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Levels of miR-21and miR-182 in Unexplained Recurrent Spontaneous Abortion

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Abstract: MicroRNAs are small non-coding RNAs involved in regulating gene expression. Recurrent spontaneous abortion (RSA) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation. The aim of this study was to explore the expression level of three pregnancy-associated miRNAs in maternal plasma in normal pregnancy and RSA cases. We conducted a case control study on a 100 Palestinian women: 60 patients with at least two unexplained consecutive pregnancy losses (half of them were in first trimester pregnant and the other half were non-pregnant) and 40 healthy controls with at least two live births and no history of pregnancy loss (half of them were in first trimester of pregnancy and the other half were non-pregnant). We investigated the relative expression of miR-21, miR-155 and miR-182 using quantitative real-time polymerase chain reaction and Ct method experiments. Differential expression was evaluated using Student t-test and fold change analyses. The expression difference of miR-21 and miR-182 between patients and controls in the pregnant subjects showed statistically significant difference (p-value ≤ 0.05) with fold decrease of 1.5 and 5.6, respectively. In the non-pregnant women miR-21 expression was also significantly different with fold decrease of 2.4. In conclusion, miR-21 could be a novel marker for idiopathic RSA as its level was significantly lower in patients before being pregnant and during pregnancy.

Keywords: miRNA; miR-21; recurrent spontaneous abortion.

Introduction

miRNAs are a family of 21–25-nucleotide small RNAs that, at least for those few that have characterized targets, negatively regulate gene expression at the post-transcriptional level [1]. miRNAs function as guide molecules in post-transcriptional gene silencing by base pairing with target mRNAs, which leads to mRNA cleavage or translational repression [2]. It is predicted that over one third of all human genes are targeted by miRNAs. Consequently, the unique combination of miRNAs that are expressed in each cell type might affect the utilization of thousands of mRNAs [3]. Since miRNAs have key roles in diverse regulatory pathways, including control of developmental timing, hematopoietic cell differentiation, apoptosis, cell proliferation, embryonic development and organ development, dys-regulation of miRNA has been associated with many diseases like cancer, cardiac hypertrophy, ischemic heart disease, alzheimer's disease, parkinson's disease, schizophrenia [4] and preeclampsia [5].

In the year of 2008, Chim *et al*., reported the presence of placental microRNAs in maternal plasma [6]. Those miRNAs can be used to obtain valuable information about the fetus or pregnancy, either for prenatal diagnosis or monitoring or for the detection of pregnancy disorders such as fetal growth restriction [7].

RSA is the occurrence of two or more consecutive pregnancies that end in miscarriage of the fetus, usually before 20 weeks of gestation. RSA affects about 1–3% of women who conceive. Today, clinical practice includes testing of several factors potentially increasing the risk of RSA, e.g., parental chromosomal anomalies, maternal thrombophilic, anatomic, endocrine, and immunological disorders [8]. However, around 50% of the RSA cases have no deviations in any of the currently applied diagnostic tests and are considered idiopathic, i.e., of unexplained origin. In addition to clinical, environmental, and life-style risk factors, there is growing evidence that RSA has also genetic susceptibility [9].

The current study was carried out in order investigate the relation between the expression level of three selected pregnancy-associated miRNAs (miR-21, miR-155 and miR-182) in maternal plasma and RSA in a group of Palestinian women residing in Gaza strip.

Materials and Methods

After having the subjects' written informed consent and the approval of the local ethics committee, samples of peripheral blood (4 mL) were collected into EDTA tubes from a 100 women: 60 patients with at least two unexplained consecutive pregnancy losses half of them were pregnant at the first trimester and the rest were non-pregnant and 40 healthy controls with at least two live births and no history of pregnancy loss; half of them were at their first trimester of pregnancy and the rest were non-pregnant. Both groups were recruited from Gaza strip private Ob/Gyn clinics, IVF centers and

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hospitals. All subjects were in the age 18-35 years and their husbands were not their family relatives. To harvest cell-free plasma, whole blood samples were centrifuged twice at 1200×g for 10 min at room temperature. RNA was extracted immediately after sample collection using miRNeasy RNA isolation kit (Qiagen, USA), according to the manufacturer protocol.

For this study three miRNAs were selected (miRNAs 21, 155 and 182). This set of miRNAs was analyzed using the real-time RT–PCR scheme for miRNA quantification according to the protocol of Applied Biosystems (P/N: 4364031); this two-steps protocol consists of reverse transcription with a miRNA-specific primer, followed by real-time PCR with TaqMan probes. The TaqMan miRNA assays used were also purchased from Applied Biosystems. In brief, for each RT–PCR 10ng RNA was reverse transcribed to cDNA using µl specific looped RT primers (Applied Biosystems, USA). The 15µl reactions were incubated in a BioRad thermo cycler for 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C and then kept at 4 °C. Real-time PCR was performed in duplicate using a standard protocol on the Applied Biosystems 7500 real time PCR System. The reactions were incubated in a 96-well plate at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. In each sample the relative amount of miRNA was calculated using the comparative threshold method using miR-223 as the endogenous control with ΔCt= Ct (miRNA) – Ct (miR-223). Relative quantification of miRNA expression was calculated $2^{-\Delta Ct}$ method which was calculated using the following formula

$2^{-\Delta Ct}$ = (2^{-}(Ct selected miRNA – Ct endogenous control)).

The data was analyzed by SPSS software (version 14). The independent sample t-test was used for mean comparisons.

Results

The results indicated that the expression difference of miR-21 and miR-182 between the pregnant patients and controls is statistically significant with fold decrease of 1.5 and 5.6, respectively. Tables 1 illustrate the mean difference and the fold change comparison of the three investigated miRNAs between patients and controls in the pregnant group.

Table.1: The mean difference and fold change in expression of the selected miRNAs in the pregnant group.

miRNA	Expression Difference	Mean Difference ± Std. Error	Fold Decrease (-1 / expression difference)	95% C.I.	p-value
miR-21	0.68	-0.138 ± 0.068	-1.5	-0.276 to -0.001	0.049
miR-155	0.44	-0.157 ± 0.223	-2.3	-0.621 to 0.306	0.488
miR-182	0.18	-0.292 ± 0.142	-5.6	-0.578 to -0.006	0.046

*p-value was calculated using independent sample t-test, the proportion difference is statistically significant at ≤ 0.05 level; C.I: confidence interval

In the non-pregnant group only miR-21 expression was significantly different with fold decrease of 2.4. In regard to miR-155 and miR-182, both of them were also lower in the RSA group with respective fold decrease of 12.5, 1.06 but their decline was not statistically significant (Tables 2).

Table.2: The mean difference and fold change in expression of the selected miRNAs in the non-pregnant group

miRNA	Expression Difference	Mean Difference ± Std. Error	Fold Decrease (-1/expression difference)	95% C.I.	p-value
miR-21	0.42	-0.239 ± 0.909	-2.4	-0.422 to -0.562	**0.011**
miR-155	0.08	-0.282 ± 0.142	-12.5	-0.568 to 0.466	0.054
miR-182	0.94	-0.685 ± 0.115	-1.06	-0.239 to 0.225	0.953

*p-value was calculated using independent sample t-test, the proportion difference is statistically significant at ≤ 0.05 level; C.I: confidence interval

Discussion

The success of pregnancy depends, to a great extent, on events occurring during the early stages of gestation, such as implantation of the blastocyst, trophoblast differentiation, invasion of the endometrium by the trophoblasts, establishment of feto-maternal vascular circuitry and enhanced blood supply through the maternal arteries to the placenta. These events require coordination and regulation of expression of a diverse array of genes simultaneously, a task that, we suggest here, could be, at least partly, achieved by the involvement of specific miRNAs. Especially that, one particular miRNA can regulate the post-transcriptional expression of many genes at the same time.

Over the past three years only few studies pointed to the potential role of miRNAs in RSA. For example, Manaster *et al.,* have shown that the expression of HLA-G (a gene expressed predominantly at feto-maternal interface) is modulated by a 3` UTR polymorphism that alter the HLA-G mRNA affinity to microRNAs (miR-148a, miR-148b, and miR152) and hence its differential degradation and translation suppression processes [10]. Rull *et al.,* have reported an association between two SNPs in pre-miR-125a and increased risk to RSA [9]. Moreover, Noack *et al.,* have indicated that the profile of several placenta-specific miRNAs are altered in pregnancy complications [11].

The three miRNAs investigated in the current study were selected because earlier studies have shown that they are involved in cellular proliferation, invasion and migration abilities, differentiation, adhesion, apoptosis and angiogenesis [12-14], events that are essential for pregnancy.

In the present study miR-21 was found to be significantly associated with increased risk of idiopathic RSA, as its level was highly decreased in pregnant and non-pregnant RSA subjects when compared to corresponding healthy women (Tables 1 and 2). A result that points to a potential role of miR-21and its target genes in maintaining a healthy pregnancy. Consistent with our results, Maccani *et al.,* in their analysis of term human placentas observed that the expression of miR-16 and miR-21 were markedly reduced in infants with the lowest birth weights [15].

Various genes have been reported to be the target of miR-21 but we believe that *PTEN* gene is the candidate that might be involved in the etiology of RSA as it has been implicated in cellular proliferation, invasion and migration abilities [16, 17]. Interestingly, Tokyol *et al.,* (2008) have shown that altered patterns of *PTEN* expression may be associated with abortion [18]. miR-182 was found to be 5.6 fold deceased in the pregnant RSA patients (p=0.046) but its decline in the non-pregnant group was not significant. miR-182 regulates a plenty of genes most of which function in cell invasion, cell migration and cell cycle regulation which are essential for a healthy pregnancy [19-21]. Thus, dys-regulation of this miRNA might also be responsible for increasing the risk of RSA.

Although the level of miR-155 was also decreased in the RSA patients it did not, however, reach statistical significance. The fold decrease was 2.3 (p= 0.488) in pregnant group and 12.5 (p= 0.054) in non-pregnant group. Several targets and activities pertinent to pregnancy have been associated with this miRNA including: placental development and vascular integrity through regulation of *CYR61(CCN1)* gene [22,23], regulating endothelium-dependent vaso-relaxation by targeting endothelial nitric oxide synthase gene [24], remodeling of human-trophoblast-derived HTR-8/SVneo cells [25] and pathogenesis of severe pre-eclampsia [26].

In light of the obtained results we can conclude that proper levels of miR-21 and miR-182 are important in pregnancy. The level of miR-21 could serve as a potential marker for predicting RSA as its level was significantly reduced not only in the pregnant but also in the non-pregnant RSA subjects. Moreover, restoration of the normal level of miRNAs might be a novel treatment strategy in unexplained RSA.

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References

1. He L and Hannon GJ (2004). MicroRNAs: small RNAs with a big role in gene regulation. Nature Reviews: Genetics, **5**: 522-531.

2. Chen K and Rajewsky N (2007). The evolution of gene regulation by transcription factors and microRNAs. Nature Reviews-Genetics, 8: 93-103.

3. Lewis B, Burge C and Bartel DP (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell, 1: 15-20.

4. Sayed D and Abdellatif M (2011). MicroRNAs in development and disease. Physiol Rev, 91 (3): 827-887.

5. Pineles BL, Romero R, Montenegro D, Tarca AL, Han Y, M, Kim YM, Draghici S, Espinoz J, Kusanovic JP, Mittal P, Hassan SS and Kim CJ (2007). Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol, 196 (3): 261 e261-266.

6. Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW and Lo YM (2008). Detection and characterization of placental microRNAs in maternal plasma. Clin Chem, 54 (3): 482-490.

7. Morales Prieto DM and Markert UR (2011). MicroRNAs in pregnancy. J Reprod Immunol, 88 (2): 106-111.

8. Christiansen OB, Steffensen R Nielsen, HS and Varming K (2008). Multifactorial etiology of recurrent

miscarriage and its scientific and clinical implications. Gynecol Obstet Invest, 66 (4): 257-267.

9. Rull K, Nagirnaja L and Laan M (2012). Genetics of recurrent miscarriage: challenges, current knowledge, future directions. Front Genet, 3: 34.

10. Manaster I, Goldman-Wohl D, Greenfield C, Nachmani D, Tsukerman P, (2012). MiRNA-Mediated Control of HLA-G Expression and Function. PLoS ONE 7(3): e33395.

11. Noack F, Ribbat-Idel J, Thorns C. (2011). miRNA expression profiling in formalin-fixed and paraffin-embedded placental tissue samples from pregnancies with severe preeclampsia. Journal of Perinatal Medicine, 39(3) :267-71.

12. Malumbres M and Carnero A. (2003). Cell cycle deregulation: a common motif in cancer. Prog Cell Cycle Res, 5: 5-18.

13. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, . (2009). Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor." Proc Natl Acad Sci (USA), 106 (6): 1814-1819.

14. Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X and Tang H (2012). MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. FEBS J, 279 (7): 1252-1260.

15. Maccani M, Padbury J and Marsit C (2011). miR-16 and miR-21 expression in the placenta is associated with fetal growth." PLoS One, 6 (6): e21210.

16. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST and Patel T (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer." Gastroenterology, 133 (2): 647-658.

17. Lou Y, Yang X, Wang F, Cui Z and Huang Y. (2010). MicroRNA-21 promotes the cell proliferation, invasion and migration abilities in ovarian epithelial carcinomas through inhibiting the expression of PTEN protein." Int J Mol Med, 26 (6): 819-827.

18. Tokyol C, Aktepe F, Husniye Dilek F and Yilmazer M. (2008). Comparison of placental PTEN and beta1 integrin expression in early spontaneous abortion, early and late normal pregnancy. Ups J Med Sci, 113 (2): 235-242.

19. Zhang L, Liu T, Huang Y, Liu J (2011). microRNA-182 inhibits the proliferation and invasion of human lung adenocarcinoma cells through its effect on human cortical actin-associated protein. International Journal of Molecular Medicine, 28(3):381-388.

20. Hirata H, Ueno K, Shahryari V, Deng G, Tanaka, Y (2013). MicroRNA-182-5p Promotes Cell Invasion and Proliferation by Down Regulating FOXF2, RECK and MTSS1 Genes in Human Prostate Cancer. PLoS ONE 8(1): e55502.

21. Bin Y, Tianmei Q, Yongjun W, Songlin Z, Guohui D, Fei D, and Xiaosong G. (2012). miR-182 inhibits Schwann cell proliferation and migration by targeting FGF9 and NTM, respectively at an early stage following sciatic nerve injury. Nucleic Acids Research, 40(20): 10356–10365.

22. Zhou D, Herrick DJ, Rosenbloom J and Chaqour B (2005). Cyr61 mediates the expression of VEGF, alphav-integrin, and alpha-actin genes through cytoskeletally based mechanotransduction mechanisms in bladder smooth muscle cells. J Appl Physiol, **98** (6): 2344-2354.

23. Mo FE, Muntean AG, Chen CC, Stolz DB, Watkins SC, Lau LF. (2002). CYR61 (CCN1) is essential for placental development and vascular integrity. Mol Cell Biol, 22(24):8709-20.

24. Sun HX, Zeng DY, Li RT, Pang RP, Yang H, Hu YL, Zhang Q, Jiang Y, Huang LY, Tang YB, Yan GJ, Zhou JG. (2012). Essential role of microRNA-155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension, 60(6):1407-1414.

25. Dai Y, Diao, Z, Sun H, Li R, Qiu Z., Hu Y. (2011). MicroRNA-155 is involved in the remodelling of human-trophoblast-derived HTR-8/SVneo cells induced by lipopolysaccharides. Human Reproduction, 26(7):1882-1891.

26. Cheng W, Liu T, Jiang F, Liu, C, Zhao X, Gao Y, Wang H, Liu Z. (2011). microRNA-155 regulates angiotensin II type 1 receptor expression in umbilical vein endothelial cells from severely pre-eclamptic pregnant women. International Journal of Molecular Medicine, 27(3):393-399.

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