Circulating MicroRNAs in the Screening of Prenatal Down Syndrome

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Objective: Screening tests are recommended to identify genetic defects, chromosomal aneuploidies, and structural birth defects. Sonographic and maternal serum–based options are available for the risk assessment of aneuploidy in the first and/or second trimester. Also, invasive diagnostic methods, such as amniocentesis, are used for prenatal diagnosis, but these methods carry a tangible risk to the fetus. However, in recent years, circulating fetal nucleic acids have a promising moleculer tool in the noninvasive prenatal diagnosis of fetal chromosomal aneuploidies. In this study, we aimed to explore the usability of microRNAs (miRNAs) in this process of prenatal diagnosis.

Methods: Fourteen pregnant patients who were found to be carrying fetuses with congenital anomalies were designated as the patient group; 16 pregnant women identified as being at risk of carrying children with such anomalies—but whose fetuses were later found to be anomaly-free—were assigned to control group 1; and 13 pregnant women who had been screened and who had not been identified as being at risk made up control group 2. An analysis of miRNA expression, isolated from maternal plasma and amniotic fluid samples, was performed by quantitative real-time polymerase chain reaction.

Results: It was found that hsa-miR-629-5p, hsa-miR-320c, hsa-miR-21-5p, hsa-let-7c-5p, hsa-miR-98-5p, hsa-miR-486-5p, hsa-miR-4732-5p, and hsa-miR-181a-5p levels increased in the patient group's maternal plasma compared to that of the control group.

Conclusion: In light of these data, we believe that miRNAs may have an important role in the noninvasive prenatal diagnosis of fetal birth defects, especially Down syndrome. [*P R Health Sci J 2023;42(3):219-225*]

Key words: MicroRNAs, Down syndrome, Prenatal diagnosis, Pregnancy, Congenital anomaly

Congenital anomalies are among the top 20 causes of disease burden worldwide. However, they have also been shown to be significant causes of stillbirths and neonatal deaths (1,2). Although the pattern and prevalence of congenital anomalies may change over time or by geographic location, the global birth prevalence is around 2% to 3%. Congenital anomalies can be classified based on clinical, etiologic, and pathogenetic criteria (3,4). According to World Health Organization data, the most common major congenital anomalies are congenital heart defect, neural tube defect, and Down syndrome (DS) (2,5).

The most common chromosomal disorder is DS (trisomy 21), and although the majority of embryos and fetuses are lost in the first and second trimesters, the incidence 1 in 800 births. Individuals with DS have an extra complete or partial copy of chromosome 21. The mechanisms underlying this condition are meiotic nondisjunction (95% of cases), Robertsonian translocation (4% of cases) and mosaicism (1% of cases) (6–11).

The most common reason for prenatal diagnostic testing is elevated maternal age, which is associated with DS. The combined screening tests recommended for all pregnant women are based on risk calculation, maternal age, nuchal translucency (determined by sonography), and the measurement of pregnancy-associated plasma protein-A and free beta-human chorionic gonadotropin (β-hCG), which last two items are 2 serum markers. Although there is a false positive rate of 5% to 9% (versus an 85% to 90% detection rate), these tests have no diagnostic power. Invasive procedures are necessary to obtain

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fetal cells and to detect fetal aneuploidy and other chromosomal abnormalities. The risks of fetal loss and fetal limb anomalies increase when procedures such as amniocentesis and chorionic villus sampling are performed. Therefore, the use of techniques that have the same specificity and sensitivity, provide a definitive diagnosis, and enable the targeting and analysis of fetal DNA and RNA in maternal plasma is gradually increasing in the field of noninvasive prenatal testing (12–19).

Recent studies on noninvasive prenatal testing suggest that microRNAs (miRNAs) represent another class of promising molecular markers. MicroRNAs are small, 18- to 22-nucleotide long, non-protein-encoding RNA families that emerge as key post-transcriptional regulators of gene expression (19). In recent years, studies on placenta-specific miRNAs have increased, and their release into maternal circulation by exosomes or syncytiotrophoblasts has been demonstrated (20). Furthermore, miRNAs are highly stable and allow real-time quantification, making these molecules an ideal candidate for noninvasive diagnostic purposes (21,22).

Therefore, the aim of this study was to establish a miRNA profile as a noninvasive, predictive biomarker for the prenatal diagnosis of congenital anomalies by evaluating the correlation of miRNAs in amniotic fluid (AF) and maternal plasma.

Materials and Methods

Study group

This study included 43 pregnant women aged 19 to 43 years without any additional medical problems who were admitted to the Obstetrics and Gynecology outpatient clinic of the Medical Faculty Hospital.

All the participants were screened with a physical examination and double- and triple-marker tests. In the study, 14 women whose fetuses had a congenital anomaly made up the patient group. After having been screened, these women were determined to likely be carrying a child with chromosomal abnormalities, which was then confirmed by amniocentesis. Sixteen women made up control group 1. The results of their screening indicated that their fetuses were at risk of suffering from a genetic anomaly, but later testing (amniocentesis) ascertained that they were not. The final group, control group 2, comprised 13 women whose screening showed no risk of anomalies but who nevertheless underwent amniocentesis at the request of their families; all the women were of advanced maternal age and/or had a family history of DS. For these women, the final test confirmed the absence of any abnormalities. Patients with comorbid diseases and who had been pregnant at least once before were excluded from the study.

This study was approved by the Mersin University Clinical Research Ethics Committee, with the board's decision dated January 14, 2016, and numbered 2016/17; a written consent form was obtained from each of the pregnant women who participated in the study.

Sample Collection and Storage

In this study, 49 miRNAs were analyzed, as shown in Table 1. The miRNAs to be analyzed were selected by searching for the terms/phrases "microRNA" or "miRNA," "DS," "trisomy 21," "congenital anomalies," and "prenatal" in articles in the PubMed (MEDLINE) and iRbase databases. For the analysis of miRNA, AF and venous blood were taken from the 43 pregnant women. The blood (in EDTA tubes) and AF samples were centrifuged at 2000 g for 10 minutes without delay. The separated samples were again centrifuged at 2000 g for 10 minutes, and the resulting plasma was collected intosterile microcentrifuge tubes. The obtained samples were stored in a -80 °C freezer for miRNA isolation until the day of study.

MicroRNA Expression analysis

MicroRNA Isolation

In the miRNA isolation stage, the miRNeasy Serum/Plasma Advanced Kit (QIAGEN, catalog no. 217204) was used, and the protocol recommended by the manufacturer was followed. The quality of the total RNA obtained was measured with a Thermo Scientific NanoDrop Spectrophotometer (Delaware, USA), using the recommended protocols.

Preparation of Complementary DNA Reaction

Complementary DNA (cDNA) was obtained using the miScript II RT Kit (QIAGEN, catalog no. 218161). The cDNA master mix was prepared according to the manufacturer's instructions. First, the RNA samples were pipetted into wells. After the wells in which the samples had been loaded were checked, the cDNA mixture was collected and reloaded. The plate was mixed for 2 seconds and then It was centrifuged for 60 seconds in a plate centrifuge and was added to the thermal cycler (37°C 60 minutes, 95 °C 5 minutes). The cDNA samples were diluted at a ratio of 1/5 and prepared for the next process.

Preparation of Preamplification Reaction

The miScript Microfluidics PreAMP Kit (QIAGEN, catalog no. 331455) was used in the preamplification step to increase the amount and quality of cDNA.

In this study, a total of 51 primers, 49 miRNAs, 1 miRNA reverse transcription control, and 1 spike-in control(Cel-miR-39), were explored. First, the PreAMP primer pool was prepared, and the preAMP was added to the mixture. Diluted cDNA samples and the PreAMP mixture were pipetted. The following thermal cycling parameters for preamplification; 12cycles at 95 °C for 15 minutes, at 94 °C for 30 seconds, at 60 °C for 3 minutes, and at 4 °C for at least 10 minutes.

At the end of the thermal cycling the plate was centrifuged for 60 seconds. A mixture of Exonuclease I was then prepared and pipetted. After the mixing, vortexing, and centrifugation processes, the PCR program applied to the mixture is as follows; 37 °C for 15 minutes, 95 °C for 5 minutes, and 4 °C for at least 10 minutes. The plate was centrifuged at the end of the PCR program, and the PreAMP samples were diluted at a ratio of 1/5.

Real-time Polymerase Chain Reaction Mix and Primer Preparation

At this stage, the miScript Microfluidics PCR Kit (QIAGEN, catalog no. 331431) was used, and the primers were made ready for loading into the BioMark HD System (Fluidigm, San Francisco, USA), following the manufacturer's protocol.

2 µl of diluted preamplified sample together with 4 µl of the real-time polymerase chain reaction (RT-PCR) mixture were pipetted. The plate was subjected to mixing, vortexing, and centrifugation processes.

Dynamic Array Preparation

The control fluids were injected into small cells in a 96.96 Dynamic Array and primed in the Dynamic Array IFC Controller. The primers prepared were pipetted into the wells in the assay inlets of the Dynamic Array and the PCR mixture into the wells in the sample inlets of the 96.96 Dynamic Array Loading was started after the 96.96 Dynamic Array placed in the IFC Controller. While this process was taking place, the BioMark Data Collection software was started. After the loading of the Dynamic Array was completed, and the Dynamic Array was placed in the BioMark.

Statistical analysis

The analysis of the RT-PCR results (cycle threshold [Ct] values) was calculated by 2-ΔΔCt, and after these values were calculated, a basic student's t test method was used to make comparisons between groups. In the statistical analysis, the QIAGEN data analysis website (http://www.qiagen.com/us/shop/genes-and-pathways/data-analysis-center-overview-page/) was utilized, and the analysis was performed with global Ct mean normalization.

Spearman's correlation coefficient was calculated to determine the correlation between Ct values obtained from AF and plasma. The analyses were performed using the Statistica v.13.3 software. The level of statistical significance was set at a p value less than .05.

Results

Results of the Descriptive Information of the Pregnant Women in the Study Group

The mean age of the participants was 34.442 (±5.0535) years and the mean gestational age of the fetuses of the 43 pregnant women who met the inclusion criteria was 17.256 (±1.4325) weeks. The distribution of the reasons for amniocentesis (by group) is given in Table 2.

MicroRNA Expression Levels in Plasma Samples

In this study, all the participants in the patient group were pregnant women with chromosomal anomalies, meaning that all the data obtained from the samples that were acquired from the women in this group were analyzed within the framework of chromosomal anomalies, which are the most common congenital anomalies. The sample size of pregnant women carrying fetuses with DS was large enough to be statistically evaluated, thus enabling us to explore which miRNAs might possibly be used for a noninvasive diagnosis of this syndrome.

Compared to the Control groups, expression changes of 43 miRNAs in the patient group were detected in the plasma samples taken from the pregnant women. The miRNAs that showed significant differences after the the comparison are given in Table 3.

MicroRNA Expression Levels in Amniotic Fluid Samples

Compared to the control groups, the expression changes of 43 miRNAs in the AF samples (obtained from the same pregnant women from whom the plasma samples were obtained) of the patient group were also analyzed. The miRNAs that showed significant differences upon comparison can be seen in Table 4.

Results of the Correlation Between MicroRNA Expression Levels in Plasma and Amniotic Fluid Samples. The correlations between the expression levels of hsa-miR-629-5p, hsa-miR-320c, hsa-miR-21-5p, hsa-let-7c-5p, hsa-miR-98-5p, hsa-miR-486-5p, hsa-miR-4732-5p, and hsa-miR-181a-5p, which showed significant upregulation in the plasma samples of the patient group with chromosomal anomalies, were evaluated in the AF samples.

Table 1. List of miRNAs analyzed

hsa-miR-155-5p	hsa-miR-125a-5p	hsa-miR-483-5p	hsa-miR-196b-5p	hsa-miR-1301-3p
hsa-miR-126-3p	hsa-miR-92a-2-5p	hsa-miR-20a-5p	hsa-miR-181a-5p	hsa-miR-16-5p
hsa-miR-629-5p	hsa-miR-1298-5p	hsa-miR-10b-5p	hsa-miR-320b	hsa-miR-30c-5p
hsa-miR-23c	hsa-let-7c-5p	hsa-miR-98-5p	hsa-miR-363-3p	hsa-miR-210-3p
hsa-miR-19b-2-5p	hsa-miR-223-3p	hsa-miR-513a-3p	hsa-miR-141-3p	hsa-let-7f-2-3p
hsa-miR-320c	hsa-miR-124-5p	hsa-miR-99a-5p	hsa-miR-188-3p	hsa-miR-504-5p
hsa-miR-199b-3p	hsa-miR-335-5p	hsa-miR-486-5p	hsa-miR-92b-5p	hsa-miR-1233-3p
hsa-miR-802	hsa-miR-374b-3p	hsa-miR-4732-5p	hsa-miR-498	ahsa-miR-374a
hsa-miR-21-5p	hsa-miR-891b	hsa-miR-222-5p	hsa-miR-221-3p	hsa-miR-3156-5p
hsa-miR-362-3p	hsa-miR-125b-2-3p	hsa-miR-133b	hsa-miR-135b-5p	

Table 2. Distributions of the reasons for amniocentesis in the patient, control 1, and control 2 groups.

Reason for ordering amniocentesis	Patient (n = 14) n (%)	Control 1 (n = 16) n (%)	Control 2 (n = 13) n (%)
Double risk	3 (21.4%)	10 (62.5%)	---
Triple risk	---	2 (12.5%)	---
Quadruple risk	1 (7.1%)	4 (25.0%)	---
Harmony test results	1 (7.1%)	---	---
USG (NFT, CP cyst, congenital heart anomaly, etc.)	7 (50.0%)	---	3 (23.1%)
Family history of Down syndrome	----	---	7 (58.2%)
Advanced maternal age	2 (14.2%)	---	3 (23.1%)
Total	14 (100%)	16 (100%)	13 (100%)

USG: ultrasonogram; NFT: nuchal fold thickness; CP: choroid plexus

The miRNAs expressed in the plasma and AF samples with significant correlations between them were hsa-miR-629-5p (R = -0.900; *P* = .037), hsa-miR-320c (R = -0.738; *P* = .037), and hsa-miR-486-5p (R = -0.829; *P* = .042).

Discussion

Studies have shown that the use of nucleic acids in maternal blood to detect fetal birth defects is easier and less complex than are the current noninvasive methods (23). Pettit et al. (24) evaluated the usability of circulating cell-free fetal DNA as a noninvasive prenatal test instead of amniocentesis and routine chorionic villus sampling, both of which are performed in the presence of advanced maternal age. The results of this study indicated that the participating pregnant women of advanced maternal age preferred the noninvasive method that uses fetal DNA in maternal blood. However, with the discovery of placental miRNAs in the maternal plasma, miRNAs have also garnered attention in much the same way that fetal DNA has and have been proposed as biomarkers that can be used in noninvasive diagnoses (19,25). The first study on this was published by Go et al. (26) in 2008, and an in vitro method of screening for syncytiotrophoblast-derived RNA products representing placental RNA associated with trisomy 21 was described.

Kotlabova et al. (27) showed that plasma concentrations of chromosome 21–derived miRNAs (miR-99a, let-7c, miR-125b-2, miR-155, and miR-802) were significantly increased in pregnant women compared to non-pregnant women. However, there was no significant difference in the expression levels of extracellular chromosome 21–derived miRNAs in the at-risky group of pregnant women compared to those women with a normal pregnancy. In our study, in the patient group according to our inclusion criteria and who had fetuses diagnosed with DS, there was a significant difference in the expression level of only let-7c from these 5 miRNAs.

Although these 5 chromosome 21–derived miRNAs have been shown to be associated with the variable phenotypes of DS, the genome-wide changes in the miRNA expression of fetuses with DS have not yet been fully determined (28). Rather than continuing to conduct studies to identify the correlation between the numerical chromosomal anomalies that are associated with DS and miRNAs expressed on chromosome 21, it has been proposed to investigate not only the miRNAs located in chromosome 21 but also miRNAs of all other types, because miRNAs regulate multiple genes and more than 1 miRNA can regulate the same gene (29).

Since the cause or causes of changes in miRNA expression in maternal plasma has not been fully determined, genome-wide changes in miRNA expression in fetal placentas in DS, and the function of these changes have not yet been fully determined. A study reported that 34 miRNAs were expressed significantly

Table 3. Significantly varying plasma miRNA expression levels in the patient group compared to the control groups.

miRNA	DS compared to C2		DS compared to C1		DS compared to control groups	
	FR	P	FR	P	FR	P
hsa-miR-629-5p	2.34	.064	1.77	.0572	2.00	.008
hsa-miR-320c	1.21	.165	1.24	.107	1.22	.0416
hsa-miR-21-5p	1.27	.108	1.27	.0597	1.27	.020
hsa-let-7c-5p	1.57	.131	1.55	.089	1.56	.022
hsa-miR-98-5p	1.44	.141	1.43	.081	1.43	.035
hsa-miR-486-5p	1.47	.150	1.49	.095	1.49	.030
hsa-miR-4732-5p	1.57	.183	1.99	.125	1.79	.040
hsa-miR-181a-5p	1.64	.162	1.59	.062	1.62	.045

miRNA: microRNA; DS: Down syndrome; C1-2; control 1-2 group; FR: F ratio

Table 4. Significantly varying miRNA expression levels in amniotic fluid from the patient group compared to the control groups.

miRNA	DS compared to C2		DS compared to C1		DS compared to control groups	
	FR	P	FR	P	FR	P
hsa-miR-155-5p	1.49	.087	1.25	.194	1.35	.049
hsa-miR-126-3p	-1.55	.241	-2.32	.028	-1.94	.065
hsa-miR-19b-2-5p	1.67	.075	2.03	.016	1.86	.003
hsa-miR-199b-3p	1.74	.058	2.09	.129	1.93	.043
hsa-miR-21-5p	-5.25	.061	-8.08	.003	-6.66	.008
hsa-miR-362-3p	-1.69	.120	-2.03	.035	-1.87	.044
hsa-miR-92a-2-5p	1.63	.083	1.75	.138	1.69	.043
hsa-miR-1298-5p	1.83	.053	1.95	.019	1.89	.003
hsa-miR-124-5p	1.51	.107	1.92	.025	1.73	.009
hsa-miR-335-5p	1.69	.036	1.30	.070	1.46	.007
hsa-miR-374b-3p	1.72	.063	1.99	.016	1.86	.003
hsa-miR-125b-2-3p	1.88	.018	2.67	.003	2.28	.000
hsa-miR-20a-5p	-4.53	.082	-5.25	.015	-4.91	.019
hsa-miR-10b-5p	-1.79	.435	-3.18	.010	-2.46	.050
hsa-miR-513a-3p	1.83	.053	2.05	.015	1.94	.002
hsa-miR-222-5p	1.55	.120	1.82	.056	1.70	.015
hsa-miR-133b	1.61	.045	1.44	.063	1.51	.009
hsa-miR-196b-5p	-3.02	.007	-3.84	.001	-3.45	.001
hsa-miR-141-3p	-2.15	.030	-2.22	.045	-2.19	.029
hsa-miR-1301-3p	2.25	.034	2.18	.014	2.21	.001
hsa-miR-30c-5p	-3.14	.129	-4.34	.004	-3.75	.011
hsa-miR-210-3p	1.02	.857	1.39	.031	1.21	.399
hsa-let-7f-2-3p	1.64	.088	1.76	.027	1.71	.007
hsa-miR-504-5p	1.69	.070	1.73	.034	1.72	.006

miRNA: microRNA; DS: Down syndrome; C1-2; control 1-2 group; FR: F ratio

differently in the placenta carrying a fetus with DS than in the placenta carrying a fetus with no such anomaly. It has been reported that these miRNAs are not chromosome 21–derived miRNAs but that their target genes are localized on chromosome 21 and that these target genes are significantly associated with DS and DS-related disorders, such as mental retardation, neurobehavioral symptoms, and congenital abnormalities (30). In DS, the potential effects of the unbalanced expression of genes on chromosome 21 are considered to be the main etiology of this disease. Nevertheless, genes that are potentially regulated by miRNAs are not limited to a particular chromosome and may affect the expression of various genome-wide target genes. In other words, other chromosome-derived miRNAs can contribute to DS phenotypes through the target genes located in chromosome 21.

In this conducted study, in line with this was not limited to a panel including only miRNAs of placental origin or chromosome 21–derived miRNAs while determining the miRNAs that can be used as a noninvasive method. Along with these miRNAs, miRNAs thought to be associated with chromosomal abnormalities and located in different chromosomes were also included in the analysis.

In the study by Kamhieh-Milz et al. (31), miRNA levels were analyzed in the maternal plasma of 7 pregnant women carrying fetuses with DS and in that of a control group. The miRNAs analyzed were found to be associated with mucin-type-O-glycans (associated with β-hCG), extracellular matrix-receptor interactions, the transforming growth factor beta signaling pathway, and endocytosis. Mucin-type O-glycan was demonstrated to be closely associated with hsa-miR-498, hsa-miR-30c-5p, hsa-miR-629-5p, and hsa-miR-195-3p. As a result of these data, the researchers suggest that miRNAs might be promising and stable biomarkers that could form a reliable, cost-effective diagnostic tool for the noninvasive screening of DS. In our study, of these miRNAs, miR-629-5p, miR-486-5p, and has-miR-181a-5p were found to be upregulated in the plasma of the pregnant women carrying a fetus with DS.

Apart from chromosome 21–derived miRNAs associated with DS, the investigation of the genome-wide miRNA expression profile associated with DS will provide a better understanding of the role of miRNAs in the development of a DS fetus. Numerous identified and differently regulated miRNAs, which are not chromosome 21–derived but have been found to be associated with DS, have been reported to be involved in various cellular processes, such as cell proliferation, differentiation, apoptosis, granulocyte maturation, and activation (32).

When the target genes of miRNAs, the expression levels of which vary in DS, were analyzed, it was found that the majority of the miRNA targets—such as superoxide dismutase-1, which is targeted by hsa-miR-1, miR-320b, miR-196b, miR-4732-5p, and miR-486-5p, respectively—were genes associated with immune modulation (28,33). These miRNAs have been shown to be the cause of hemopoietic abnormalities and immune defects in DS neonates and fetuses (28).

Lim et al. (34) aimed to establish a panel of miRNAs that could be used as potential biomarkers in the noninvasive prenatal testing of DS and to determine the biological functions of these biomarkers that were detected using bioinformatic tools. According to the results of the study, Lim and her team suggested that placental-specific miRNAs may be potential biomarkers for the noninvasive diagnosis of fetal DS.

In our study, which simultaneously used plasma and AF samples from the patient and 2 control groups (all of which having been determined according to the inclusion criteria), the intergroup analyses of the plasma and AF samples were performed separately, and different miRNAs and different expression levels were obtained. Given the correlation between the plasma and AF samples, the correlations between hsa-miR-629-5p, hsa-miR-320c, and hsa-miR-486-5p were significant.

In the AF samples, which were studied simultaneously with the maternal plasma samples, it was found that there was a significant change in the expression levels of a total of 22 miRNAs (15 miRNAs upregulated and 7 miRNAs downregulated), comparing the patient group with the control groups. Our aim in using AF samples obtained by amniocentesis, which is an invasive method but is required for diagnosis, was to determine a miRNA expression profile in the AF and to evaluate its correlation with the plasma samples that were taken simultaneously. Since there are very limited data on this subject, we think that ours should be added to by further studies and will lead to the establishment of a miRNA expression profile in AF. In addition, we anticipate that screening using miRNAs will reduce the amount of the amniocentesis-collected sample necessary for chromosome analysis and will yieldresults in a much shorter period.

The primary objective of our study was to establish a miRNA profile that could be used in the detection of DS in the fetuses of pregnant women, and do so using an early, easy, and noninvasive method. Of course, the reason(s) for the change in the expression levels of identified miRNAs should also be determined. While our study results are generally similar with those of other, similar studies, different results can be obtained because of the numerous differences in study design, including different sample sources (cord blood cells and fetal tissue), different sample collection procedures, different methods of sample preparation, and different methodologies used to determine expression levels.

In conclusion, although noninvasive prenatal testing (NIPT), in its current form, is particularly specific to conditions involving chromosomes 13, 18, and 21, it is necessary to broaden the scope of this method and improve screening programs. Although there is still a need for more data if we are to use miRNAs in a NIPT test that can be integrated into a fast, inexpensive, accurate, and routine clinical procedure (especially for the noninvasive detection of chromosomal abnormalities in the early stages of pregnancy), we think that miRNAs, in particular, may have an important role in the noninvasive prenatal diagnosis of DS.

Resumen

Objetivo: Se recomiendan pruebas de detección para identificar defectos genéticos, aneuploidías cromosómicas y defectos congénitos estructurales. Existen opciones ecográficas y basadas en suero materno para la evaluación del riesgo de aneuploidía en el primer y/o segundo trimestre. Además, los métodos de diagnóstico invasivos, como la amniocentesis, se utilizan para el diagnóstico prenatal, pero estos métodos conllevan un riesgo tangible para el feto. Sin embargo, en los últimos años, los ácidos nucleicos fetales circulantes se han convertido en moléculas prometedoras en el diagnóstico prenatal no invasivo de las aneuploidías cromosómicas fetales. En este estudio se tuvo como objetivo indagar sobre la usabilidad de los microARN (miARN) en el diagnóstico prenatal. Métodos: Catorce pacientes embarazadas que se encontró que tenían un feto con anomalías congénitas como grupo de pacientes, y 16 mujeres embarazadas que fueron identificadas como grupo de riesgo y 13 mujeres embarazadas cuyo riesgo no fue identificado como resultado de la prueba de detección como grupo de control se incluyeron en el estudio. El análisis de las expresiones de miARN, aislado de muestras de líquido amniótico y plasma materno, se realizó mediante qRT-PCR. Resultados: Se encontró que las niveles de hsa-miR-629-5p, hsa-miR-320c, hsa-miR-21-5p, hsa-let-7c-5p, hsa-miR-98-5p, hsa-miR-486-5p, hsa-miR-4732-5p y hsa-miR-181a-5p aumentaron cuando el plasma materno del grupo de pacientes se comparó con el del grupo de Control. Conclusión: En la dirección de estos datos, los miARN pueden tener un lugar importante en el diagnóstico prenatal no invasivo, especialmente en el síndrome de Down.

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