

# Expression of microRNA (miR126\*, miR155, miR21, miR29a) in breast milk cell fraction in women with hypertension: a comparative analysis with women without hypertension

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## ABSTRACT

**Objectives:** The ideal option of food for a newborn's nourishment has traditionally been human breast milk (HBM). Previous studies have demonstrated a connection between the length of exclusively breastfeeding and its preventive effects on several conditions in neonates. Considering the significance of HBM, the study aimed at detecting the expression of microRNA (miR126\*, miR155, miR21, and miR29a) in the breast milk cell fraction of women with hypertension. This was a cohort study of 35 postpartum women.

**Material and methods:** Five ml of milk was collected into a sterile container from patients in the morning on the second and third days after the labor. The collected milk has been centrifuged, total cellular RNA has been isolated from cell fraction from the collected milk, isolated RNA has been subject to qualitative and quantitative analysis, next reverse transcription has been conducted, followed by that, evaluation of the expression of the selected microRNA has been conducted using the synthesized cDNA. Finally, the tested microRNA's relative expression level has been calculated.

**Results:** Among patients with hypertension, the analysis of cell fraction of breast milk reported lower mean expression of miR126\*, miR155, miR21, and miR29a as compared to patients without hypertension. Strong and very strong positive correlation between the expression of miR126\* and miR155, miR126\* and miR21, miR155 and miR21, miR 155 and miR29a, and miR 21 and miR29a have been noted.

**Conclusions:** Comparing patients with and without hypertension, it has been noted that patients with hypertension had lower mean expression of miR126\*, miR155, miR21, and miR29a.

**Keywords:** microRNAs; breast feeding; mil; human; hypertension

Ginekologia Polska 2024; 95, 1: 52–61

## INTRODUCTION

Human breast milk (HBM) has always been regarded as the best choice of food for the nutrition of newborns [1]. The process of feeding a young child, specifically under the age of two years, directly from the breast of a woman at the time of lactation is considered breastfeeding. It has been

reported by World Health Organization (WHO) that feeding a newborn with the own milk of the mother during the first six months without any additional external supplements provides the optimum nutrition for the infants, which provides them with the benefits associated with health outcomes and immunity [2]. It has also been recommended

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Received: 14.06.2023 Accepted: 24.06.2023 Early publication date: 19.07.2023

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in this context that children continue breastfeeding in addition to supplemental food till the age of two years. Studies have revealed that there is an association between exclusive breastfeeding duration and associated protective impacts against various diseases among newborns, for instance, type 1 diabetes (T1D), neurocognitive behavior changes and intelligence, malocclusions, cardiorespiratory disorders, pediatric sleep-disordered breathing, among others. Various studies have been conducted on understanding how HBM provides the mentioned protective effects. It has been revealed in these studies that HBM is a complex integration of various bioactive molecules and the contribution of all the components is not yet clear [3]. The presence of miRNAs in HBM was only not identified until 2010 [4]. It was suspected by the researchers that the molecules play a role in the regulation of significant aspects of the development of infants for instance the immune function. Considering the mentioned role of the molecules in HBM, it was also hypothesized that miRNAs can be incorporated in infant formula for ascertaining that infants receive the required benefits and do not miss out on the associated health benefits. Studies have also put forward the fact that HBM is highly rich in circulating RNA molecules, unlike other body fluids. In recent years studies have identified a wide range of circulating miRNAs in HBM, approximately 1400 miRNAs have been identified in HBM [5]. Based on the above-discussed aspects, it can be inferred that the studies on the expression of microRNA are at a considerably elementary stage. In addition to that, no major studies have been conducted on the impact of "Pregnancy-induced hypertension, or peripartum hypertension or hypertensive disorders of pregnancy" on the expression of microRNA in HBM, which affects 15% of women [6]. The reason for considering the expression of microRNA (miR126\*, miR155, miR21, miR29a) in the current study is because of the metabolic significance that these microRNAs hold in the functioning of the human body. MiR 126 has been identified to play a pivotal role in the regulation of the metabolism of blood glucose and the development of T2DM. Similarly, MiR-21 has been identified to be associated with the metabolic alteration of cancer-associated fibroblasts (CAFs) along with the development of cancer cells. MiR-155, under usual physiological conditions has been identified to be associated with maintaining standard glucose levels through its association with the regulation of insulin sensitivity and blood glucose homeostasis. MiR-29 family is associated with altered substrate oxidation and insulin resistance and overexpression of miR-29a in adipocytes has been reported to cause insulin resistance. Thus, considering the above-mentioned aspects, the focus of the current study would be on identifying the expression of microRNA (miR126\*, miR155, miR21, miR29a) in breast milk cell fraction in women with hypertension.

The most prevalent class of extremely short regulatory non-coding RNA molecules, miRNAs, comprises 20 to 24 nucleotides and can regulate 40% to 60% of post-transcriptional gene expression [7, 8]. The miRNAs can be created endogenously, supplied exogenously by neighboring cells as cell-cell communication, provided from foods like plants and human HBM as cell-free miRNAs, or given via milk exosome. All milk fractions, including lipids, cells, and skim, have been discovered to contain greater levels of miRNAs than other bodily fluids, such as plasma. While skim milk contains the lowest concentration and diversity of miRNAs, milk cells have the highest concentration and variety [9]. According to the study conducted by Alsaweed et al. [10], in milk cells, there be 1467 known miRNAs, 1996 unique miRNAs, and 308 miRNAs have been detected in milk lipids. MiRNAs extracted from HBM fat globules are affected by a maternal high-fat diet, and these miRNAs can alter the metabolic pathways in babies who are given HBM. Because HBM has the greatest concentration of miRNAs (47,240 g/L compared to 308 g/L in plasma and 94 g/L in urine), which is due to the presence of stem cells and miRNAs produced from HBM exosomes, the miRNA is an essential component of HBM [11].

According to another study conducted by Alsaweed et al. [12], it has been observed that HBM miRNA expression varies according to the stage of lactation, as seen by the significant decline in miR-181a and miR-155 expression levels after six months of breastfeeding. The total miRNA concentration in the proportion of colostrum whey was 87.78 ng/L in the study conducted by Xi et al. [13], which included 33 matched samples, wherein it was reported to be considerably higher than the total miRNA concentration in the proportion of mature milk whey (33.15 ng/L) (445–449). Colostrum (4.64, 4.05, and 2.58, respectively) and mature milk both had high expression levels of miRNA-378, miRNA-30B, and Let-7a (3.62, 4.92, and 2.39, respectively). While levels of miRNA-30B in mature milk were greater as compared to colostrum, levels of miRNA-378 and let-7a dramatically reduced with lactation. The difference in miRNA composition between pre- and post-feeding results from the change in milk composition during breastfeeding (such as an increase in the cells and fat content), with post-feeding showing a high content and composition of miRNAs, suggesting that breastfeeding increases the content of miRNAs in HBM. More miRNAs are found in the fat and milk cells [13]. Associated with cell turnover during breast sucking, epithelial cell migration into milk channels, and the process of milk production, those components are enhanced in post-feeding. Elevated miRNAs associated with milk fat are substantially connected with newborn milk volume consumption, in contrast to miRNAs related to cell content.

A distinctive profile of HBM miRNA with adaptive metabolic targets and roles for growth in premature newborns was reported in premature infant delivery. Given that they have different dietary requirements than fully developed newborns, premature infants may experience a variety of physiological difficulties. 113 miRNAs' expression in skim and lipid samples from mothers of preterm infants (pMBM) and term infants (tMBM) have demonstrated some noticeable variances [14]. MiRNA expression is changed during pregnancy by a high-fat diet. According to target pathway analysis, changes in food consumption-related miRNA expression may have an impact on either mothers' or babies' metabolic processes [15]. High glucose and galactose diets showed no discernible impact on the miRNA species found in the mother's milk.

In addition to the above-mentioned aspects of miRNAs expression in breast milk, in various studies, miRNAs have also been reported to play a significant role in regulating endothelial function, which is an integral aspect of hypertension. In the study conducted by [16], the researchers opined that miR-126 is endothelial cell-enriched. In the study, circulating miR-126 was measured in rats with NTN [nephrotoxic nephritis] and among humans with acute endothelial and renal injury. The findings have been compared with patients with CKD (chronic kidney disease) and ESRD (end-stage renal disease) wherein the association between miR-126 and vascular dysfunction has been studied. The findings of the study revealed that in the case of NTN miR-126 was reported to be reduced. In the case of ANCA vasculitis, the findings revealed that pre-treatment miR-126 was reduced compared to health. A 3.4-fold increase in miR-126 was recorded post-treatment. The researchers concluded that between CKD and health, miR-126 did not differ, however, with endothelial dysfunction the concentration of miR-126 has been reported to be correlated. In ESRD miR-126 has been observed to be reduced ~350 fold and the researchers believe that for vascular inflammation miR-126 can be considered a marker. In this alignment, in the study conducted by [17], the findings of the study demonstrated high-density lipoprotein (HDL) when isolated from CHF [chronic heart failure] patients reduce the expression of pro-angiogenic miRs, for instance, miR-21 and miR-126, which the researchers considered to be contributing factor to endothelial dysfunction and atherogenesis. The study conducted by [18] which focused on understanding the role of miR-19b as an influencing factor in Atherosclerosis (AS) revealed that the inflammation associated with the condition is prevented by HDAC3 upregulation for inhibiting it by inactivating NF- $\kappa$ B/p65 through upregulation of miR-19b which is mediated by PPAR $\gamma$ .

## Objectives

The study aims to analyze the expression of microRNA (miR126\*, miR155, miR21, miR29a) in breast milk cell fraction in women with hypertension and compare the findings with women without hypertension.

## MATERIAL AND METHODS

### Study design

This was a cohort study. The study group comprised of two groups, one group comprised of patients with hypertension, and the second group comprised of patients without hypertension. 5 mL of milk was collected into a sterile container from patients in the morning (up to two hours after the meal) on the second and third day after the labour. The samples were collected from June to October 2022. The collected milk has been centrifuged, total cellular RNA has been isolated from cell fraction from the collected milk, isolated RNA has been subject to qualitative and quantitative analysis, next reverse transcription has been conducted, followed by that, evaluation of the expression of the selected microRNA has been conducted using the synthesized cDNA. Finally, the tested microRNA's relative expression level has been calculated.

### Setting

The milk samples collected from patients hospitalized in The Department of Obstetrics and Pathology of Pregnancy SPSK no.1 in Lublin were transported to the Department of Clinical Genetics, Medical University of Lublin. Directly after the collection of the samples, they were transformed to the university, where further analyses were conducted.

### Participants

In this study, a total of 35 postpartum patients have been considered. The participants selected for the study are within the age range of 22–43 years. The selected patients were divided into two groups based on the presence of gestational hypertension in patients. 29 out of 35 patients were without hypertension which formed one group of the participants, while only 6 out of 35 patients qualified for the group with hypertension (the patients were taking medications for hypertension; hypertension in these patients occurred between 18 and 36 weeks of gestation, with a mean of 29 weeks of gestation). One of the mentionable inclusion criteria for the participants for the study was patients free of addiction, who were considered eligible for the trial group. The adaption was effective since all the patients who were looked at gave birth to healthy babies who obtained an Apgar score of 10/10. Additionally, some individuals with hypertension also had hypothyroidism.

### Variables

The variables for the study were the status of gestational hypertension among the patients [that is the presence or absence of the condition], the mode of delivery [cesarean section (CS) vs normal delivery (ND)], the number of pregnancies, previous miscarriages, the age of the patient, week of gestation at delivery, the weight of the newborn and gender of the newborn.

### Data sources/Measurement

Milk samples were collected from the participants to collect data associated between the expression of microRNA in HBM and the impact of hypertension on it. To collect data related to the relative expression level of the tested microRNAs, the Livak method was used for the analysis of relative gene expression. The approach assumes that both the target and reference genes are amplified with rates close to 100% and within 5% of one another.

### Study size

The sample size selected for the study is based on clinical parameters, which include hypothyroidism in pregnancy, number of pregnancies, gender of newborn, previous miscarriages, and the mode of delivery. In addition to that, to calculate the sample size for the study, the researcher has used a sample size calculator, wherein the margin of error has been considered to be 95%, with a 5% margin of error. Considering the mentioned parameters, the researcher selected the sample size for the current study.

### Methodology for analyzing miRNAs in breast milk

Using an 805 g speed and 15°C temperature, 5 mL of collected milk has been centrifuged for 20 minutes (5810R Eppendorf centrifuge). The cell fraction has been washed using double centrifugation and buffered saline after centrifugation, with the supernatant and fat phase being collected. The whole cellular RNA has been extracted from the cell fraction using the miRVana™ miRNA isolation Kit (Invitrogen by ThermoFisherScientific, Vilnius, Lithuania). The procedure for the isolation followed the manufacturer's instructions for the reagent. ThermoScientific's NanoDrop 2000c and an electrophoretic technique were used to perform qualitative and quantitative analysis on isolated RNA. In the following step, reverse transcription has been conducted using commercial reagent kits (High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor and TaqMan MicroRNA Reverse Transcription Kit, AppliedBiosystems by ThermoFisherScientificVilnius, Lithuania). Ready-to use primer kits Human Pool A and B (AppliedBiosystems by ThermoFisher Scientific, CA, USA), Megaplex™ RT Primers have been used in the process, wherein the amount of RNA

recommended by the manufacturer has been used. In the following step, to evaluate the expression of the chosen microRNA synthesized cDNA has been used, wherein the qPCR method has been implemented. In the StepOnePlus system (AppliedBiosystems) the reaction has been conducted, in which, commercial reagents including TaqMan probes (TaqMan MicroRNA Assays, Applied Biosystems by ThermoFisherScientific, CA, USA: hsa-miR-126\* (Assay ID: 000451); hsa-miR-155 (Assay ID: 002623); hsa-miR-21 (Assay ID: 000397); has-miR-29a (Assay ID: 002112) have been used. In this regard, an endogenous control 18S (Hs99999901\_s1) (Applied Biosystems by ThermoFisherScientific, CA, USA) has been used. By the recommendation of the manufacturer, the volume of the reagents used and the reaction protocol along with the test material, have been maintained. To calculate the relative expression level of the tested microRNAs the Livak method has been used in the study, for which, ExpressionSuiteSoftware Version 1.3 (LifeTechnologies) software has been expressed in the form of RQ value.

### Statistical analysis

Statistica v.13 was used to statistically analyze the findings that were collected. Mann-Whitney the U Test was employed to evaluate the variations among the research subgroups. To determine and analyze the correlation between the expression of miR126\* and miR155; miR126\* and miR21, miR 126\* and miR 29a, miR155 and miR21, miR 155 and miR29a, and miR 21 and miR29a Spearman rank correlation coefficient of the tested microRNAs in milk cells have been conducted. The significance level was  $p < 0.05$ .

### Ethical consideration

The study was conducted based on the approval of the Bioethics Committee at the Medical University of Lublin (No. KE-0254/88/04/2022). Each patient provided written consent to collect the material and conduct tests.

## RESULTS

### Characteristics of the study group

The study comprised a total of 35 participants who were postpartum patients. The participants were within the age range of 22–43 years. 6 out of the 35 participants were having hypertension and taking drugs for hypertension; hypertension in these patients occurred between 18 and 36 weeks of gestation, with a mean of 29 weeks of gestation. The other group of participants which included the remaining 29 patients was not suffering from hypertension. For the group of patients with no gestational hypertension, the mean age of the patients, week of pregnancy (hbd), and weight of the newborn were 31.41, 39.27, and 3402.00 respectively, while for the group with gestational hypertension has been

recorded to be 30.667, 36.667, and 2820.00 respectively. The weight of newborns, among the group of patients with gestational hypertension, is significantly low as compared to the group without gestational hypertension. Furthermore, in regards to the mode of delivery [cesarean section (CS) vs normal delivery (ND)], it can be noted that among the group with gestational hypertension, the percentage of patients is significantly higher as compared to the group of patients with no gestational hypertension, which has been recorded to be 66.7% and 44.8% respectively. When both the subgroups are compared based on the clinical parameter of miscarriages, it can be observed that among the group with no gestational hypertension, the percentage of respondents having miscarriages is significantly less when compared to the group with gestational hyperten-

sion, which has been recorded to be 20.7% and as high as 50%, respectively. Regarding the clinical parameter of the number of pregnancies, it has been noted that for both subgroups, the majority of the respondents had two or three pregnancies. In context to the mentioned parameter, for the sub-group of patients with no gestational hypertension, for two pregnancies, it is slightly high as compared to the sub-group of patients with gestational hypertension, which has been recorded to be 38% and 33.3% respectively. For the clinical parameter of hypothyroidism in pregnancy, it has been noted that among the sub-group of patients with gestational hypertension, as high as 50% of the respondents had hypothyroidism, which was significantly low for the group of patients with no gestational hypertension, which has been recorded to be as low as 13.8% (Tab. 1 and 2).

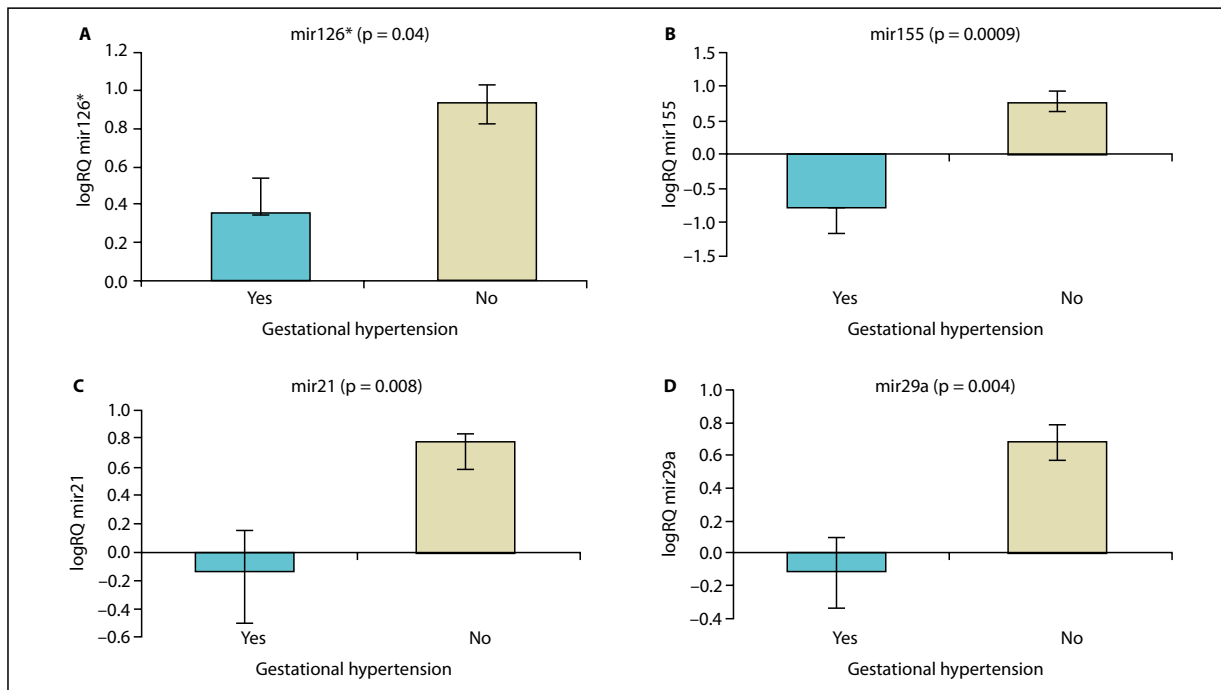
**Table 1.** The characteristics of the study group based on the patient's age, week of gestation at delivery, and the weight of the newborn

Group of patients	Parameter	n	Mean	Median	Minimum	Maximum	SD
No gestational hypertension	Patient's age [years]	29	31.414	31.000	22.000	43.000	4.9463
	hbd		39.273	39.500	35.000	42.000	1.5486
	Weight of the newborn [g]		3402.000	3410.000	2530.000	4050.000	354.8356
With gestational hypertension	Patient's age [years]	6	30.667	31.000	28.000	33.000	1.862
	hbd		36.667	37.500	31.000	40.000	3.0768
	Weight of the newborn [g]		2820.000	2920.000	1150.000	3850.000	937.1873

SD — standard deviation; hbd — week of pregnancy

**Table 2.** The characteristics of the study group based on the mode of delivery, history of miscarriages, number of pregnancies, hypothyroidism in pregnancy, and gender of the newborn

Parameter	Group of patients	
	With gestational hypertension, n = 6	No gestational hypertension, n = 29
<b>Delivery</b>		
Cesarean section	4 (66.7%)	16 (44.8%)
Normal delivery	2 (33.3%)	13 (55.2%)
<b>Miscarriages</b>		
Yes	3 (50%)	6 (20.7%)
No	3 (50%)	23 (79.3%)
<b>Number of pregnancies</b>		
1	1 (16.7%)	9 (31%)
2	2 (33.3%)	11 (38%)
3	2 (33.3%)	8 (27.7%)
4	1 (16.7%)	1 (0.3%)
<b>Hypothyroidism in pregnancy</b>		
Yes	3 (50%)	4 (13.8%)
No	3 (50%)	25 (86.3%)
<b>Gender of newborn</b>		
Male	3 (50%)	15 (52%)
Female	3 (50%)	14 (48%)



**Figure 1.** The mean expression level of miR126\* (A), miR155 (B), miR21 (C), and miR29a (D) (logRQ ± SE) in breast milk cell fraction depends on the presence of gestational hypertension. Mann-Whitney U Test

### Main results

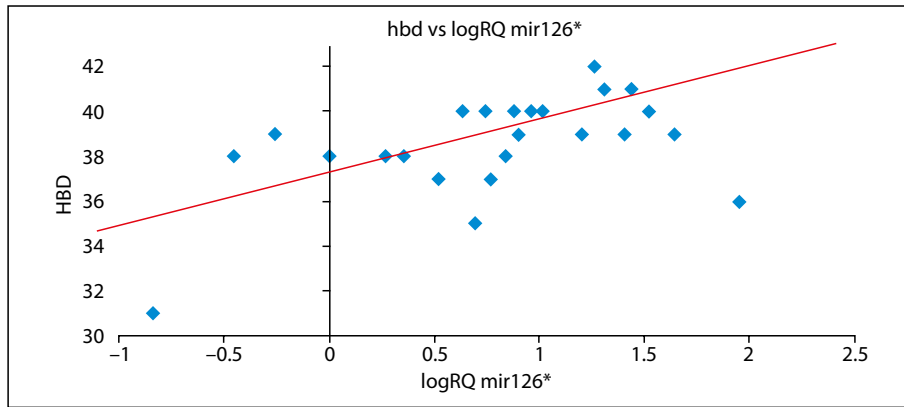
The results of the experiments demonstrated that the miR126, miR155, miR21, and miR 29a genes are expressed in the cell fraction of the breast milk that was collected on the second and third day after delivery. In patients with gestational hypertension, the expression of the tested microRNAs was significantly different from the expression in patients without hypertension, according to a statistical analysis of the obtained expression results of the tested microRNA along with clinical data regarding the patients, such as the course of gestation and delivery, and data regarding the newborn. The mean expression of miR126\* was reported to be 3 times lower ( $p = 0.04$ ) in the cell fraction of breast milk from patients with hypertension, 10 times lower ( $p = 0.0009$ ) in the cell fraction of miR155, 4 times lower ( $p = 0.008$ ) in the cell fraction of miR21, and 7 times lower ( $p = 0.004$ ) in the cell fraction of miR29, compared to patients without hypertension (Fig. 1).

No significant correlations between the expression level of miR126\*, miR155, miR21, and miR29a in breast milk cell fraction and the mode of delivery (CC vs ND), the number of pregnancies, prior miscarriages, the age of the patient, week of gestation at delivery, the weight of the newborn, and gender of the newborn were found after statistical analysis of the obtained results of the expression of microRNAs along with the available clinical data.

Based on the analysis of the Spearman rank correlation coefficient of the tested microRNAs in milk cells and the selected clinical characteristics in the whole study group ( $n = 36$ ) it has been noted that there is a significantly strong and positive correlation between the expression of miR126\* and miR155 ( $r = 0.775$ ,  $p < 0.05$ ); miR126\* and miR21 ( $r = 0.776$ ,  $p < 0.05$ ), miR126\* and miR29a ( $r = 0.775$ ,  $p < 0.05$ ), miR155 and miR21 ( $r = 0.938$ ,  $p < 0.05$ ), miR155 and miR29a ( $r = 0.947$ ,  $p < 0.05$ ), miR 21 and miR29a ( $r = 0.955$ ,  $p < 0.05$ ) (Tab. 1). It has been further noted in this regard that there exists a moderate positive correlation between miR126\* expression and milk cells and week of gestation at delivery ( $r = 0.501$ ,  $p < 0.05$ ) (Fig. 2, Tab. 3) and a weak positive correlation between the expression of miR155 in milk cells and the weight of the newborn ( $r = 0.358$ ,  $p < 0.05$ ) (Fig. 3, Tab. 3). On conducting a similar analysis in the group of patients with hypertension ( $n=6$ ) it has been noted that there are no significant correlations.

### Outcome data

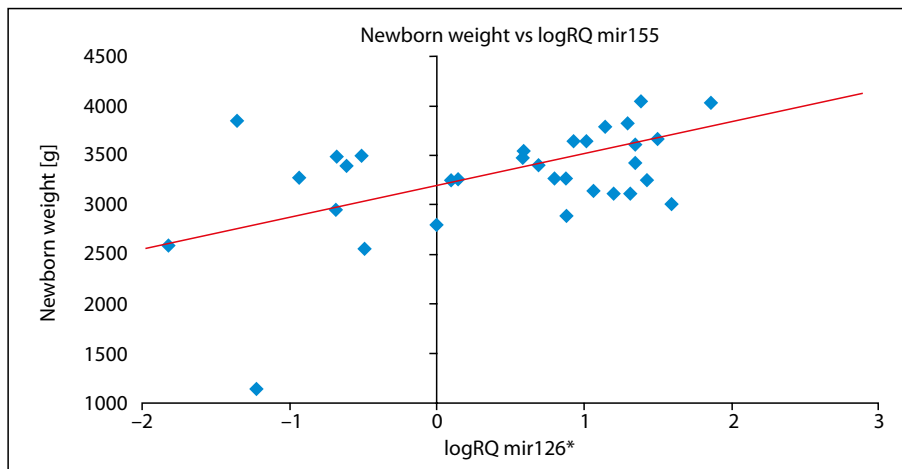
Based on the findings from the study, it can be inferred that lower mean expression of miR126\*, miR155, miR21, and miR29a among patients with hypertension can be observed when compared to patients without hypertension. Strong and very strong positive correlation between the expression of miR126\* and miR155, miR126\* and miR21, miR155 and miR21, miR155 and miR29a, and miR21 and miR29a have been observed in this study.



**Figure 2.** Scatter plot between the week of gestation at delivery and the expression of miR126\* in breast milk cell fraction ( $r = 0.501$   $p < 0.05$ ). Spearman rank correlation; hbd — week of pregnancy

Parameter	logRQ miR126*	logRQ miR155	logRQ miR21	logRQ miR29a
logRQ miR126*	1.000	0.775*	0.776*	0.775*
logRQ miR155	0.775*	1.000	0.938*	0.947*
logRQ miR21	0.776*	0.938*	1.000	0.955*
logRQ miR29a	0.775*	0.947*	0.955*	1.000
Number of pregnancies	-0.035	-0.082	0.035	0.022
hbd	0.501*	0.318	0.345	0.308
Patient's age	-0.213	-0.188	-0.173	-0.175
newborn weight	0.269	0.358*	0.326	0.300

\* $p < 0.05$ ; hbd — week of pregnancy



**Figure 3.** Scatter plot between the newborn weight and expression of miR155 in breast milk cell fraction ( $r = 0.358$   $p < 0.05$ ). Spearman rank correlation

## DISCUSSION

### Interpretation of the literature

In the previous literature that has been considered for reviewing no major studies could be found that fo-

cused on analyzing the association between expressions of microRNA in breast milk cell fraction in women with hypertension. However, in endothelial dysfunction which plays an integral role in hypertension in various considered

studies in this paper, the significant role of miRNAs in regulating endothelial function has been identified. The study conducted by [19], which focused on determining the expression profiles of serum microRNAs significance in the function of ECs [Endothelial cells] revealed that miR-221-3p and miR-222-3p demonstrates a decreasing expression trend between the subclinical hypothyroidism (SCH) + + atherosclerosis (ATH) groups and the SCH group. For miR-126-3p and miR-150-5p a stepwise decrease was recorded from the normal control (NC) subjects to SCH groups, and SCH + ATH or ATH group. In the SCH, SCH + ATH, and ATH groups, miR-21-5p upregulation was recorded. Increased levels of miR-21-5p in the SCH + ATH group were recorded, which was observed to be higher as compared to SCH and ATH groups. The researchers, based on the findings concluded in the study that miR-21-5p can be associated with the atherosclerosis process among SCH patients and miR-150-5p can be considered to be sensitive risk markers to predict endothelial dysfunction in patients with ATH. According to the study conducted by [20], the findings of the researchers opined that human umbilical vein endothelial cells (HUVECs) highly expressed miR-155 which might co-target AT1R and Ets-1 and miR-221/222 targets Ets-1, which regulate the expression of various inflammatory molecules of ECs indirectly. In the study conducted by [20], the findings of the study demonstrated that among heart failure with preserved ejection fraction (HFpEF) patients, as compared to healthy controls miR-126, miR-342-3p, and miR-638 were significantly downregulated whereas miR-21 and miR-92 were observed to be upregulated. Followed by 3-month treatment with empagliflozin, among HFpEF patients, significant reduction in miR-21 and miR-92 was recorded. In the case of patients treated with metformin or insulin no major differences in the profile of endothelial miRs. The findings of the study [21] demonstrated circulating miRs associated with the regulation of endothelial function are highly regulated in frail HFpEF patients with diabetes mellitus as response to SGLT2 inhibition, indicating the association of novel microRNA signature with the regulation of endothelial function that is significantly regulated in patients with diabetes and HFpEF.

In addition to that, in recent studies, it has been revealed that HBM has many interconnected defensive elements that act as an "innate immune response" to defend against viruses [22]. Whey and casein are the two primary protein subgroups that have been identified to be present in HBM. These two groups are present in early and late lactation, respectively, with ratios changing from 70/30 to 80/20 and 50/50 [23]. Infants are protected against bacterial infections by lactoferrin, one of the primary proteins in the whey family. However, "cathelicidin-derived antimicrobial peptides",

"folate-binding protein", and "α-lactalbumin" are some of the other proteins present in HBM. In the stomach, the main protein in HBM, "α-lactalbumin", is transformed into "human α-lactalbumin rendered deadly to tumor cells" (HAMLET). [23] HBM cells generate antimicrobial peptides derived from cathelicidin. They provide maternal protection against the risks associated with infection, BC, and allergy and newborn protection against autoimmune illnesses. Additionally, HBM controls inflammation by inhibiting the interleukins that regulate the production of "proinflammatory mediators" such as cytokine genes for instance IL-8 gene.

The previous studies have revealed that mature milk (3.62, 4.92, and 2.39, respectively) and colostrum (4.64, 4.05, and 2.58, respectively) both displayed high expression levels of miRNA-378, miRNA-30B, and Let-7a. While miRNA-30B levels in mature milk were higher than in colostrum, miRNA-378 and let-7a levels sharply decreased throughout lactation [13]. After six months of nursing, miR-181a and miR-155 expression levels significantly decreased, which demonstrated how HBM miRNA expression varies depending on the stage of lactation. In the current study among patients with hypertension, the analysis of cell fraction of breast milk has been observed to have the lower mean expression of miR126\*, miR155, miR21, and miR29a as compared to patients without hypertension. Thus, based on the findings from the study, it can be inferred that the expression of miR126\*, miR155, miR21, and miR29a in HBM is impacted if the mother is suffering from hypertension.

### Strength and limitations

The major strength of the current study is the new finding associated with the expression of miRNA in HBM and the associated impact of hypertension on the expression level, which have not been studied in major studies conducted previously. The study is unique in its nature in identifying the association between the expression of miRNA in HBM and the impact of hypertension, wherein the finding of the current study can further contribute to the existing knowledge of the impact of the health conditions of the women on various aspects associated with pregnancy, along with its impact on the fetus, which leaves with new arenas of research. However, the small sample size and lack of literature associated with the research topic, for reviewing can be considered as two mentionable limitations. The consideration of a small sample size in this study may result in impacting the accuracy of the findings which can be considered as a major limitation of the existing study. The need to collect samples from the participants during the specific period of their pregnancy might have resulted in expedience bias in the current research. The consequence of this bias can be dependence of the researcher on one data point and



not taking the required time to receive clarity and understanding of the different aspects of the participants and the samples collected in this study.

## CONCLUSIONS

Based on the findings from the study it has been noted that in context to clinical parameters, among patients with no gestational hypertension, the prevalence of miscarriages is significantly low as compared to patients with gestational hypertension. A similar pattern, with a significantly low rate of hypothyroidism during pregnancy, was observed among patients with no gestational hypertension. Regarding the delivery type, among patients with no gestational hypertension, normal delivery has been observed to be comparatively higher than patients with gestational hypertension. Among patients with hypertension, the analysis of cell fraction of breast milk reported lower mean expression of miR126\*, miR155, miR21, and miR29a as compared to patients without hypertension. Strong and highly strong positive correlation between the expression of miR126\* and miR155, miR126\* and miR21, miR155 and miR21, miR155 and miR29a, and miR21 and miR29a have been noted.

## Article Information and declarations

### Data availability statement

The data presented in this study are available on request from the corresponding author.

### Ethics statement

The study was conducted on the basis of the approval of the Bioethics Committee at the Medical University of Lublin. Each patient provided written consent to collect the material and conduct tests.

### Author contributions

Conceptualization — A.K., M.W.-M., and B.K.; methodology — P.G.-K., A.P., and J.K.; validation — A.K.; formal analysis — P.G.-K., M.W.-M., A.K., and A.P.; writing — original draft preparation — A.K., M.W.-M., P.G.-K., A.P., and A.K.; writing — review and editing — B.K. and J.K.; supervision — B.K. and J.K. All authors have read and agreed to the published version of the manuscript.

### Funding

This study was supported in part by funds from the project “The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases” within the Operational Program Development of Eastern Poland 2007–2013, Priority Axis I Modern

Economy, Operations I.3 Innovation Promotion and in part by National Centre for Research and Medical University grant “DS 222/2022.

### Acknowledgements

None.

### Conflict of interest

Authors declare no conflict of interest.

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