-3, indexed in Pubmed: 25135655.

11. Pantham P, Soncin F, Zhang-Rutledge K, et al. Innovations in Placental Pathology. Benirschke's Pathology of the Human Placenta. 2021: 837–867, doi: 10.1007/978-3-030-84725-8_31.

12. Martínez-Ibarra A, Martínez-Razo LD, Vázquez-Martínez ER, et al. Unhealthy Levels of Phthalates and Bisphenol A in Mexican Pregnant Women with Gestational Diabetes and

Its Association to Altered Expression of miRNAs Involved with Metabolic Disease. Int J Mol Sci. 2019; 20(13), doi: 10.3390/ijms20133343, indexed in Pubmed: 31284700.

13. Hromadnikova I, Kotlabova K, Ivankova K, et al. First trimester screening of circulating C19MC microRNAs and the evaluation of their potential to predict the onset of preeclampsia and IUGR. PLoS One. 2017; 12(2): e0171756, doi: 10.1371/journal.pone.0171756, indexed in Pubmed: 28182660.

14. Bounds KR, Chiasson VL, Pan LuJ, et al. MicroRNAs: New Players in the Pathobiology of Preeclampsia. Front Cardiovasc Med. 2017; 4: 60, doi: 10.3389/fcvm.2017.00060, indexed in Pubmed: 28993808.

15. von Elm E, Altman DG, Egger M, et al. STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008; 61(4): 344–349, doi: 10.1016/j.jclinepi.2007.11.008, indexed in Pubmed: 18313558.

16. Jaszczuk I, Koczkodaj D, Kondracka A, et al. The role of miRNA-210 in pre-eclampsia development. Ann Med. 2022; 54(1): 1350–1356, doi: 10.1080/07853890.2022.2071459, indexed in Pubmed: 35543206.

Table 1. Characteristics of the study group

	Control group			**Study group**			
	Median	**q1**	**q3**	**Media n**	**q1**	**q3**	**p**
miRNA 210ddct	0.79	0.577	1.32	1.19	0.66	2.23	0.13
miRNA 517ddct	0.158	0.128	0.284	2.14	0.603	3.36	**0.00**
miRNA 526ddct	0.178	0.093	0.42	1.3	0.465	3.94	**0.00**
bHCG_[MoM]	1.02	0.921	1.82	1.03	0.75	1.82	0.60
BMI	23.2	20.8	25.9	22.7	21	25.2	0.68
Gestational age of delivery [weeks]	38	38	39	39	38	40	**0.03**
Mean miRNA 210	33.2	32.8	34	32.6	32	33.7	0.19
Mean miRNA 517	34.7	33.7	35.9	35.3	20.3	36.1	0.73
Mean miRNA 526	35.1	33.8	36.4	35.4	33.7	36.5	0.91
Mean C425	27.6	26.9	28.5	27.4	26.7	29.3	0.98
PAPP-A_[MoM]	1.01	0.716	1.54	0.924	0.668	1.52	0.54
Age [years]	34.5	32.8	36.2	33	29	36	0.21

BMI — body mass index

Table 2. Univariate analysis of risk factors of hypertension or preeclampsia

	Control group		**Study group**					
	n	**%**	**n**	**%**	**OR**	**95% CI**		**p**
Hypothyroidism								
0	14	58.33	17	68.00				
1	10	41.67	8	32.00	0.66	0.17	2.46	0.561
Diabetes mellitus								
0	21	87.50	21	84.00				
1	3	12.50	4	16.00	1.33	0.20	10.18	0.100
Gravidity								
1	1	4.17	12	48.00				
> 1	23	95.83	13	52.00	0.05	0.00	0.41	**0.001**
Parity								
1	4	16.67	13	52.00				
> 1	20	83.33	12	48.00	0.19	0.04	0.81	**0.016**
Hypertension								
0	24	100.00	23	92.00				
1	0	0.00	2	8.00	–	–	–	0.490
Premature								
0	23	95.83	22	88.00				
1	1	4.17	3	12.00	3.067	0.23	171.51	0.609
Stillbirth								
0	23	95.83	25	100.00				
1	1	4.17	0	0.00	–	–	–	–
Age group								
0	12	50.00	15	60.00				
1	12	50.00	10	40.00	0.672	0.19	2.38	0.571

OR — odds ratio; CI — confidence interval

Table 3. Univariate analysis of risk factors of hypertension or preeclampsia

	Control group		Study group		OR	95% CI		p
	n	%	n	%				
R210s								
–	14	58.33	8	32.00	0.344	0.09	1.25	0.088
+	7	29.17	11	44.00	1.883	0.51	7.43	0.378
0	3	12.50	6	24.00				
R517s								
–	17	70.83	6	24.00	0.088	0.01	0.47	**0.001**
+	1	4.17	10	40.00	19.44	2.20	957.22	**0.001**
0	2	8.33	3	12.00				
R526s								
–	17	70.83	9	36.00	0.120	0.02	0.59	**0.004**
+	2	8.33	12	48.00	9.288	1.62	100.96	**0.004**

0	1	4.17	2	8.00				

OR — odds ratio; CI — confidence interval

Table 4. Multivariate model of risk factors of hypertension or preeclampsia

	estimate	**aOR**	**95% CI**		**p**
(Intercept)	0.6620	1.94	1.52	2.47	**0.001**
primapara	0.5340	1.71	2.21	1.32	**0.001**
R526s_+	0.4327	1.54	1.18	2.01	**0.003**
R517s_+	0.1487	1.16	0.86	1.57	0.339

CI — confidence interval

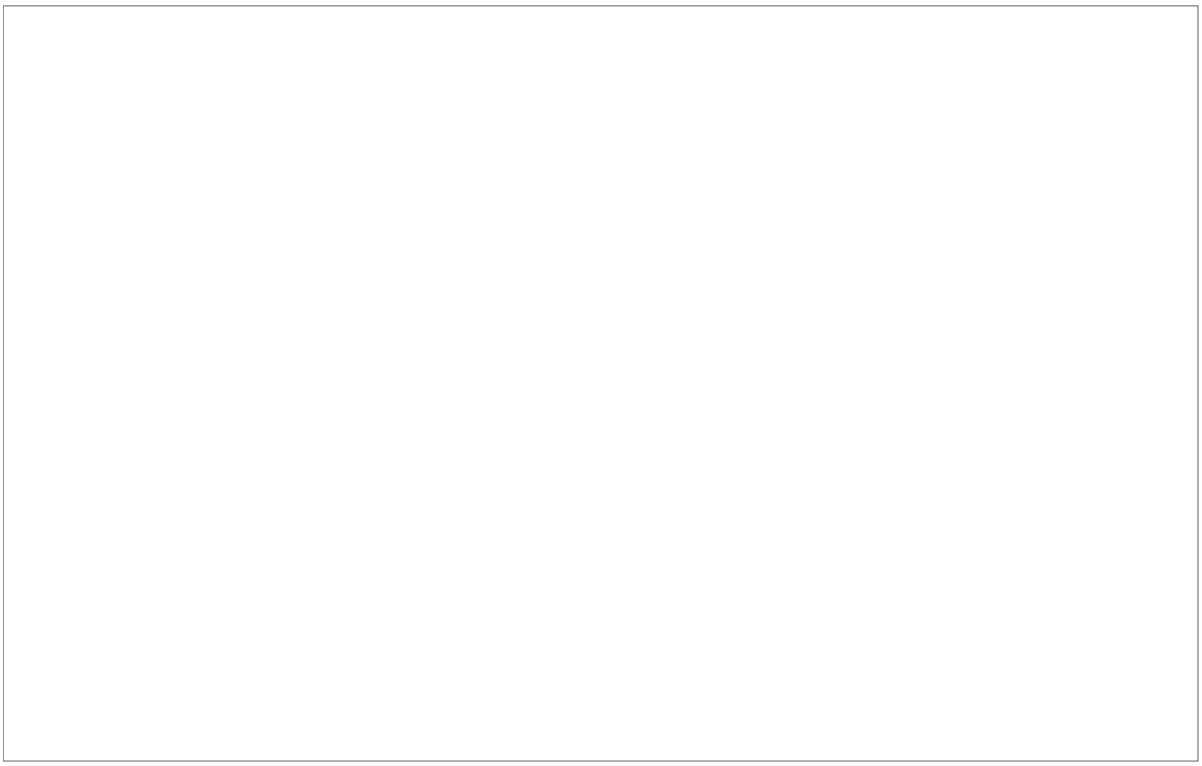

Figure 1. Receiver operating characteristic (ROC) curve for the multivariate model; AUC — area under the curve